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Seven-Arms Starfish "*Luidia maculata*" Characteristics of Coelomocytes and Promising GSK3-β Protein for Wound Healing: Cellular and Chemical Analyses

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ABSTRACT

The coelomic fluid (haemocoel) and coelomocytes of echinoderms were scarcely studied in Egypt. Remarkably, the most popular species is the five-arms starfish. While, in this work, the microscopical and chemical characteristics of the 7-arms giant starfish "***Luidia maculata***" were addressed. The starfish were gathered at 7 meters' depth in the seawater of Ras Sedr, Gulf of Suez, Red Sea, Egypt. As far as we know, no other microscopical data have been published on this species in Egypt. The extracted coelomic fluid of this giant starfish "***Luidia maculata***" was microscopically and chemically analyzed using TEM and GC/MS, respectively. The exhibited data showed the presence of glycogen synthase kinase3-β protein by protein docking, which is considered a promising constituent for wound healing. Finally, this work spotted the light on novel documented data on the microscopical and chemical characteristics of barely studied species of starfish "***Luidia maculata*",** in which the studied coelomocytes showed an amoeboid state resembling the human white blood cells. The examined flagella displayed a typical microtubule arrangement "**9+2** array". The coelomic fluid extracted from "***Luidia maculata***" exhibits glycogen synthase kinase3-β (**GSK3-β**) protein, which could be considered a promising biovital constituent for many medicinal applications including wound healing in diabetics.

INTRODUCTION

The binomial nomenclature of the concerned animal in this search is *Luidia maculata* **(Müller & Troschel, 1842)**. Few articles were published on *Luidia maculata*, and none of the published data from Egypt addressed the microscopical or chemical characteristics of this animal. At the same time, the data published about *Luidia maculata* is promising and concerned with the extraction of bioactive materials from the starfish's whole body **(Kawatake *et al.*, 1997, 1999, 2002, 2004; Inagaki *et al.*, 2006; Higuchi *et al.*, 2007)**. Other publications were concerned with the population distribution of this starfish, its relation with certain crabs, antioxidant properties from their dried bodies and the preliminary phylogenetic

analysis in China with cytochrome oxidase subunit I (COI) sequences **(Bos *et al.*, 2008; Rajan *et al.*, 2012; Chamundeeswari *et al.*, 2013; Ning *et al.*, 2013; Suguna *et al.*, 2014; Yaghmour & Whittington-Jones, 2018)**. The officially-documented geographical distribution of *Luidia maculata* is in parts of the Mediterranean Sea and the Atlantic Ocean, these starfish can withstand both tropical and temperate or sub-boreal environments, the coast of Europe (including the British Isles), the north of Shetlands and the south such as Cape Verde **(Cooke, 1971; Grzimek, 1972; Parker, 1982; Piction, 1996)**.

Phylum Echinodermata was found in the Red Sea, which makes it pharmacologically a distinctive fortune due to its usage as a potent antioxidant, anticancer and antimicrobial **(Youssef *et al.*, 2013; El-Hossary *et al.*, 2020; Abuhijjleh *et al.*, 2021; Khalil *et al.*, 2022)**. There is no doubt that echinoderms showed high regenerative properties of their amputated parts. Most bioactive materials were extracted from the echinoderms' dried bodies **(Kawatake *et al.*, 1997, 1999, 2002, 2004; Inagaki *et al.*, 2006; Higuchi *et al.*, 2007; Dai *et al.*, 2016; Khalil *et al.*, 2022**), except few published articles that addressed the coelomic extract (**Soleimani *et al.*, 2021; Abdel-Ghaffar & Youssef, 2022; Abdel-Ghaffar *et al.*, 2022)**. The bioactive materials of certain starfish were extracted, and the authors investigated their effect on the wound healing of zebrafish; the end result of this trial was highly-promising (**Dai *et al.*, 2016**).

Based on the trial of **Dai *et al.* (2016)** who used starfish tissue extract for regeneration/wound healing, together with **Vaz and Anura (2016)** who worked on the crucial function of the human blood in delivering nutrients and oxygen to all cells in the body, the current study was designed for working on the coelomic fluid. The idea of studying the coelomic fluid stimulated the idea of studying the characteristics of *Luidia maculata* and how to use it optimally for human benefit. In addition, and in order to be on the right track, molecular docking was listed in our consideration and methodology due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. Characterization of the binding behavior plays an important role in the rational design of drugs as well as to elucidate fundamental biochemical processes **(Kitchen *et al.*, 2004; Mostashari-Rad *et al.*, 2019)**.

The main purpose of this work was to describe and identify the basic cellular structure of the obtained coelomocytes of *Luidia maculata* microscopically. In addition, this investigation addressed the chemical composition of the coelomic fluid of the starfish "*Luidia maculata*" using GC/MS analysis, analyzed the obtained data using protein docking and evaluated the usage of such extract medically for human benefits.

MATERIALS AND METHODS

1- Materials

A- Chemicals

In the current study, all the used chemicals, solvents, and reagents were of analytical and pure grade. Additionally, all chemicals, solvents, and reagents utilized in the TEM and GC/MS methods were of analytical and pure quality.

B-Animals: *Luidia maculata* (7-arms, starfish)

Starfish were collected from Ras Sedr, Red Sea, Egypt. This species was recorded from the Red Sea more than 50 years ago in the studies of **Clark and Rowe (1971)** and **Fouda and Hellal (1987)**. Moreover, it could be the first time in Egypt to collect the animal for microscopical and chemical studies on this species "*Luidia maculata"*.

This work started in August 2022. The starfish were collected from 7-meter depths from the coast of Ras Sedr, Gulf of Suez, Red Sea, Egypt. The collected starfish specimens were identified by **Prof. Dr. Ahmed Mitwaly Hellal** (Zoology Department, Faculty of Science, Al-Azhar University "Boys-Branch") and **Prof. Dr. Magdy T. Khalil (**Zoology Department, Faculty of Science, Ain Shams University**)** according to World Asteroidea database published by **Mah (2009)** as *Luidia maculata* **Müller and Troschel (1842)**. Accordingly, the classification of this starfish is as follows:

Kingdom:	Animalia
Phylum:	Echinodermata
Class:	Asteroidea
Order:	Paxillosida

Family:	Luidiidae
Genus:	*Luidia*
Species:	***L. maculata***
Binomial name	***Luidia maculata***

The starfish specimens were collected to extract their own coelomic fluid as described in the methods used in this study.

2- Methodology

A. Samples collection and morphology of the starfish *"Luidia maculata"*

Specimens of the starfish were collected from the coast of Ras Sedr, Gulf of Suez, Red Sea, Egypt. Attempts were previously made by the researchers of the present study to collect specimens from Hurghada, Red Sea, Egypt, for two seasons; however, this effort was not successfully accomplished.

B. Coelomic fluid extraction, procedure and experimental design

The preparation of the starfish coelomic fluid (CF) was performed by applying the same procedure described in the study of **Baveja *et al.* (2018, 2019)**; additional lengthy details were published by **Abdel-Ghaffar and Youssef** (**2022)**. After centrifugation of the coelomic fluid, a pellet of cells was formed and prepared for microscopical examination.

Concomitantly, the supernatant (representing the acellular part) was prepared for GC/MS application and protein docking.

1-The preparation of the separated cellular content of the coelomic fluids for microscopical examination:

The isolated cellular part of the coelomic fluid of *Luidia maculata* was processed for ultrastructure examination by applying the preparation procedures for transmission electron microscope (TEM) according to **Williams and Carter (2009)**. For lengthy details and steps, kindly refer to **Abdel-Ghaffar (2023)**.

The semithin sections of starfish "*Luidia maculata*" coelomocytes stained with toluidine blue were photographed using a Leica DM1000 LED research trinocular microscope. The microscope is supported by Leica FlexaCam (C1 12M 4K/FHD) camera and image Morphometry system (LASX software). Finally, the stained ultrathin grids were examined and photographed using a JEOL 1200 EX II Electron Microscope, E. M. Unit, at the Faculty of Science, Ain Shams University.

