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# Coelomic fluid of *Echinometra mathaei*: The new prospects for medicinal antioxidants

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ABSTRACT

Echinoid pigments have various biological properties such as antioxidant, cytotoxic, and antibacterial activities. We aimed to evaluate the extraction of cell-free coelomic fluid (CFCF) and coelomocyte lysate (CL) as well as qualitatively and quantitatively identify the coelomic fluid of *Echinometra mathaei* as a new source of polyhydroxylatednaphthoquinone (PHNQ) antioxidant pigments. Based on the High Performance liquid chromatography–electrospray mass spectrometry (HPLC-MS) analysis in negative mode, the main quinonoid (PHNQ) pigments were identified and quantified. This study also illustrated the total ion current chromatograms and related mass spectra of Spinochrome A, Spinochrome B, Spinochrome C, and Echinochrome A in CL and SpinochromeC in CFCF samples. The ions at 221, 279, 265 and 263 m/z correspond to the pseudo-molecular [M − H] ions of Spinochrome B, Spinochrome C, Echinochrome A, and Spinochrome A, respectively. These components have previously been noted from the shells and spines of sea urchins but identification of PHNQs pigments in CL and CFCF of *E. mathaei* using LC-MS was introduced for the first time. The results also showed that, the highest DPPH radical scavenging activity of CFCF (88.12 DPPH% scavenging at 70 μg/mL, $IC_{50}$ = <10 μg/mL). The findings clearly suggest that the coelomic fluid of *E. mathaei* could be served as the promising as well as potential natural antioxidants in the medical and pharmaceutical industries and could replace the increasing prices of the commercial antioxidants products.

## 1. Introduction

Sea urchin belongs to the marine invertebrate Echinoidea [1], found in a sequential distribution from the intertidal zone to the deepest depths of the ocean (Smith et al., 2010) which plays major roles in the marine ecosystem [2,3]. In ancient times, there are multiple uses of sea urchins in traditional folk medicine systems, especially in China, for treating various diseases [4]. But, recently it has been researched to be a source of bioactive components, known to be effective in treating diabetes, heart disease, Alzheimer's disease and Parkinson's disease [5,6].

At present, there are many large-scale programs operating allover the world for sea urchin cultivation (Echinoculture) based on their biomedical applications [7]. Over a century of science, sea urchins have served as model organisms for understanding various biological processes and part of this is also referred to as their close phylogenetic relevance to humans. Sea urchins are nonchordsdeuterostomes and are more closely relevant to humans than other invertebrate model organisms such as flies (e.g. *Drosophila melanogaster*) and worms (e.g. *Caenorhabditiselegans*) [8].

Through dissection of the internal part of the animal body, it is evident that the body cavity of echinoderms is filled with coelomic fluid, which bathes the interior organs where the coelomocytes get suspended. The composition of coelomic fluid (CF) is similar to seawater in terms of minor soluble salts, other minerals, and proteins [9]. Based on the coelomocytes and humoral factors in the coelomic fluid, sea urchins have shown the ability to immune responses against wounding and

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pathogenic microorganisms [10]. Coelomic fluids play a critical role in host response by undergoing reactions such as opsonisation, coagulation, encapsulation, and phagocytosis [11]. The coelomic fluid extracted from sea urchins proposes three basic categories of coelomocytes namely 76% phagocytes, 12% vibratile cell, and 12% red and uncoloured spherulocytes or amoebocytes [9,12]. Many studies also reported the immunological functions of amoebocytes and uncoloured spherulocytes. Amoebocytes and spherulocytes demonstrate the basic coelomocyte communities that seem responsible for a wide repertory of immuno functions: cellular recognition, phagocytosis, graft reaction, inflammatory reactions, prophenoloxidase activity, capsule formation, and reactive oxygen intermediates (ROI) production [13]. In addition, Echinoid pigments have various biological properties such as antioxidant activity, especially, radical scavenging activity of 1, 1-diphenyl 2-picryhydrazyl (DDPH) radical [14], cytotoxic activity based on the presence of phagocytes [9], as well as, antibacterial activity against both Gram-negative and Gram-positive bacteria [15].

Polyhydroxylatednaphthoquinone (PHNQ) pigments in echinoids were first observed and recorded by MacMunn [16]. Further, crystalline Echinochrome A was isolated [17,18], and the structure was specified subsequently by Ref. [19]. The first quinoid pigment (Spinochrome A) from sea urchin (*Paracentrotus lividus*) spines were identified by Lederer and Glaser [20]. In recent times, the dimeric Naphthoquinone pigments from sea urchins are believed to be more active and largely responsible for observed pharmacological and clinically proven activities [21]). Despite of its numerous biochemical studies of PHNQs, based on the safety concerns and limitations in cosmeceuticals, there are only a few medical approval (e.g. Echinochrome A) [21]. The biological properties of sea urchins red spherule can be attributed to their EchinochromeA [22–25]. Other report by Li et al. [26] and Coates et al. [27] showed that Echinochrome A plays a role in the immune response in sea urchins. Till date, about thirty quinoid pigments have been isolated from the spines and shells of the different sea urchin species [1].

