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Unique heterocyclic phenolic compounds from shrimp (*Pandalus borealis*) and beyond

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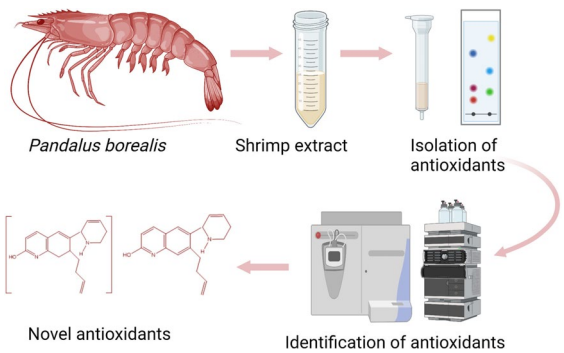
Abstract

Shrimps are the most consumed species among crustaceans and are well appreciated for their flavor and high nutritional value. This work investigated the antioxidative compounds of Northern shrimp (*Pandalus borealis*) and its processing discards (shells and heads) using various solvents and chromatographic techniques as well as mass spectrometry. Ethanol served as the best extraction medium, and the extracts obtained demonstrated strong antioxidant activity in a β-carotene-linoleate emulsion system. The antioxidative compounds of the extract were isolated using a silica gel column chromatography, followed by thin layer chromatography. This led to the identification of two highly polar and two low polarity compounds. These compounds, for the first time, were isolated and purified by using reversed-phase HPLC. Their structures were tentatively elucidated using electrospray mass spectrometry. These unique compounds were heterocyclic phenolic compounds and identified as 7-(3-butenyl)-2-hydroxy-6-(1,2,5,6-tetrahydropyridin-2-yl) quinoline and 7-(3-butenyl)-2-hydroxy-6-(1,2,5,6-tertahydropyridin-2-yl)-7,8-dihydroquinoline and their isomers. The proposed novel compounds in shrimp provide a reason for their oxidative stability and potential benefits, as well as possibly serving as substrates for the discoloration of shrimp.

Keywords *Pandalus borealis*, Processing discards, Antioxidants, HPLC, Mass spectrometry

Graphical Abstract



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Introduction

Shrimp species are a good source of proteins and amino acids (e.g., histidine, proline, and arginine), lipids and omega-3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid), minerals (e.g., copper, calcium, magnesium, zinc, and phosphorus), and vitamins along with various other components such as chitin and carotenoids (AlFaris et al. 2022). The consumption of shrimp has increased remarkably over the past few decades, generating up to 50% of by-products/ waste (e.g., heads, shells, and tails), which are a challenge for fish processing industries due to their potential environmental impact upon disposal. Shrimp shells are mainly composed of 35% minerals, 18–40% protein, and 14–30% chitin (Ambigaipalan & Shahidi 2017). Therefore, there is an increasing interest in utilizing these discards in the production of value-added products with desirable functional properties. So far, efforts have been made to utilize these by-products for the extraction of carotenoids (astaxanthin, canthaxanthin, and zeaxanthin), proteins, and chitin and chitosan (Heu et al. 2003; Lira et al. 2017). Northern shrimp (*Pandalus borealis*) is distributed in the Northwest Atlantic from Baffin Bay south to the Gulf of Maine. The meat recovery of Northern shrimp is about 25% (w/w), while the rest of the body parts are often considered as waste/ by-products. The percentage of shrimp (*P. borealis*) shells is around 25% (dm), which contain 33–40% protein, 17–20% chitin, 34% ash, and 0.3–0.5% lipid (Rødde et al. 2008). Despite much-concerted efforts, shells have not been highly utilized, but some have been used as a protein meal or as a feed additive or processed to produce chitin/ chitosan and glucosamine (Ambigaipalan & Shahidi 2017).