2-Chemical compositions of *n*-hexane fraction obtained from starfish "*Luidia maculata*" coelomic fluid using GC/MS

Gas Chromatography coupled with Mass Spectrometry (GC/MS) analysis was performed on the coelomic fluid extracted from the 7-arms starfish "*Luidia maculata*". After several steps (Kindly, for lengthy steps, refer to **Abdel-Ghaffar and Youssef (2022**), the identification of compounds was done depending on the retention indices of the detected compounds with regard to a homologous series of *n*-alkanes (C8–C28) that were injected under the same conditions and *via* comparison mass spectra of the detected compounds with those recorded in Wiley library database as well as the National Institute of Standards and Technology (NIST) and together with the literature (**Youssef *et al.*, 2014; Ayoub *et al.*, 2015; Mamadalieva *et al.*, 2019; Youssef *et al.*, 2021**). The run of the specimens GC/MS analysis was done in the Department of Pharmacognosy (GC/MS unit), Faculty of Pharmacy, Ain Shams University.

***C- In silico* molecular docking studies**

Molecular docking analysis was done on the major chemical constituents detected in the *n*-hexane fraction obtained from starfish "*Luidia maculata"* coelomic fluid, using GC/MS on glycogen synthase kinase3-*β* protein (PDB ID 5K5N; 2.20 Å) that is considered among enzymes incriminated in the process of wound healing. This protein was downloaded from the protein data bank, and docking experiments were carried out using Discovery Studio 4.5 (Accelrys Inc., San Diego, CA, USA), using C-Docker protocol as previously reported (**Labib *et al.*, 2017; Talaat *et al.*, 2018; Thabet *et al.*, 2018; Altyar *et al.*, 2020)**, where binding energies (Δ G) were calculated *via* the following equation:

$\Delta G_{binding} = E_{complex} - (E_{protein} + E_{ligand})$ Where;

$\Delta G_{binding}$: The ligand–protein interaction binding energy,

$E_{complex}$: The potential energy for the complex of protein bound with the ligand,

$E_{protein:}$ The potential energy of protein alone and

E_{ligand}: The potential energy for the ligand alone.

RESULTS

1. Samples collection and morphology of the starfish *Luidia maculata* (7-arms)

This 7-arms starfish was caught from a depth of 7 meters along the coast of Ras Sedr, Gulf of Suez, the Red Sea, Egypt. The global positioning system (GPS-GP80) is **30°05'17.1"N and 31°20'13.6"E.** Notably, the starfish morphology is formed of upper and lower surfaces,

which are known as aboral and oral surfaces, respectively. The aboral part in this echinoderm is totally dark olive and glossy-darkened in color. The huge caught animals weight was about 2.652, 2.705, 2.918, 3.000 and 3.032 kg. The length measurement of the arm is shown in Fig. 1 (B- D) is 29.1 cm. From the oral surface, the arm was observed to compose spicules and tube feet (Figs. **1B & C**).

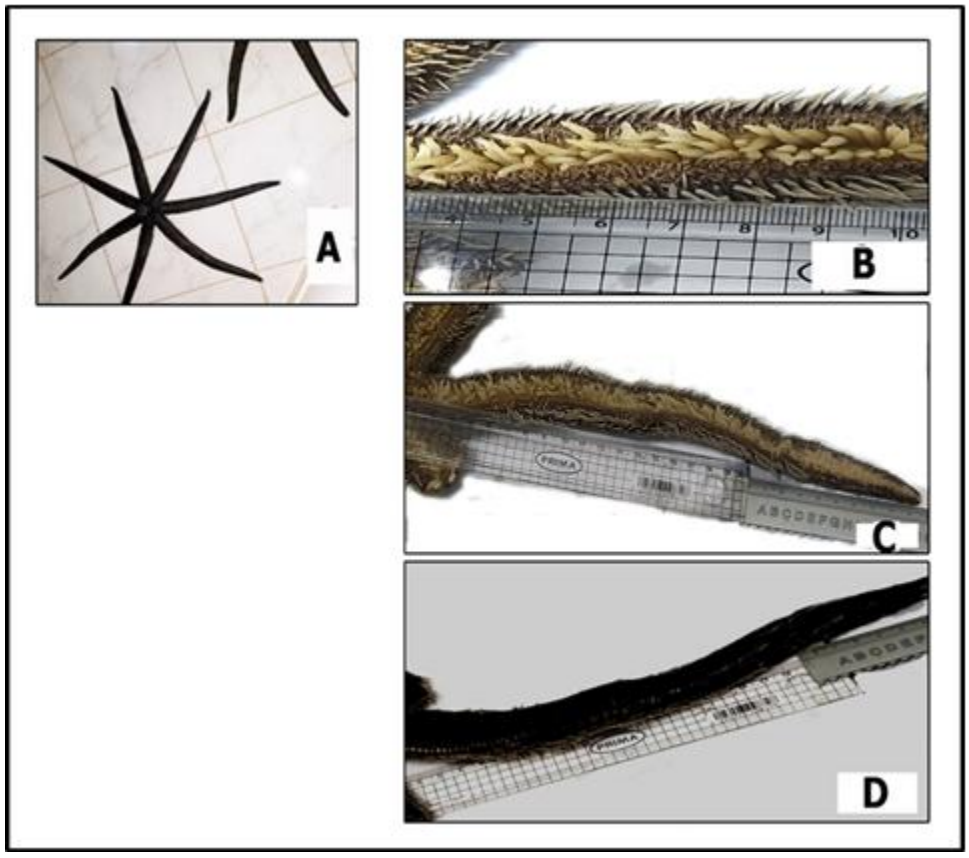

Fig. 1 (A-D). Photos representing the whole sample of a freshly-hunted 7-arms starfish "***Luidia maculata***". **A)** represents the whole animal. {Each ceramic block {the background of the photo) that forms the floor measures 25*25cm}. **(B, C)** represent the oral surface, in which **"B"** shows the tube feet and spinules. Moreover, (**C)** shows the length of the starfish (29 cm), starting from the mouth to the tip of the arm. **D)** shows the aboral surface of this giant starfish with its dark olive and glossy-darkened color. The photos were captured using the cam of a Samsung Galaxy A9 (2018) device.

This animal species is predatory. In addition, one of our critical documented observations is that it owns the capability to fragment each part of its body once realized being hunted, or the surrounding habitat is changed, or in case of getting out from the seawater for more than one hour.

2. The microscopical study

A- The semithin sections of the coelomocytes of *"Luidia maculata"*

After centrifugation, the coelomic fluid was separated into cellular and acellular parts. The cellular portion was processed for TEM. Semithin sections were prepared and stained with

toluidine blue. The obtained semithin sections were examined and from which we observed that the sections exhibited two main cells: small cells or progenitor cells and other large cells. The small cells are found either solitary (Figs. 2D, F) or as syncytium (Fig. 2A, B). The coelomocytes were recorded in two states either aggregated/assembled (Fig. 2F) or separated/dissembled (Figs 2D, E). The large cells are differentiated into two subtypes, which are the secretory granular cells (Fig. 2D, E) and the secretory mucous cells (Fig. 2C). The latter size records ranged from 17- 27 µm in diameter (Fig. 2C). As shown in Fig. (2F), young coelomocytes of this species are aggregated/assembled with each other, which might be done to prevent the leakage of the cytoplasmic fluid with its cells.

B*- The ultrathin sections of the coelomocytes of *"Luidia maculata"

The examination of the individual coelomocytes of this giant starfish surprised us, in which the examined progenitor cells were almost shown in the amoeboid state, as well as the two subtypes of the secretory mucous and secretory granular coelomocytes (Figs. 3A-C, 5A). Moreover, these coelomocytes exhibited typical arrangement of flagellum, *i.e.*, microtubules typical arrangement "9+2 array", described as a ring of nine microtubule doublets, surrounding a single microtubule doublet in the center (Figs. 3B, 4A-D). Coincidently, we found different stages of spermatogenesis collected with the coelomocytes; and their flagella showed typical microtubules arrangement of 9+2 array as in human spermatozoa", *i.e.*, a ring of nine microtubule doublets, surrounding a single microtubule doublet in the center (Figs. 5A-D).