However, *Echinometra mathaei* is a very plentiful sea urchin on the Iranian coasts of the Persian Gulf, their coelomic fluid pigments were less considered worthy of attention. Whereas, extracting coelomic fluids from sea urchins (with a syringe) is non-invasive, low-cost, and eco-friendly technique which dose not require the killing of an organism in the echinoculture and/or environment. Although, current data on PHNQs distribution, in the coelomic fluid, and other organs, is scanty, and probably shows considerable variation as pigments are widely dispersed by amoebocytes [18]. Nevertheless, sea urchin PHNQs pigments have demonstrated significant bioactivity with differences in origin, compositions, and interactive mechanisms [28,29]. From there search perspective, *E. mathaei* coelomic pigments will evoke renewed interest as a promising biomaterial for pharmaceutical industries.

The present study aims to report the following things: (1) to introduce a new source of polyhydroxylated antioxidant pigments for the development of natural drugs in an eco-friendly/sustainable manner, (2) attempt to separate and identify *E. mathaei* coelomic fluid quinonoid pigments especially in cell-free coelomic fluid (CFCF) and coelomocyte lysate (CL) and (3) qualitative and quantitative characterization of the pigments.

## 2. Materials and methods

### 2.1. Chemicals

Ascorbic acid (Vit C), 1,1-diphenyl 2-picrylhydrazyl (DPPH), and Butylatedhydroxytoluene (BHT), was obtained from Sigma (St. Louis, MO). All other chemicals and reagents used in the experiments were of analytical grade.

### 2.2. Animals and laboratory maintenance

The specimens of sea urchins, *E. mathaei* were manually obtained during low tide, from the beaches of Qeshm Island (26°55′N, 56°16′E), Hormozgan province, Persian Gulf, Iran in July 2015, thus outside the reproductive period that reported by Ref. [30]. Urchin samples (all adults, n = 30) were brought to the laboratory and were kept in a holding tank (100 L) with constant aeration considering suitable conditions i.e. 42.6 ppt salinity, 27 °C±1° temperature, 7.5–8.4 pH for two days. The average weight of the specimens used was 51.15 ± 2.5 g (n = 30).

### 2.3. Collection of coelomic fluid and samples preparation

Collection, handling, and analysis of sea urchin coelomocytes were assessed using the method followed by Smith et al. (2019). To perform this process, coelomic fluid from each individual of sea urchin *E. mathaei*, withdrawn from the coelomic cavity using syringes with needle range from 18 to 22 gauge, preloaded with isosmotic anticoagulant buffer (ISO-EDTA) containing 20 mM Tris, 0.15 M NaCl, 70 mM EDTA pH = 7.5. In view of sea urchin survival, the optimal volume of withdrawn fluid from adult *E. mathaei* is 0.5 mL CF [31]. The coelomic fluid was centrifuged at 900×*g* for 10 min at 4 °C, the supernatant as CF was separated from coelomocytes and other acellular components and stored at −20 °C [12,31].

### 2.4. Preparation of coelomocyte lysate supernatant (CLS)

Coelomocyte pellets were resuspended in 10 mL of 1 mL of ISO–$Ca^{2+}$ containing 10 mM of $Ca^{2+}$ and conducted to sonication for 4 min at 0 °C (1 pulse per second) and centrifuged at 27,000×*g* for 30 min at 4 °C. After ensuring no precipitate in the tube, these suspensions referred to as coelomocyte lysate (CL), and stored at −20 °C [31,32].

### 2.5. Liquid chromatography–mass spectrometer (LC–MS)

In order to accurately separate and determine the major *E. mathaei* quinonoid pigments in CFCF and CL, the optimized liquid chromatography coupled with ion trap mass spectrometry (LC-MS) was performed using the standard protocol [33]. Prepared coelomic fluid (20 μL) were analyzed on an LCQ-DECA system, comprising an Agilent LC 1200 series liquid chromatography coupled to a Thermo Finnigan mass spectrometer ion trap (Thermo Scientific, Hemel Hempstead, UK). HPLC condition including C18 column (250 9 4.6 mm, 5 lm), a solvent system delivered at a flow rate of 0.5 mL/min and consisted of a mixture of solvent (A) formic acid/water (0.1:100, v/v) and solvent (B) MeOH/acetonitrile (5: 9, v/v) were applied, so that the mobile phase was 50% mixture of solvents (A) and (B) in an isocratic elution. ESI was set in a negative mode in a condition including sheath gas: 60 mL min-1, auxiliary gas: 20 mL min-1, spray voltage: 4.5 kV, capillary temperature: 200C, capillary voltage: 46 kV, and tube lens: −60 kV). The Xcalibur 2.0 SR2 software (copyright Thermo Electron Corporation 1998–2006) was used. To HPLC method validation, the linearity of the method was established by using stock solutions of standard samples of Echinochrome A and Spinochrome A-E in methanol at different levels of 100–1300 ng/mL. Calibration curves were plotted by drawing the height of the negative peak vsconcentration [34]. The limits of quantification (LOQ) and detection (LOD) of standard samples were measured as levels at which the signal-to-noise ratio (SNR) is < 10 and < 3, respectively.

### 2.6. Quantification of the quinonoid compounds in CFCF and CL

When the collecting pigments in CFCF and CL were concentrated by evaporation and dissolved in ethanol, the quantity (μg/mL) of identified quinonoid compounds was calculated on the basis of molecular extinction coefficients and the absorbance (OD) of pigments by using formula [14,18]:

$$A = \varepsilon cl \quad (1)$$

where.

Λ = the absorbance of the solution (1 mL).
ε = the molar extinction coefficient (mol.$L^{-1}$. $cm^{-1}$) for standard samples of Spinochrome A (ε = 3311 at 520 nm), Spinochrome B (ε = 4898 at 480 nm), Spinochrome C (ε = 5888 at 463 nm) and Echinochrome A (ε = 7413 at 490 nm) [14].
c = the concentration of sample (mol.$L^{-1}$).
l = the light path length in centimeters (1 cm).