Phenolic compounds are powerful natural antioxidants that exhibit a wide range of biological properties, such as anticancer, anti-inflammatory, antihypertensive, antidiabetic, and antimicrobial activities, among others (Shahidi & Hossain 2023). In particular, phenolic antioxidants obtained from natural sources have received significant attention from the food, nutraceutical, pharmaceutical, cosmeceutical, and packaging industries due to their antioxidant and antimicrobial characteristics (Shahidi & Hossain 2022). However, most of the antioxidants found in the marketplace originate from terrestrial environments, while less attention has been paid to aquatic ones. In the last few years, a series of promising new marine-derived antioxidative compounds, including phenolic compounds, have been isolated and launched to the world market due to their unique attributes (Hossain et al. 2020; Hossain et al. 2023). To date, shrimp and its by-products/ waste (shells, tails, heads, and paws) have been characterized for their antioxidant activity due to the presence of carotenoids, proteins (protein hydrolysates/ peptides), and indolocarbazole type compounds (AlFaris et al. 2022; Ambigaipalan & Shahidi 2017; García-Romo et al. 2020; Lira et al. 2017). However, no information is yet available on the phenolic compounds of shrimp and its processing discards. Shrimp contains phenolase; thus, the presence of phenolic compounds in shrimp is well expected. Therefore, the aim of this study was to isolate and identify phenolic compounds in Northern shrimp and its processing by-products using various chromatographic techniques and mass spectrometry for the first time. Moreover, the contents of total phenolics and carotenoids were determined, and their antioxidant activities were evaluated using a β-carotene-linoleate model system.

Materials and methods

Chemicals and reagents

Chloroform, methanol, ethanol, n-hexane, acetone, n-butanol, acetic acid, phosphomolybdic acid, sodium tungstate, sodium carbonate, phosphoric acid, gallic acid, β-carotene, Tween 40, and butylated hydroxyanisole (BHA) were procured from the Fisher Scientific Co. (Nepean, ON, Canada) and Sigma-Aldrich Ltd. (Oakville, ON, Canada).

Preparation of shrimp extracts

Northern shrimp (*Pandalus borealis*) were collected from a local seafood processing plant in Newfoundland, Canada. Shrimp flesh (without the shell), shells, and heads were separated, and then extracts were prepared using various solvents, namely chloroform, acetone, diethyl ether, and ethanol. For that, 50 g of samples (heads or flesh) were homogenized with 100 mL of solvent at 1200 rpm for 2 min using a Polytron homogenizer, while shells were homogenized with a sample to solvent ratio of 1:3 (w/v). A Whatman No. 1 filter paper was used to filter the slurry, and the resultant filtrates were evaporated to dryness using a rotary evaporator under vacuum at 40 °C. The residues were dissolved in 100 mL of absolute ethanol and stored at − 20 °C for subsequent analysis.

Determination of total phenolic content (TPC) of shrimp extracts

The Folin-Denis reagent was used to determine the TPC in the ethanolic extract of shrimp samples (Swain & Hillis 1959). The Folin-Denis reagent was prepared using 20 g phosphomolybdic acid, 100 g of sodium tungstate, and 50 mL phosphoric acid in 750 mL distilled water. The

mixture was allowed to stand for 2 h, followed by cooling. After that, 0.25 mL of Folin-Denis reagent, 0.1 mL of shrimp ethanolic extract, 0.5 mL of sodium carbonate, and 2 mL distilled water were mixed and then allowed to stand for 30 min. The absorbance was measured at 725 nm using a UV – visible spectrophotometer (HP8452 A diode array spectrophotometer, Agilent Technologies, Palo Alto, CA, USA), and the TPC was calculated in gallic acid equivalents (GAE).