3. Chemical compositions of *n*-hexane fraction obtained from seven-arms starfish "*Luidia maculata*" coelom using GC/MS

GC/MS analyses of the *n*-hexane fraction obtained from seven-arms starfish acellular coelomic fluid extract using GC/MS led to the identification of only three compounds namely *n*-Tridecane, 5-Butylhexadecane and (22E)-Ergosta-5,22-dien-3-yl acetate that belong to fatty acid derivatives and steroidal compounds (Table 1). A scheme showing the identified chemical constituents in the *n*-hexane fraction is illustrated in Fig. (6).

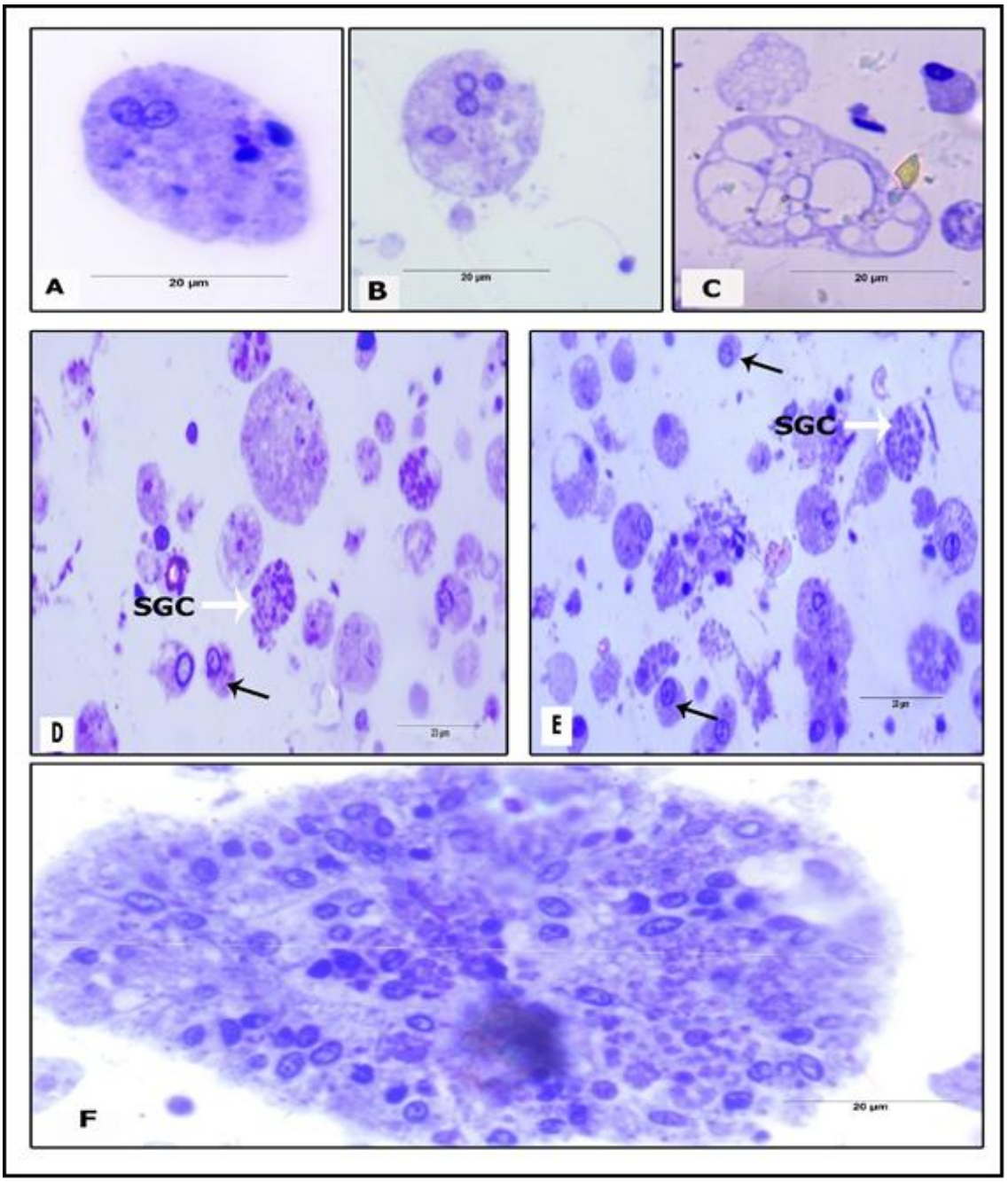

Fig. 2. (A-F) Photomicrographs of semithin sections of the coelomocytes extracted from the coelomic fluid of the 7-arms starfish *"Luidia maculata"*. The sections are stained with toluidine blue. The coelomocytes of this starfish are large in size. (A & B) represent a syncytium of the small or the progenitor cells; C) shows the secretory mucous cell. (D & E) represent various types and subtypes of the coelomocytes in dissembled phase, i.e., the solitary form of the small cells is shown (black arrows), and secretory granular cells (SGC, white arrow). F) represents the assembled phase of the coelomocytes that measure about 99.1µm; such aggregated form might prevent the leakage of the cytoplasmic fluid with its cells. X 1000.

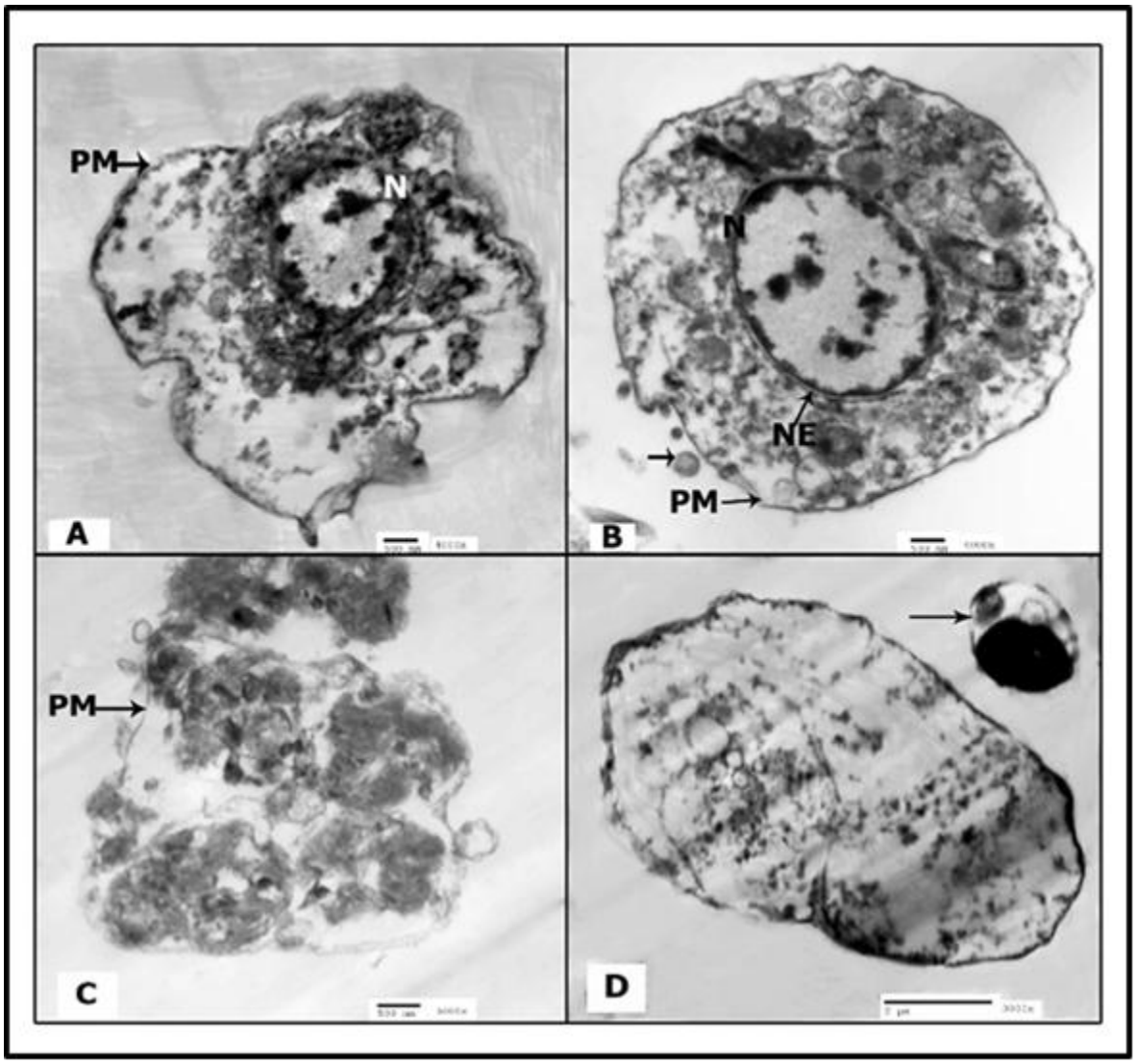

Fig. 3(A-D). Electron micrographs of ultrathin sections of *Luidia maculata* coelomocytes. (A&B) represent the small cells or the flagellar cells that exhibit flagellum (arrow in B), Moreover, the coelomocyte amoeboid appearance is shown. A prominent centrally-located nucleus (N) is shown with a well-recognized nuclear envelope (NE). PM: plasma membrane. C) shows the secretory granular coelomocyte "SGC" discharging apocrine secretion. D) shows the secretory mucous coelomocyte "SMC in addition to a stage of spermatocyte (arrow).