### 2.7. DPPH radical scavenging activity

The free radical scavenging activity of the CFCF and CL against 1,1-diphenyl-2-picrylhydrazyl (DPPH) was assessed using the method of Pozharitskaya et al. [34] and Duan et al. [35]. Briefly, the volumes of samples at gradient final concentrations (10–70 μl), and 0.1 mL of a 0.5 mM DPPH solution (in methanol), was mixed. After vigorous mixing and 30 min incubation in the dark at room temperature, the absorbance of the sample was taken at 490 nm by using an Elisa reader. For evaluation its ability to trap the DPPH free radicals, in parallel, blanks containing methanol instead of DPPH solution and controls containing methanol instead of the samples [35,36].

DPPH scavenging activity in the samples was calculated according to the following formula [36]:

$$\text{DPPH scavenging activity (\%)} = 100\% \text{ x } [1\text{-}(A_s\text{-}A_0/A)], \quad (2)$$

where, $A_s$ is the absorbance of the sample, $A_0$ of the blank, and A of the control. Butylatedhydroxytoluene (BHT) was used as a positive control.

### 2.8. Determination of reducing power

The reducing power of CFCF and CL was assessed according to the method of Oyaizu [37]. In brief, different volumes of various concentrations of CFCF and CL (12.5, 25, 50, 100, 200, 400 μl) was mixed 1 mL of phosphate buffer (0.1 M, pH 6.6) and 1 mL of potassium ferricyanide (1% w/v). The mixture was incubated at 50 °C for 20 min. Then, 1 mL of trichloroacetic acid (TCA, 10% w/v) was added and mixed with 1 mL of distilled water and 0.2 mL of ferric chloride (0.1%, w/v). The absorbance of this mixture was measured at 700 nm. Greater reductive potential is indicated by higher absorbance of the reaction mixture. BHT was used as the standard [38].

### 2.9. Determination of total antioxidant capacity (TAC)

The total antioxidant capacity of the pigments extract was determined according to the method described by Mitsuda et al. [39]. For evaluation TAC, 7.45 mL of sulfuric acid (0.6 M), 0.99 g of sodium sulphate was mixed with 1.23 g of ammonium molybdate in 250 mL with distilled water. Different volumes of samples (50, 100, 250, 500, 1000 μL) were dissolved in 1 mL of the TAC solution. After 15 min incubation at room temperature, the absorbance was read at 695 nm. Ascorbic acid (vitamin C, at 50–1000 μg/mL) was used as standard [39]. Increased absorbance of the reaction mixture indicated increased antioxidant activity.

### 2.10. Statistical analysis

All experiments were conducted in triplicate and all data are present in terms of means ± SD. The SPSS 21 (IBM, SPSS) software package and Excel 2010 software for Windows were used to analyze variance of the raw data. A one-way analysis of variance (ANOVA) and Duncan's new multiple-range tests were used to determine any significant differences among the means. Values of $p < 0.05$ were assumed significant.

## 3. Results

In the present study, the compositions of the quinonoid pigments of CFCF and CL fraction of *E. mathaei* coelomic fluid have been studied. To accurately quinonoid pigment identification, the absorption spectra of methanol extracts of the CFCF and CL comparing with the spectrum of standard samples of Spinochromes and Echinochrome by HPLC-MS analysis. Based on PHNQs molecular weight and LC-MS in negative mode, the quinonoid pigments content in sea urchins extracts and further confirmation was investigated. Figs. 1 and 2 illustrated the total ion current chromatograms and related mass spectra of Spinochrome A-C, and Echinochrome A in both CL and CFCF samples. Due to the optimization of the mass spectrometer in negative mode, all pigments appeared as deprotonated pseudo-molecular ions ([M − H]). The ions at 221, 279, 265, and 263 m/z corresponding to the pseudo-molecular [M − H] ions of Spinochrome B, Spinochrome C, Echinochrome A, and Spinochrome A, respectively. The results in Fig. 1 revealed that the LC-MS profile, total ion chromatogram, extracted ion chromatogram, and mass spectrum corresponding to Spinochrome B (221 m/z), Spinochrome C (279 m/z), Echinochrome A (265 m/z), and Spinochrome A (263 m/z) in the CL extract (Fig. 1), while in the CFCF extract (Fig. 2), it was corresponding to Spinochrome C (279 m/z). According to HPLC-MS analysis, Spinochrome C was the only pigment detected in the CFCF extract.

To quantification of quinonoid pigments content in the isolated CFCF, and CL fraction of *E. mathaei* coelomic fluid, we conducted a spectrophotometric approach. The results were displayed that the highest and lowest quantity pigment in the CL is Spinochrome A and Echinochrome A, respectively (Fig. 3). While moderate amounts of Spinochrome C were observed in the CFCF sample (Fig. 3). In this regard, Table 1 showed the percentages of identified quinonoid pigments in *E. mathaei* CFCF and CL. As a result, in 1 mL of CFCF extracts, Spinochrome C (up to 9.4%), and in 1 mL of CL extracts, Spinochrome A (up to 12.6%), as well as Spinochromes B (up to11.1%),C (up to 10%), and Echinochrome A (approximately 7%) were mainly present.

The DPPH radical has a characteristic absorption maximum between 78 and 88% in CFCF, and 38–67% in CL (Fig. 4a), which is abstracts a hydrogen atom from the hydroxyl group of PHNQs to become a constant diamagnetic structure. By losing two hydrogen atoms sequentially, PHNQs could become naphthosemiquinone as an intermediate product and naphthotetraketone as the final reaction product [1]. As can be seen in Fig. 4a, the coelomic fluid (CFCF and CL) of *E. mathaei* exhibited a dose-dependent DPPH radical scavenging activity at concentration of 10–70 μg/mL, whereas CFCF (88.12 DPPH% scavenging at 70 μg/mL, $IC_{50}$ = <10 μg/mL) was higher than that of CL (67.11 DPPH% scavenging at 70 μg/mL, $IC_{50}$ = 39.34 μg/mL) scavenging activity. However, their value approximately close to the positive control, BHT (89.92 DPPH% scavenging at 70 μg/mL, $IC_{50}$ = 20.84 μg/mL).

From the analysis in Fig. 4b, it was found that the coelomic fluid (CFCF, CL) *E. mathaei* was able to convert the oxidized form from $Fe^{2+}$ into $Fe^{3+}$. All the samples possessed the obvious chelating ability to reduce iron ($Fe^{3+}$) and also in a linear dose-dependent fashion at a level of 12.5–400 μg/mL. Among the samples, CFCF at a maximum volume (400 μl) was significantly ($p < 0.05$) higher than iron ($Fe^{3+}$) which was more reduced compared to CL. It appeared that the lowest reducing activity was possessed by CL.

The total antioxidant capacity of coelomic fluid (CFCF, CL) possessed a substantial dose-dependent effect at a concentration range of 50–1000 μg/mL. The results were displayed that the TAC capacity of the CFCF excellent and significantly ($p < 0.05$) higher than that of the well-known synthetic antioxidant, vitamin C (Vit C). Though results showed at a low concentration, the TAC capacity of CL was weaker than that of VitC. However, at concentrations of 1000 μg/mL, it was comparable to that of Vit C (Fig. 4c).

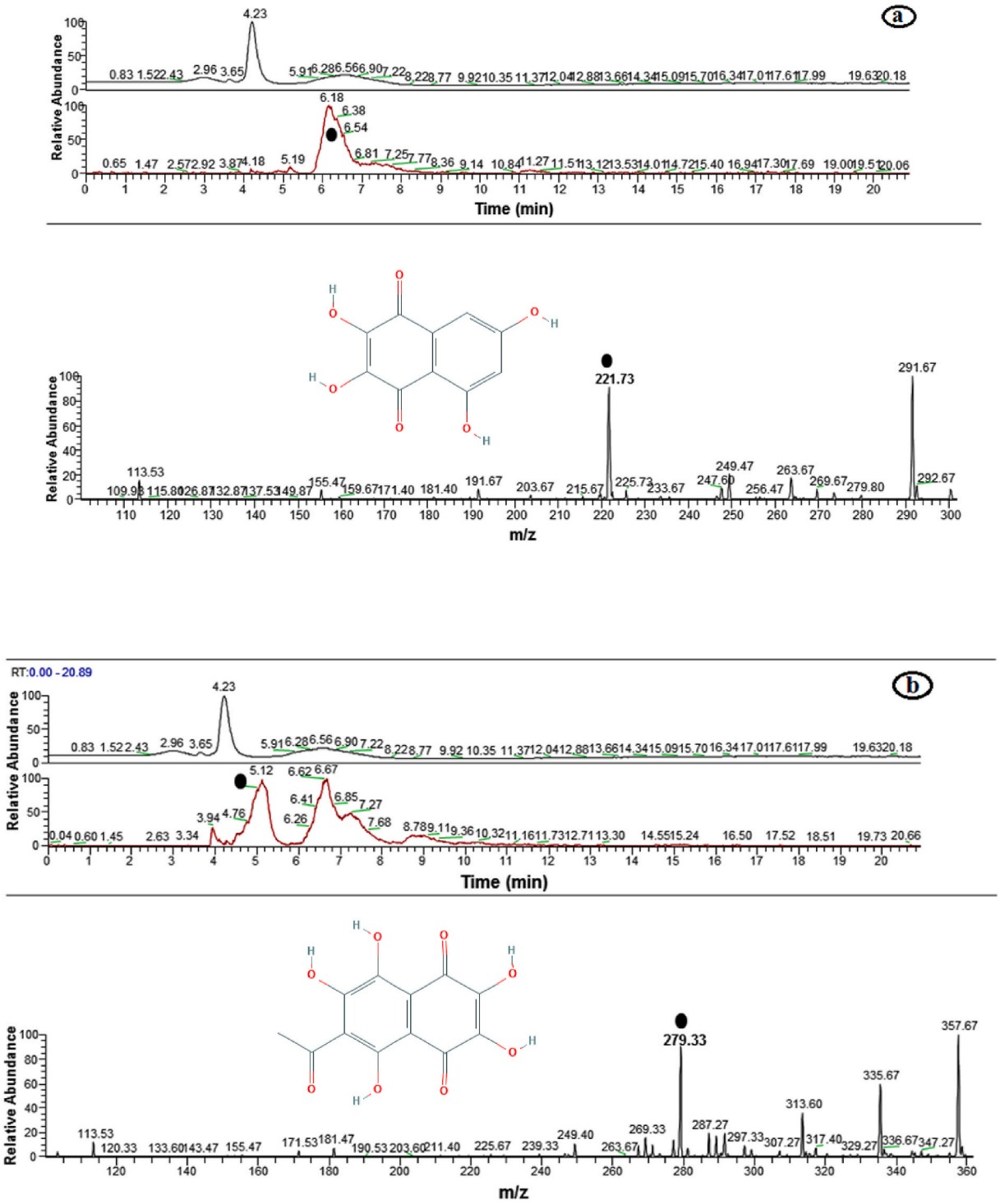



**Fig. 1.** LC-MS profile, total ion chromatgram, extracted ion chromatgram and mass spcetrum corresponding to Spinochrome B (a), Spinochrome C (b), Echinochrome A (c) and Spinochrome A (d) in CL extract.