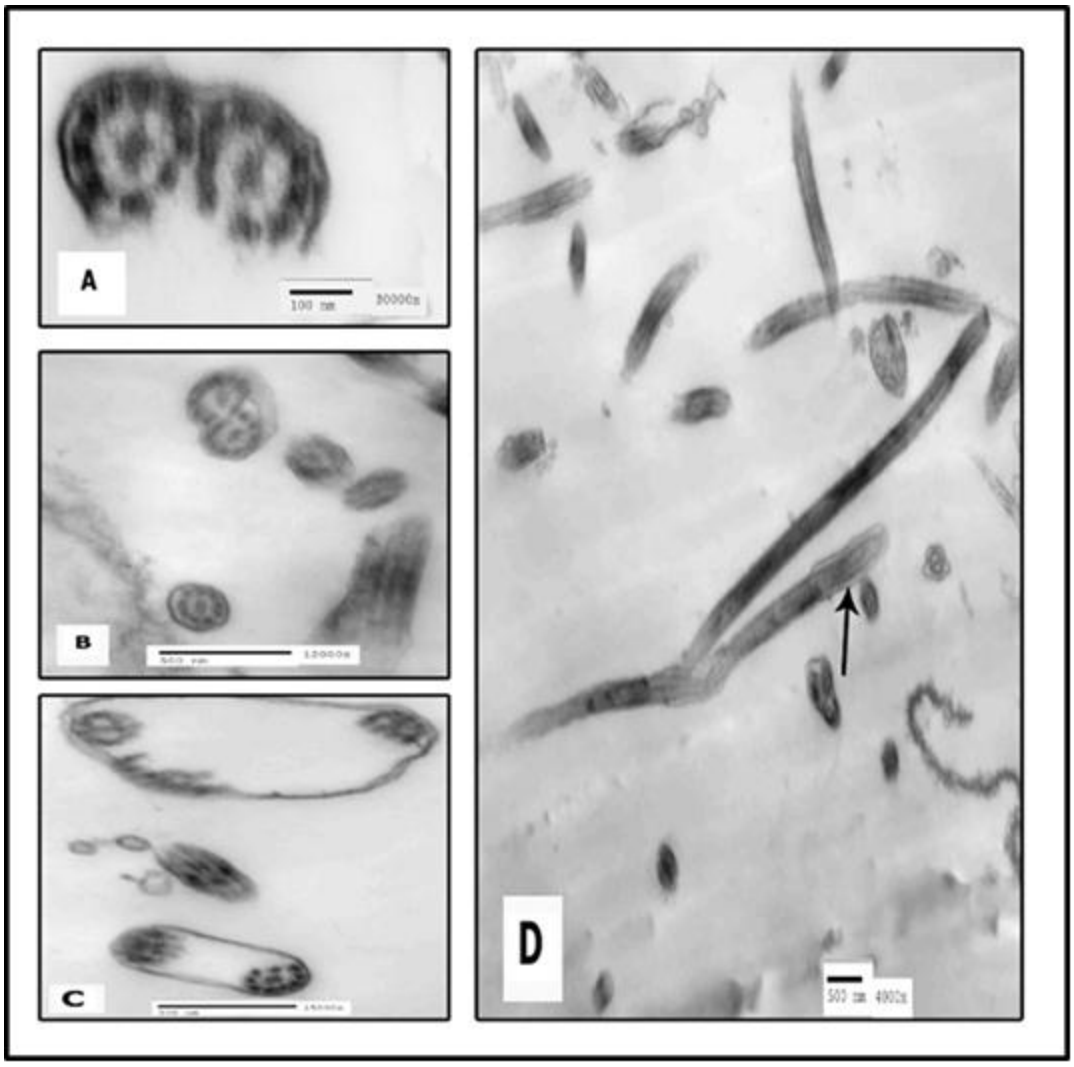

Fig. 4(A-D). Electron micrographs of T.s. and L.s. of *Luidia maculata* coelomocytes' flagella are shown. (A-C) represent transverse sections showing the microtubules' typical arrangement "9+2 array", *i.e.*, a ring of nine microtubule doublets surrounds a single microtubule doublet centrally. D) illustrates variable longitudinal and transverse sections of flagella. As the flagellum tapers toward its end, the plasma membrane surrounds it become thinner (arrow).

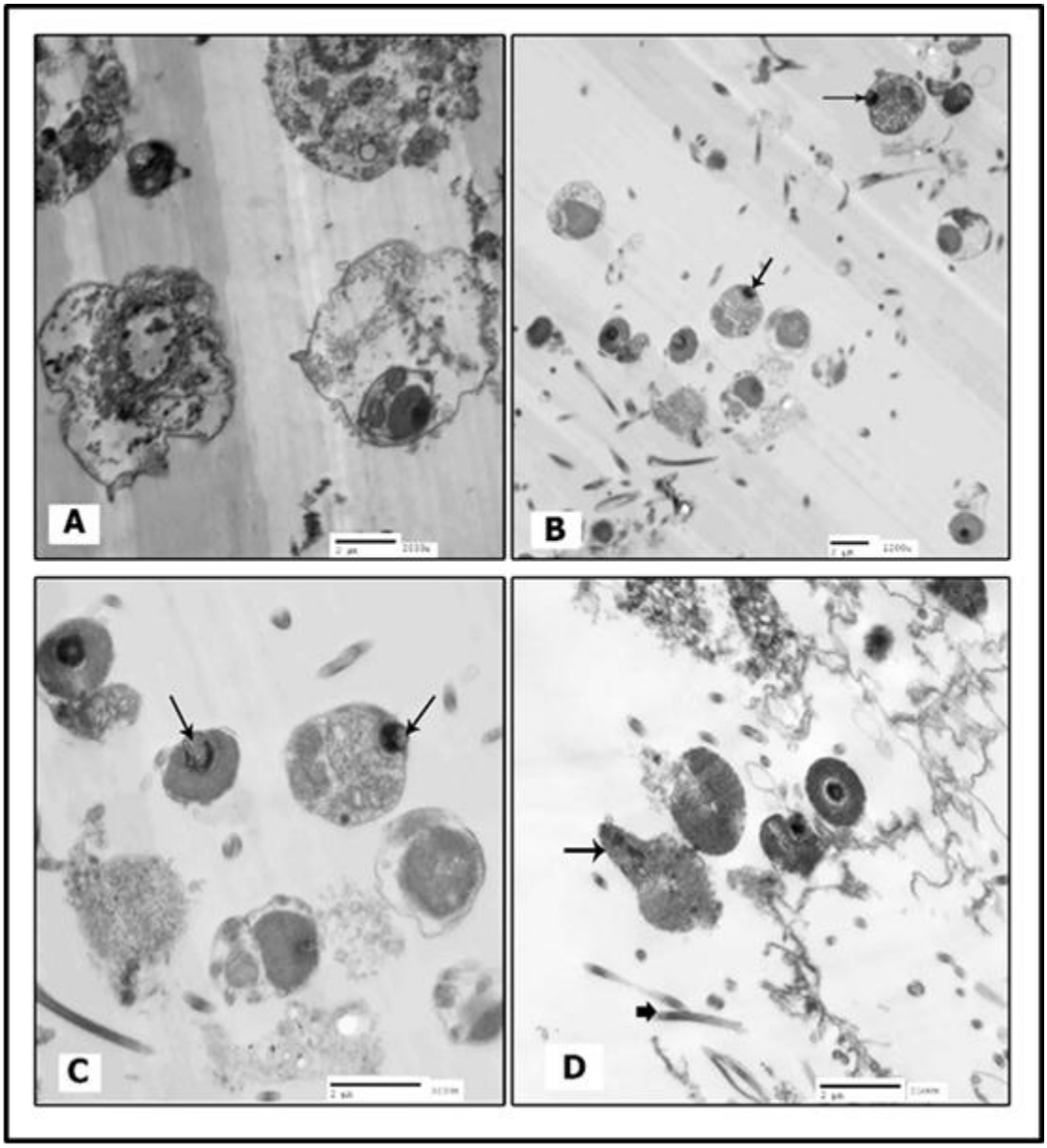

Fig. 5 (A-D). Electron micrographs of cellular ultrastructure of *Luidia maculata*. A) shows numerous coelomocytes, which agree in the amoeboid cell state to facilitate its migration. (B-D) represent different stages of spermatogenesis, which indicates that the examined species' sex is male. The cells' nuclei are directed towards the apex of each cell during spermatogenesis (arrows). The cells exhibit flagella (Thick arrow) shown in (D) consisting of microtubules with typical arrangement "9+2 array", i.e., a ring of nine microtubule doublets, surrounding a single microtubule doublet in the center.

Table 1. Chemical compositions of *n*-hexane fraction obtained from seven-arms starfish "*Luidia maculata*" coelomic fluid using GC/MS, supplied withRtx-5MS column

Compounds	R_I		% Composition	References
	Measured	Reported		
1. *n*-Tridecane	1300	1300	2.49	MS, RI
2. 5-Butylhexadecane	1924	1897	7.92	MS, RI
3. (22E)-Ergosta-5,22-dien-3-yl acetate	2748	2779	8.81	MS, RI

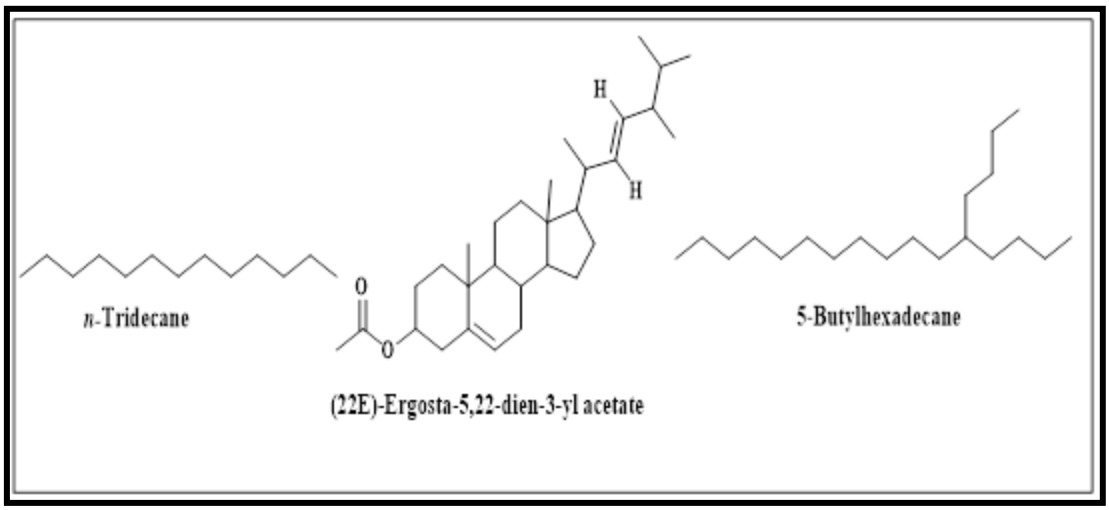

Fig. 6. Scheme showing the chemical compositions of *n*-hexane fraction obtained from seven-arms starfish "*Luidia maculata*" coelomic fluid using GC/MS, supplied withRtx-5MS column

4. *In silico* molecular docking studies

Regarding metabolites identified in the seven-arms starfish *Luidia maculata* coelomic fluid, results presented in Table (2) reveal that, 5-butylhexadecane showed the best fitting within the active site of glycogen synthase kinase3-*β* protein followed by *n*-tridecane with free binding energies (Δ G) of -41.98 and -28.79 Kcal/mol, respectively, exceeding in this approach the co-crystalised ligand (ΔG = -12.10 kcal/mol). In contrast, (22E)-Ergosta-5,22-dien-3-yl acetate showed unfavorable interaction evidenced by the positive values of ΔG (41.34 Kcal/mol). 5-butylhexadecane forms three alkyl and π-alkyl bonds with Arg141, Tyr140, and Ile62 at the binding site in addition to many Van der Waals interactions (Fig. 7A); meanwhile, *n*-tridecane forms one π-alkyl bond with Phe67 together in addition to many Van der Waals interactions (Fig. 7B).

Table 2. Free binding energies (kcal/mol) of major compounds in the *n*-hexane fraction obtained from seven-arm starfish "*Luidia maculata*" coelom in glycogen synthase kinase3-*β* protein active site using *in silico* studies

Compound	Glycogen synthase kinase3-*β* protein	Number of formed hydrogen bonds	Number of other formed bonds
n-Tridecane	-28.79	1:Ile32	1 ; Phe67
5-Butylhexadecane	**-41.98**	-	3; Arg141, Tyr140, Ile62
(22E)-Ergosta-5,22-dien-3-yl acetate	41.34	2; Arg141, Arg148	7; Ile62m Arg141, Lys85, Leu132, Ala83, Val70, Cys199
Co-crystalised ligand (PF-367)	-12.10	2; Val135	8; Val70, Lys85, Ala83, Leu132, Leu188, Val110, Asp133