## 4. Discussion

Coelomocytes as the immune effectors in the coelomic fluid of the Echinoid have been studied for many decades, based on their ability to respond to injuries, host invasion, and cytotoxic agents [40–42]. In contrast, the identification and reporting of antioxidant PHNQs pigments from the coelomic fluid of the sea urchin is limited [29,43]. Though, the PHNQs pigments were previously reported for the shells and spines of the sea urchin [1,44,45], our study is the first of its kind to report the PHNQs pigments in the coelomic fluid of *E. mathaei* from the Persian Gulf.

Existing pigments in the coelomic fluid of *E. mathaei* species, such as Spinochrome A, Spinochrome B, Spinochrome C, and Echinochrome A, as well as their structural variety, are thought to represent a species specificity feature [44]. In the present study, Spinochrome C was the only PHNQs pigment detected in the CFCF, whereas, recently, Vasileva

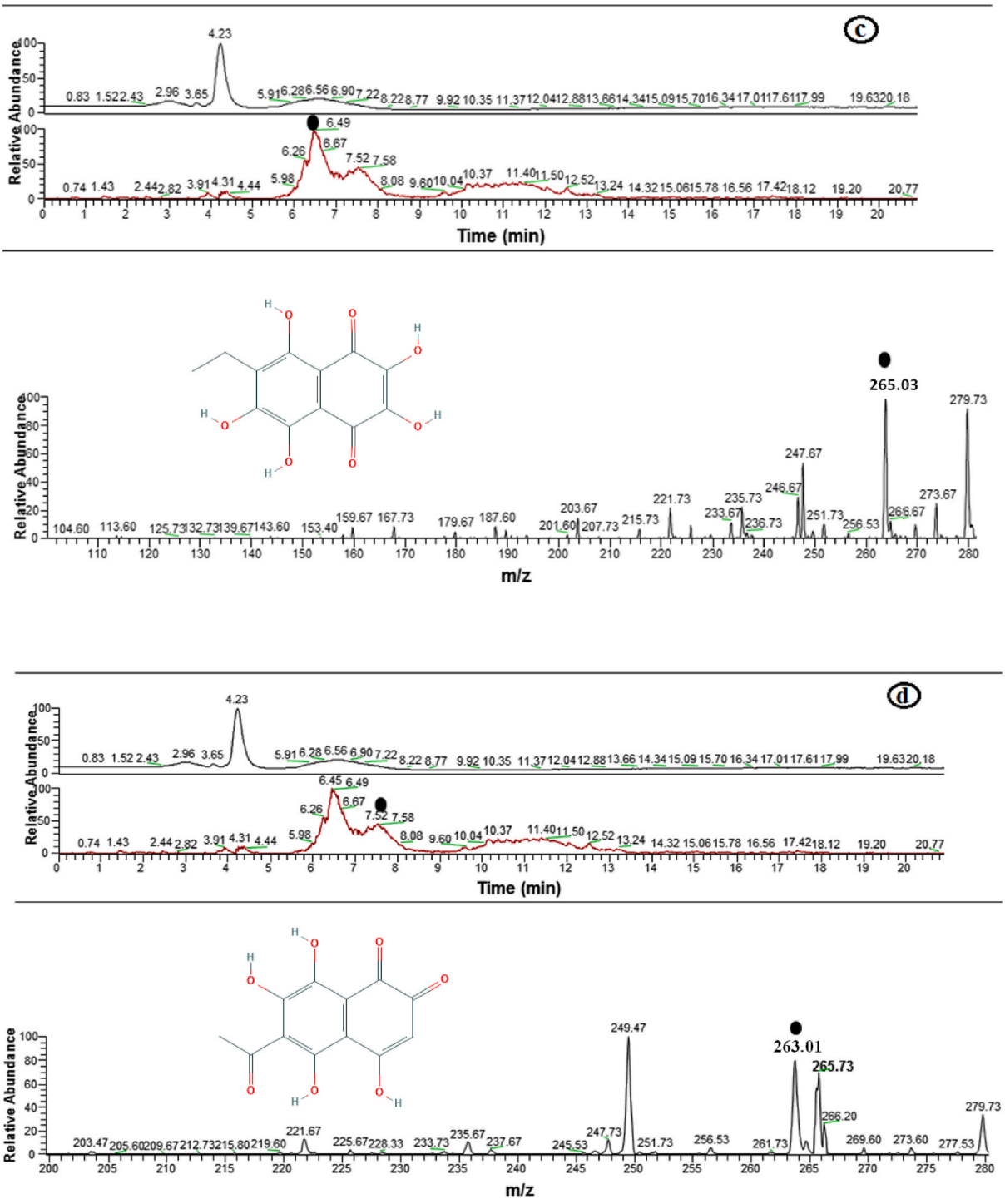



**Fig. 1.** (*continued*).

et al. [43] reported Echinochrome A as the predominant quinonoid pigments in the CFCF from the following sea urchins: *Astropyga radiata*, *Diadema setosum*, *Echinarachnius parma*, *Echinocardium cordatum*, *Echinothrix calamaris*, and *Scaphechinus mirabilis*; in the CFCF of *Mesocentrotus nudus*, *D. setosum* and *E. calamaris*, Spinochrome E was also existed; and in the CFCF of *Strongylocentrotus intermedius*, Echinochrome A, as well as Spinochromes E and D, were present. Due to the varied developmental, ecological, and seasonal roles, gender variations, and the life support of each sea urchin species, it is expected that the composition of PHNQs pigments as secondary metabolites may differ substantially [43].