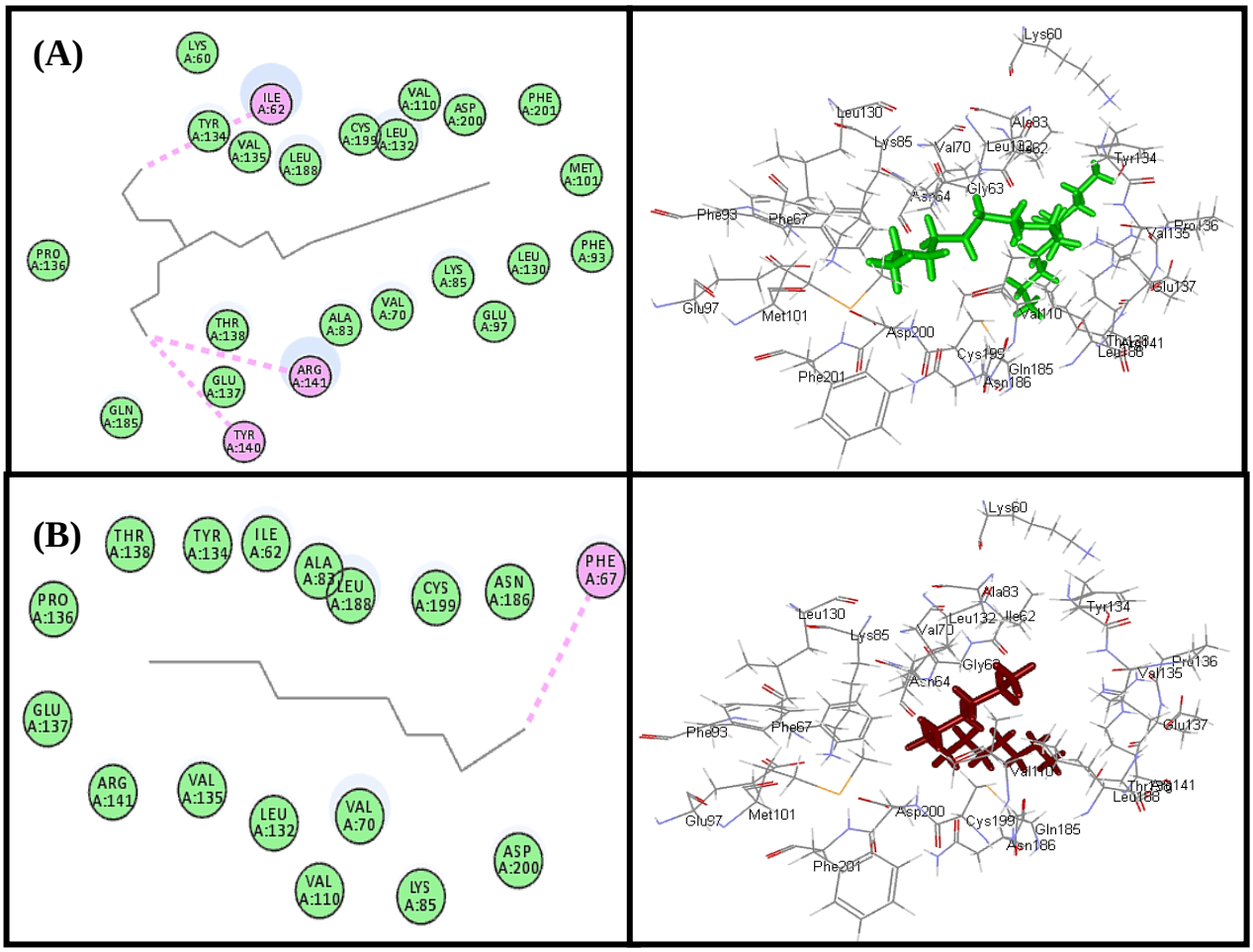

Figure 7 (A & B): 2D and 3D binding modes of 5-Butylhexadecane (A) and *n*-Tridecane (B) within the active sites of glycogen synthase kinase3-*β* protein.

DISCUSSION

Starfish are considered the dominant, widespread and most known animal in phylum Echinodermata, *i. e.*, the king of this phylum. Nevertheless, no one could imagine that our team, unfortunately, failed to hunt starfish from Sharm El-Sheik or even from Hurghada, the Red Sea, Egypt at the start of these experiments in November 2021. We refer this failure to many reasonable factors, such as the heavy presence of tourists snorkeling, winter season, overfishing of starfish by fishermen to buy it as a gift for more luck with high prices in addition to climate change. These reasons might lead to a change in the dominance of starfish, and subsequently its disappearance in certain areas accompanied by heavy migration to another calm area. Collectively, this made us decide to change the search place to Ras Sedr, Gulf of Suez, the Red Sea, Egypt. The divers found this 7-arms starfish "*Luidia maculata"* and hunted the specimens at 7 meters' depth in the seawater. The presence of these specimens till reaching giant masses may be referred to the tendency of the present tourists to relax, enjoy the scenery of the virgin nature of this place rather than snorkeling, adding to the security procedures that restrict any intended spoiling of any natural or environmental item in this area.

To our knowledge, this is the first microscopical study on this darkened 7-arms starfish "*Luidia maculata*". Moreover, microscopically, one of our surprising results is that the coelomocytes showed the amoeboid state of the progenitor cells and the two subtypes of the secretory mucous and secretory granular coelomocytes (Figs. 3A- C, 5A). Besides, these coelomocytes exhibited typical arrangement of flagellum, *i.e.*, microtubules typical arrangement "9+2 array", described as a ring of nine microtubule doublets, surrounding a single microtubule doublet in the center (Figs. 3B, 4A-D). In our viewpoint, this is not a haphazard coincidence; if we collect it with the suffix "-dermata" from Echinodermata means "skin" – to which the starfish *L. maculata* belongs – together with the typical microtubules arrangement (9+2 arrays). The similarities of starfish coelomocytes in shape and function make them generally the most suitable match to serve and cover human needs medically, specifically the animal of choice in this work "*Luidia maculata*". The human body soldiers are white blood cells; they play a crucial role in the defence system. Their amoeboid form allows them to squeeze through blood capillaries, while their pseudopodia aid in the phagocytosis process, which kills pathogens, which is scientifically known as diapedesis **(Filippi, 2016)**.

For the mature coelomocytes, **Xing *et al.* (2008)** reported that the small cells are called lymphocytes, and progenitor cells; they are called here the small coelomocytes. At the same time, he called the mature coelomocytes "amoebocytes". It appears with different shapes and variable materials inside its own cytoplasm, depending on the heterogeneous materials of variable sizes they engulfed *via* amoeboid phagocytosis. Meanwhile, according to **Gorshkov *et al.* (2009)**, the mature secretory coelomocytes differentiating into secretory granular and mucous cells represent two types of cells. In this work, as well as in our previous work **Abdel-Ghaffar and Youssef (2022)** and **Abdel-Ghaffar *et al.* (2022)**; we did not agree with

the two opinions. In addition, it is suggested that, these small cells (progenitor cells) bearing clotting granular/mucus materials formed at the site of the animal injury **(Xing *et al.*, 2008)**, harbor such secretory granules whether granular or mucous secretion to the site of injury and then they are transformed to mature or large coelomocytes variable in their size, based on the type of secretory material it bears and the quantity. Surprisingly, **Guatelli *et al.* (2022)** had the same opinion as ours, in which they stated that coelomocytes are scarcely studied. In addition, the coelomocytes are detached from the coelomic epithelium (CE). Moreover, the CE cells share the same ultrastructure and proteomic features as the detached circulating coelomocytes. Furthermore, they own the privilege in their results and conclusion to our previous work **(Abdel-Ghaffar & Youssef, 2022)** in clarifying that the CE and coelomocytes are actively involved in protein synthesis processing and massive secretion phenomena. The latter might explain the apocrine secretion of the secretory granular cells, as shown in Fig. (3C). The ultrastructure findings in this work on the starfish "*Luidia maculata*" agrees with similar previous results of **Muñoz-Chápuli *et al.* (2005)**, **Holm *et al.* (2008)**, **Gorshkov *et al.* (2009)**, **Sharlaimova *et al.* (2010)**, **Franco *et al.* (2011)**, **Andrade *et al.* (2021)**, **Sharlaimova *et al.* (2021)** and **Guatelli *et al.* (2022)**.

Focusing on the proteins resulting from the GC/MS obtained data from the extracted coelomic fluid of "*Luidia maculata*" in this work led to the identification of only three compounds; namely, *n*-Tridecane, 5-Butylhexadecane and (22E)-Ergosta-5,22-dien-3-yl acetate that belong to fatty acid derivatives and steroidal compounds (Table 1). The metabolites identified in the seven-arms starfish coelom (results presented in Table (2)) revealed that 5-butylhexadecane showed the best fitting within the active site of glycogen synthase kinase3-*β* protein; these protein factors obtained from the protein docking analyses. Additionally, it might be considered as a promising constituent for many medicinal applications including wound healing **(Abdel-Ghaffar & Youssef, 2022; Abdel-Ghaffar *et al.*, 2022)**, especially in diabetic patients. With respect to this finding, the coelomic fluid of the starfish *L. maculata* could be used as an effective or potent agent for wound healing **(Harish *et al.*, 2008; Naika *et al.*, 2015; Aksoy *et al.*, 2021; Abdel-Ghaffar & Youssef, 2022; Abdel-Ghaffar *et al.*, 2022)** due to its characteristic glycogen synthase kinase3-*β* protein, which is identified by the protein docking (Table 2, 3 & Fig. 7A, B) of this work. In **2021**, **Soleimani *et al.*** worked on the coelomic fluid of a certain type of sea urchin and listed it as a new perspective for medicinal antioxidants. **Franco *et al.* (2011)** and **Guatelli *et al.* (2022)** postulated that rearrangement of the cytoskeleton (herein, the microtubules' arrangement is "9+2", as shown in Figs. (3B, 4A- D, 5B- D); the same as in human), membrane trafficking, and cell motility in coelomocytes of starfish together with the cellular morphological similarities and the several proteinsocytes and the CE suggest the origin of the coelomocytes from the CE.

CONCLUSION

Finally, this work spotted the light on novel documented data on the microscopical and chemical characteristics of scarcely studied species of starfish "*Luidia maculata*", in which the studied coelomocytes showed an amoeboid state resembling the human white blood cells. The examined flagella exhibited a typical microtubule arrangement "9+2 array". The coelomic fluid extracted from "*Luidia maculata* exhibits glycogen synthase kinase3-β protein, which could be considered a promising biovital constituent for many medicinal applications including wound healing in diabetics.

ABBREVIATIONS

GSK3- *β*: glycogen synthase kinase3-*β* protein. **SC:** small Coelomocytes/Cells. **SGC**: secretory granular cells. **SMC:** secretory mucous cells. **TEM:** transmission electron microscope. **CE:** Coelomocytes epithelium.

AUTHOR CONTRIBUTION STATEMENT

Abdel-Ghaffar WH: Conceived, designed, and performed the experiments; Abdel-Ghaffar WH, Attia MS, and Youssef FS; analyzed and interpreted the data; contributed materials, and wrote the paper equally.

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COMPETING INTEREST STATEMENT

The authors declare no conflict of interest.

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AVAILABILITY OF DATA AND MATERIALS

The datasets supporting the conclusions of this article are included within the article.

ETHICAL APPROVAL

This study follows guidelines for the care and handling of experimental animals established by the ethical Committee belonging to the Higher Studies and Research Sector, Faculty of Science, ASU. For the purpose of the experimental design, animal accommodation, preventing contamination, animal way of dosing, handling, and getting rid of the wastes, the protocol was approved in accordance to the given code: **ASU-SCI/ZOOL/2022/8/16**.

REFERENCES

Abdel-Ghaffar, W. H. (2023). Hepatic and Renal Ultrastructural Alterations Induced by Tamiflu on Male Rats. The Egyptian Journal of Histology DOI: 10.21608/ejh.2023. 174223.1815.

Abdel-Ghaffar, W. H. and Youssef, F. S. (2022). Microscopical and Chemical Analyses of the Coelomic Fluid and the Coelomocytes Extracted from the Sea Biscuits "*Clypeaster humilis*". Egyptian Journal of Aquatic Biology and Fisheries (EJABF), 26(5): 1403-1423. DOI: 10.21608/ejabf.2022.298664.

Abdel-Ghaffar, W. H. *et al.* (2022). Sea Cucumber *"Holothuria (Thymiosycia) arenicola*" induced-autotomy for sustainable development in Egypt**: Histological, Ultrastructure, and Chemical studies**. Egyptian Journal of Aquatic Biology and Fisheries (EJABF), 26 (4). 1459-1491.

Abuhijjleh, R. K.; Shabbir, S.; Al-Abd, A. M.; Jiaan, N. H.; Alshamil, S.; El-labbad, E. M. and Khalifa, S. I. (2021). Bioactive marine metabolites derived from the Persian Gulf compared to the Red Sea: Similar environments and wide gap in drug discovery. PeerJ., 9 (e11778).

Aksoy, H.; Şekerler, T. and Mert, N. M. (2021). *In silico* investigation of wound healing potential of some compounds in tubers of *Asphodelus* species with GSK3-β protein. Journal of Research in Pharmacy (online), 25(5): 747-754.

Altyar, A. E.; Ashour, M. L. and Youssef, F. S. (2020). *Premna odorata*: Seasonal Metabolic Variation in the Essential Oil Composition of Its Leaf and Verification of Its Anti-Ageing Potential *via In Vitro* Assays and Molecular Modelling. Biomolecules, 10(6): 879.

Andrade, C.; Oliveira, B.; Guatelli, S. *et al.* (2021). Characterization of coelomic fluid cell types in the starfish *Marthasterias glacialis* using a flow cytometry/imaging combined approach. Front. Immunol., 12:641-664. https://doi.org/10.3389/fmmu.2021.641664

Ayoub, I.; Youssef, F.; El-Shazly, M.; Ashour, M.; Singab, A. and Wink, M. (2015). Volatile constituents of *Dietes bicolor* (Iridaceae) and their antimicrobial activity. Z. Naturforsch. C, 70: 217–225.

Baveja, M.; Sarkar, A. and Chakrabarty, D. (2018). Hemotoxic and wound healing potential of coelomic fluid of sea-star *Astropecten indicus*. The Journal of Basic and Applied Zoology, 79(27).

Baveja, M.; Sarkar, A.; Mondal, S.; Pathan, J. and Chakrabarty, D. (2019). AiP1, a protein from the coelomic fluid of sea star *Astropecten indicus* promotes wound healing and fibrinogenolysis *in vitro*. The Journal of Basic and Applied Zoology, 80(50). https://doi.org/10.1186/s41936-019-0120-4

Bos, A. R.; Alipoyo, J. C. E,; Cardona, L.T.; Gumanao, G. S. and Salac, F. N. (2008). Population Structure of Common Indo-Pacific Sea Stars in the Davao Gulf, Philippines. UPV J. Nat. Sci., 13: 11-24.

Chamundeeswari, K.; Saranya, S.; Shanker, S.; Varadharajan, D.; Rajagopal, S. and Balasubramanian, T. (2013). Incidental Catch and New Distributional Records of Asteroids by Bottom Trawling Activities from Southeast Coast of India. J. Aquac. Res. Development., 4(6): 1-9.

Clark, A. M. and Rowe, F. W. E. (1971). Monograph of shallow water indo west pacific echinoderms. Trstees. Br. Mus. (Nat. Hist.), London.

Cooke, N. D. J. (1971). The Oxford Book of Invertebrates. pg (181), New York: Oxford University Press.

Dai, Y.; Prithiviraj, N. and Gan, J. *et al.* (2016). Tissue extract fractions from starfish undergoing regeneration promote wound healing and lower jaw blastemal regeneration of Zebrafish. Scientific reports,6:1-11. DOI: 10.103B/srep38693.

El-Hossary. E. M.; Abdel-Halim, M.; Ibrahim, E. S.; Pimentel-Elardo, S. M.; Nodwell, J. R.; Handoussa, H. *et al.* (2020). Natural Products Repertoire of the Red Sea. Mar. Drugs, 18 (457).

Fauda, M. M. and Hellal, A. M. (1987): Fauna and flora of Egypt. The echinoderms of the northwestern Red Sea. Asteroidea. of Acad. Sci. Res. Tech. Egypt, 2: 1-71.

Filippi, M-D. (2016). Mechanism of Diapedesis: Importance of the Transcellular Route. In:Frederick, W.; editor, Advances in Immunology, Vol. 129, Burlington: Academic Press, Elsevier Inc. 25-53. ISBN: 978-0-12-804799-6.

Franco, C. F.; Santos, R. and Coelho, A. V. (2011). Proteome characterization of sea star coelomocytes–the innate immune effector cells of echinoderms. Proteomics 11:3587–3592. https://doi.org/10.1002/pmic. 201000745

Gorshkov, A.; Blinova, M. and Pinaev G. (2009). Ultrastructure of Coelomic Epithelium and Coelomocytes of the Starfish *Asterias rubens* in Norm and after Wounding. Cell and Tissue Biology, 3(5): 477–490.

Grzimek, B. (1972). Grzimek's Animal Life Encylopedia: Vol. 3 Mollusks and Echinoderms; pg. 365, 377, 387. New York: Van Nostrand Reinhold Company.

Guatelli, S.; Ferrario, C.; Bonasoro, F.; Anjo, S. I.; Manadas, B.; Carnevali, M. D.; Coelho, A. V. and Sugni, M. (2022). More than a simple epithelial layer: multifunctional role of echinoderm coelomic epithelium. Cell and Tissue Research, 390:207–227.

Harish, B. *et al.* (2008). Wound healing activity and docking of glycogen-synthase-kinase-3-b-protein with isolated triterpenoid lupeol in rats. Phytomedicine, 15: 763-767.

Higuchi, R.; Inagaki, M.; Yamada, K. and Miyamoto, T. (2007). Biologically active gangliosides from echinoderms. J. Nat. Med., 61:367-370.

Holm, K.; Dupont, S. and Sköld, H. *et al.* (2008). Induced cell proliferation in putative haematopoietic tissues of the sea star, Asterias rubens (L.). J. Exp. Biol., 211:2551–2558. https://doi.org/10.1242/jeb. 018507

Inagaki, M.; Ikeda, Y.; Kawatake, S.; Nakamura, K.; Tanaka, M.; Misawa, E.; Yamada, M. and Higuchi, R. (2006). Isolation and Structure of Four New Ceramides from the Starfish *Luidia maculata*. Chem. Pharm. Bull., 54(12): 1647-1649.