The present study investigated the PHNQs antioxidant activity of chromatographic subfractions CFCF and CL that obtained from *E. mathaei* coelomic fluid. Based on the anti-DPPH radical potency of quinonoid pigments, PHNQs become a naphthosemiquinone as an intermediate product if transmit two electrons consecutively to free radicals and then converted to naphthotetraketone [1,46]. Here, in the presence of a hydrogen donating PHNQs antioxidant of CFCF and CL, the coelomic fluid of *E. mathaei* was capable of reducing the stable radical DPPH• to the yellow coloured 1-diphenyl-2-pricrylhydrazine. CFCF from *E. mathaei*, on the other hand, has the greatest DPPH radical scavenging activity. These findings agreed with those reported

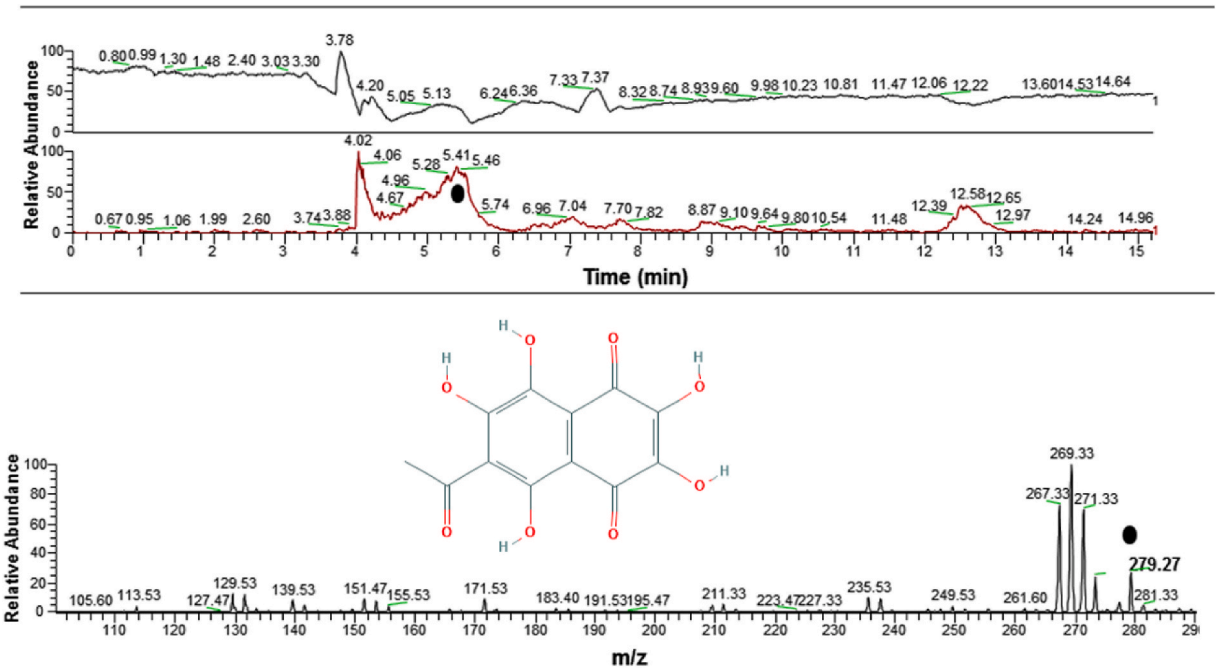



**Fig. 2.** LC-MS profile, total ion chromatgram, extracted ion chromatgram and mass spcetrum corresponding to Spinochrome C in CFCF extract.

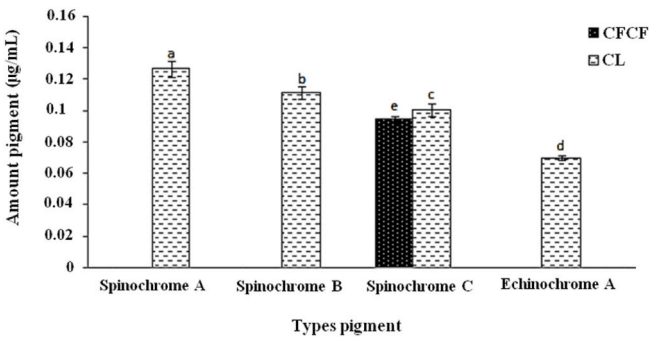
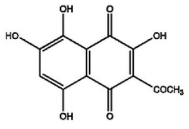
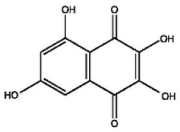
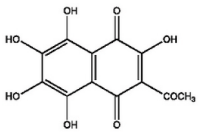
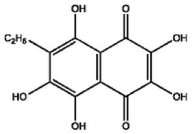



**Fig. 3.** The quantification of quinonoid compounds in CFCF and CL of sea urchin (µg/mL) on the basis of molecular extinction coefficients (Cited by Refs. [14,18] (Values with different lowercase letters are significantly different ($P < 0.05$)).

previously in shells/spine PHNQs pigments from sea urchins, *E. mathaei*, *Glyptocidaris crenularis*, *Strongylocentrotus nudus*, *Anthocidari scrassispina,* and *Evechinus chloroticus* [1,44,45,47,48]. Though the PHNQs pigments of both shells and spines illustrated previously a potent DPPH radical scavenging activity in a concentration-dependent manner [1,44,45,47,48], the current scavenging results obtained here from PHNQs of *E. mathaei* CFCF and CL were significantly stronger than those of shells/spines PHNQs pigments [44,48,49], and to be similar to that of BHT [1,45]. CFCF and CL of *E. mathaei* have greater pigment/unit weight than their spines/shells, according to the DPPH test [44]. Because, as previously reported, the percent DPPH scavenging activity of *E. mathaei* spines/shells in the concentration range of 12.5–800 µg/mL was up to 60%, whereas CFCF and CL in the concentration range of 10–70 µg/mL were 80% and 68%, respectively (Fig. 4a). Furthermore, the anti-DPPH radicalactivity of CFCF and CL were shown to differ based on the first recognition of the different quinonoid composition of Spinochrome A-C, and Echinochrome A in CL and Spinochrome C in CFCF, and the variable numbers of their hydroxyl groups of Spinochromes [45,50].

In comparison to this work, Li et al. [45] and Zhou et al. [1] showed that the DPPH scavenging activity of *G. crenularis* crude extracts, which included a combination of Spinochrome B, D, and E, was found to be greater than that of *S. intermedius* crude extracts, which only contained Spinochrome B. However, Vasileva et al. [51] reported that the scavenging activity of PHNQs pigment in North Pacific sea urchins decreased in the order Echinamine B > Spinamine E > Echinochrome A > Echinamine A > Spinochrome E > α-tocopherol. Overall, variations in the compositions [43] and molecular structures [1] of the sea urchin organ quinonoid pigments appear to affect their bioactivity and function. Because reductions were reported to be terminators of free radical chain reactions and caused the conversion of $Fe^{3+}$/ferric cyanide complex to its $Fe^{2+}$/ferrous cyanide complex, the higher absorbance at high concentration indicates a strong antioxidant activity in the case of reducing

**Table 1**
Occurrence, structure, and the percentage of identified main quinonoid pigments in 1 mL of *E. mathaei* CFCF and CL.

| Structure chemical of pigments |  Spinochrome A |  Spinochrome B |  Spinochrome C |  Echinochrome A |
|---|---|---|---|---|
| **CFCF**[a] | – | – | 9.4 | – |
| **CL**[a] | 12.6 | 11.1 | 10.0 | 6.9 |

[a] All quinonoid pigments measurements were based on [14] method.