Kawatake, S.; Inagaki, M. and Isobe, R. (1997). Biologically active glycosides from Asteroidea, 37. Glycosphingolipids from the starfish *Luidia maculata*, 1-Structure of a new sulfatide molecular species. Liebigs. Ann. Recueil., 8: 1797-1800.

Kawatake, S.; Inagaki, M.; Miyamoto, T.; Isobe, R. and Higuchi, R. (1999). Biologically Active Glycosides from Asteroidea, 38 [∞] Glycosphingolipids from the Starfish *Luidia maculata*, 2[∞] Isolation, and Structure of a GM 3-Type Ganglioside Molecular Species. Eur. J. Org. Chem., 4:765-769.

Kawatake, S.; Nakamura, K.; Inagaki, M. and Higuchi, R. (2002). Isolation and Structure Determination of Six Glucocerebrosides from the Starfish *Luidia maculata*. Chem. Pharm. Bull., 50(8): 1091-1096.

Kawatake, S.; Inagaki, M.; Isobe, R.; Miyamoto, T. and Higuchi, R. (2004). Isolation and Structure of a GD3-Type Ganglioside Molecular Species Possessing Neuritogenic Activity from the Starfish *Luidia maculata*. Chem. Pharm. Bull. 52(8): 1002-1004.

Khalil, E. A., Swelim, H., El-Tantawi, H.; Bakr, A. F. and Abdellatif, A. (2022). Characterization, cytotoxicity, and antioxidant activity of sea urchins (*Diadema savignyi*) and jellyfish (*Aurelia aurita*) extracts. Egyptian Journal of Aquatic Research, 48: 343-348.

Kitchen, D. B.; Decornez, H.; Furr, J.R. and Bajorath, J. (2004). "Docking and scoring in virtual screening for drug discovery: methods and applications". Nature Reviews: Drug Discovery. **3** (11): 935–49. doi:10.1038/nrd1549.

Labib, R. *et al.* (2017). Chemical Composition of *Pinus roxburghii* Bark Volatile Oil and Validation of Its Anti-Inflammatory Activity Using Molecular Modelling and Bleomycin-Induced Inflammation in Albino Mice. Molecules, 22(9): 1-14.

Mah, C. (2009). Mah CL (ed.). "*Astropecten polyacanthus* Müller & Troschel, 1842". World Asteroidea database. World Register of Marine Species.

Mamadalieva, N.; Youssef, F.; Ashour, M.; Sasmako,v S.; Tiezzi, A. and Azimova, S. (2019). Chemical composition, antimicrobial and antioxidant activities of the essential oils of three Uzbek Lamiaceae species. Nat. Prod. Res., 33: 2394-397.

Mostashari-Rad, T.; Arian, R.; Mehridehnavi, A.; Fassih,i A. and Ghasemi, F. (2019). "Study of CXCR4 chemokine receptor inhibitors using QSPR and molecular docking methodologies". Journal of Theoretical and Computational Chemistry, **178** (4).

Muñoz-Chápuli, R.; Carmona, R. and Guadix, J. A. *et al.* (2005). The origin of the endothelial cells: an evo-devo approach for the invertebrate/ vertebrate transition of the circulatory system. Evol Dev 7:351– 358. https://doi.org/10.1111/j.1525-142X.2005.05040.x.

Naika, H. R. *et al.* (2015). Molecular docking and dynamic studies of bioactive compounds from *Naravelia zeylanica* (L.) DC against glycogen synthase kinase-3β protein. Journal of Taibah University for Science, 9(1): 41-49.

Ning, X.; Ruiyu, L.; Shuai, Y. and Zhongli, S. (2013). A Preliminary Phylogenetic Analysis of *Luidia* (Paxillosida: Luidiidae) from Chinese Waters with Cytochrome Oxidase Subunit I (COI) Sequences. J. Ocean Univ. China (Oceanic and Coastal Sea Research), 12: 459-468.

Parker, S. (1982). Synopsis and Classification of Living Organisms: Vol. 2, pg. 796. New York: McGraw-Hill Book Co.

Piction, B. E. (1996). "Echinodermata: Starfish, Sea Urchins, etc." (online). Accessed October 2, 2001, at http://www.itsligo.ie/biomar/echinode/LUICIL.htm.

Rajan, R.; Venkatraman, C.; Aleela, G. S.; Aranthaman. D. P.; Padmanaban, P. and Venkataraman, K. (2012). Notes on the Association of *Lissocarcinus Polybiodes Adams* and White, 1848 (*Portunidae, Caphyrinae*), With Sea Star *Luidia Maculata* Muller and Troschel, 1842. Rec. zool. Sum. India, 112(Part-2): 23-26.

Sharlaimova, N.; Pinaev, G. and Petukhova, O. (2010). Comparative analysis of behavior and proliferative activity in culture of cells of coelomic fluid and of cells of various tissues of the sea star Asterias rubens L. Isolated from normal and injured animals. Cell and Tissue Biology, 4:280–288. https://doi.org/10.1134/S1990519X10030107

Sharlaimova, N.; Shabelnikov, S. and Bobkov, D. *et al.* (2021). Coelomocyte replenishment in adult *Asterias rubens:* the possible ways. Cell Tissue Res., 383:1043–1060. https://doi.org/10.1007/s00441-020-03337-z

Soleimani, S., Mashjoor, S.; Mitra, S. *et al.* (2021). Coelomic fluid of *Echinometra mathaei*: The new prospects for medicinal antioxidants.

Suguna, A.; Bragadeeswaran, S.; Natarajan, E. and Mohanraj, M. (2014). Studies on antioxidant properties of starfish *Luidia maculata* (Muller & Troschel, 1842) off Parangipettai, Southeast coast of India. Journal of Coastal Life Medicine, 2(9): 694-698.

Talaat, A. N. *et al.* (2018). Verification of the anti-inflammatory activity of the polyphenolic-rich fraction of *Araucaria bidwillii* Hook. using phytohaemagglutinin-stimulated human peripheral blood mononuclear cells and virtual screening. Journal of Ethnopharmacology, 226: 44-47.

Thabet, A. A. *et al.* (2018). Validation of the Antihyperglycaemic and Hepato-protective Activity of the Flavonoid Rich Fraction of *Brachychiton rupestris* Using *in vivo* Experimental Models and Molecular Modeling. Food and Chemical Toxicology, 114: 302-310.

Xing, k. Yang, H. S. and Chen, M. Y. (2008). Morphological and ultrastructural characterization of the coelomocytes in *Apostichopus japonicas*. Aquatic Biology, 2: 85-92.

Yaghmour, F. and Whittington-Jones, B. (2018). First Record of *Lissocarcinus polybioides* Adams & White, 1849 (Crustacea: Decapoda: Brachyura: Portunidae) for the United Arab Emirates and the Gulf of Oman. Marine Biodiversity Records, 11(11): 1-4.

Youssef, D. T.; Shaala, L. A. and Asfour, H. Z. (2013). Bioactive compounds from the Red Sea marine sponge *Hyrtios* species. Marine drugs, 11(4), 1061-1070.

Youssef, F.; Hamoud, R.; Ashour, M.; Singab, A. and Wink, M. (2014). Volatile oils from the aerial parts of Eremophila maculata and their antimicrobial activity. Chem. Biodivers., 11: 831-41.

Youssef, F. S. *et al.* (2021). Morphology, Anatomy and Secondary Metabolites Investigations of Premna odorata Blanco and Evaluation of Its Anti-Tuberculosis Activity Using In Vitro and In Silico Studies. Plants, 10(9): 1953.

Vaz, M.; Raj, T. and Anura, K. (2016). Guyton & Hall Textbook of Medical Physiology - E-Book: A South Asian Edition. Elsevier Health Sciences. 255pp. ISBN 978-8-13-124665-8.

Williams, D.B. and Carter, C. B. (2009). Transmission Electron Microscopy: A Textbook for Materials Science. Springer Science and Business Media, LLC, 233 Spring Street, New York, NY10013, USA.