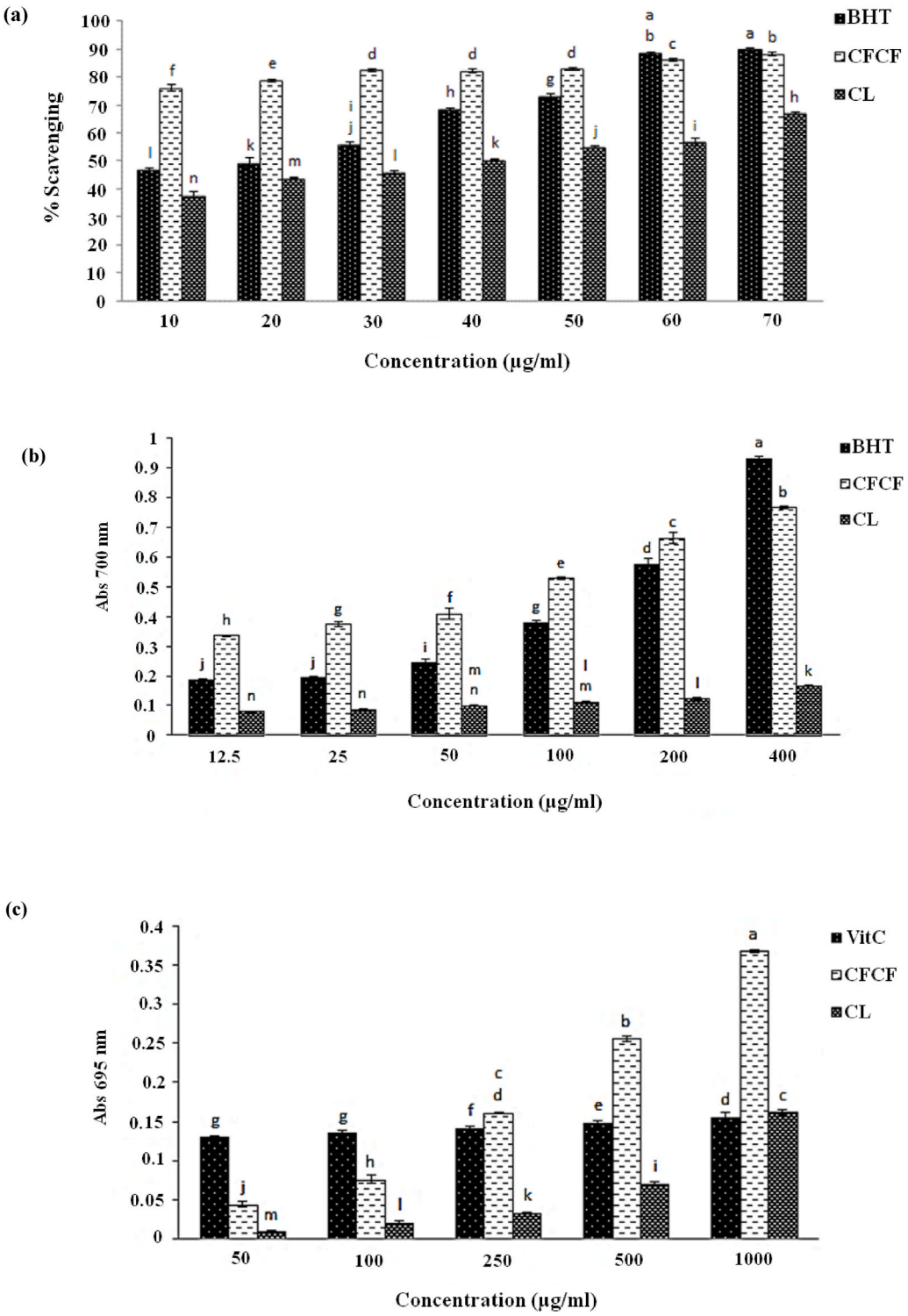



**Fig. 4.** Antioxidant activity of CFCF and CL from *E. mathaei* coelomic fluid. a) DPPH scavenging activity, b) reducing power activity, and, c) TAC. Significant differences are indicated by different letters as determined by Duncan's Post-Hok multiple comparison ($p \leq 0.05$).

the capacity of CFCF and CL PHNQs pigments [35,52]. These findings agreed with the report of Ferreira et al. [53] that exhibited the reducing power increased with an increase in specimen size. Based on di- and polyvalent anions of PHNQs, and their difference in susceptibility to oxygen and prevention of Fenton-induced oxidation, Spinochrome C in CFCF exhibited greater ferrous ion chelating ability than CL pigment extracts, as shown in Fig. 4b [1,45,54]. Though, the CFCF chelating potential was lower than that of BHT, which was comparable to those of shells/spines PHNQs pigments [44]. When compared to a conventional antioxidant, Vit C, the findings of this investigation revealed that *E. mathaei* cell-free coelomic fluid had a high TAC capacity under the circumstances studied. Given the importance of Spinochromes in PHNQ bioactivity, the antioxidant activities of CFCF and CL were shown to be considerably greater in CFCF than in CL. It can be connected to Spinochrome structure and position, as well as the quantity of double bonds, hydroxyls, and acetyl groups [55]. The theory is that the quinonoid

pigment composition and low rate of PHNQs heterogeneity in the CFCF (which only included Spinochrome C with 5 hydroxyl radicals) might affect their antioxidant capabilities [43,55]. Alternatively, as reported by Luparello et al. [56]; the cytotoxicity of *A. lixula* CFCF extracts was investigated to see if they might affect cell redox state, reactive oxygen species (ROS) production, mitochondrial depolarization, cell cycle distribution, and autophagic activity. It's worth noting that PHNQs' ROS-scavenging mechanism may be chemically comparable to those of polyphenols (e.g., flavonoids) [47,49]. The structural characteristics of natural polyphenols, such as hydroxylation, methylation, and the degree of sulfation, can function as a ROS inhibitor [57]. In AC16 cardiomyocytes, hydroxylated substances such as Spinochromes were shown to have increased ATP generation and oxygen consumption rate (OCR) in the face of oxidative stress and doxorubicin [58]. Additionally, some researcher has distinguished Echinochrome A from primary immune cells of sea urchin red spherule cells (RSC) [27,59]. RSCs are responsible for the biogenesis of naphthoquinone compounds derived from Spinochrome dimers/Echinochrome and for the regulation of their bioactivity function [59].

In relevance of marine invertebrates dietary antioxidants, differences between sea urchin species can result from a variety of natural niches, feeding habits (e.g., catching on drift algal/seaweed with high potent antioxidant activity) [60–62], and synergistic activity with other potent diversity of the mechanisms of PHNQs radical selectivity [34,63].

## 5. Conclusion

First, polyhydroxylatednaphthoquinone pigment was identified and quantified in *E. mathaei* cell-free coelomic fluid and coelomocytelysate from the Persian Gulf. The structures of the major quinonoid pigments Spinochrome A, B, C, and Echinochrome A in CL and Spinochrome C in CFCF were verified by HPLC-MS analysis. It was interesting that the PHNQs enriched coelomic fluid of *E. mathaei* contains effective natural antioxidants (especially in CFCF and to some extent in CL) and possess antiradical activity on DPPH radicals, activeiron chelation, and TAC capacity which approximately exceeds that of synthetic antioxidants such as vitamin C and BHT. The antioxidant potent of PHNQ compounds in the CFCF and CL exhibited a substantial dose-dependent effect. The *E. mathaei* coelomic pigments could find use as a new molecular targets and natural food grade antioxidants to replace and/or decreasing the prices of the commercial antioxidants products. Furthermore, the established HPLC-MS fractionation of PHNQs will be extremely beneficial for evaluating the quality and stability of medicines produced from sea urchin pigments.

## CRediT authorship contribution statement

SS, and SM1, implemented the experiments, writing, and data handling. HR assisted in LC-MS quantitation. SM2 took part in English editing the manuscript. SM1and MY critically revised and edited the manuscript for important intellectual content and approved it for submission. MY was the supervisor of this work, prepared materials, and designed experiments.

## Declaration of competing interest

The authors declare no conflict of interest.

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