Determination of total carotenoid content (TCC) of shrimp extracts

The TCC in the lipid fraction of samples was evaluated based on the method of Saito and Regier (1971) with some modifications. Lipids and carotenoids were extracted according to Bligh and Dyer (1959) using chloroform and methanol. After evaporating the solvent layer using a rotary evaporator, the samples were reconstituted in 50 mL of petroleum ether. The absorbance of this solution was measured at 468 nm using a UV – visible spectrophotometer, and the content of carotenoids was calculated according to the following equation.

$$\text{Content of carotenoid } (\mu g/\text{g}) = (\text{Absorbance} \times \text{total extract volume})/(\text{sample weight} \times \text{absorbance of standard astaxanthin})$$

Determination of antioxidant activity using the β-carotene-linoleate model system

The antioxidant activity of shrimp extracts was determined using the β-carotene-linoleate model system based on the method described by Miller (1971) with a minor modification. Briefly, 1 mg of β-carotene, 200 mg of Tween 40, 20 mg of purified linoleic acid, and 50 mL of distilled water were mixed in a flask, followed by mixing. After that, 1 mL of shrimp extract in different solvents was added to 5 mL of prepared emulsion in a series of glass tubes. A positive control (BHA) and a control (without shrimp extract) were prepared, similar to the sample preparation for comparative studies. The zero-time absorbance was read at 470 nm using a UV – visible spectrophotometer immediately after the addition of emulsion into each tube. The mixture was then placed in a hot water bath at 50 °C, and the absorbances were recorded at 15 min intervals till the color of β-carotene was fully bleached (~ 120 min).

Separation of antioxidants from shrimp using column chromatography

The isolation of phenolics from ground shrimp was carried out by extraction of the crude components using 95% ethanol (l:2, w/v). The extract was then concentrated using a rotary evaporator at 50 °C, followed by passing nitrogen gas into the vials. The first step of the separation of the components was done using a column chromatography containing 2.5 × 25 cm column packed with silica gel (60–200 mesh, J. T. Baker Chemical Co., Phillipsburg, NJ). Initially, the column was washed with methanol, followed by 400 mL of n-hexane/acetone (3:1, v/v). The crude ethanolic extract was reconstituted in 10 mL ethanol and applied to the column. Two solvent systems with different degrees of polarity were used for the elution of the two components. Solvents A and B were n-hexane/ acetone (3:l, v/v) and n-butanol/ water/ acetic acid (3:l:l, v/v/v), respectively. The resultant fractions (25 mL each) were collected, followed by tentative identification of the type of components using thin layer chromatography (TLC).

Thin layer chromatography (TLC) of shrimp isolates

The collected eluate fractions from column chromatography were examined by TLC to identify the presence of phenolic compounds. TLC plates (Silica Gel 60 with 200 µm thickness, E. Merck, Darmstadt, Germany) were constructed in a 22 × 22 × 10 cm glass chamber using solvent systems A (n-hexane/ acetone) and B (n-butanol/ water/ acetic acid) as mobile phases. After spraying with a ferric chloride reagent (1% ferric chloride in 2.5 M HCl), the plates were placed in a forced-air convection oven at 105 °C for 5 min to develop color and then cooled to room temperature. The development of a brownish-yellow color indicated the presence of phenolic compounds.

In another set of experiments, fractions 3–6 were separated by using solvent A and fractions 6-ll using solvent B, followed by evaporation for solvent removal. A preparative TLC was employed to obtain sufficient quantities of components of interest. The dried isolates obtained from the above steps were reconstituted in analytical-grade methanol and then applied to silica gel plates as mentioned above. The chromatograms were developed using solvent system A or B as the mobile phase. Components from bands with identical R_f values were scrapped off the plate, collected, and then reconstituted in methanol. The solution was centrifuged at 3500 x g for 3 min, and the supernatant was evaporated to dryness under a stream of nitrogen.

High-performance liquid chromatography (HPLC) of shrimp isolates

A semi-preparative HPLC was used to further purify the samples obtained by the preparative TLC in the

preceding section. A Shimadzu chromatographic system (Kyoto, Japan) was used: LC-6A pump, SCL-6B system controller, SPD-6AV UV-VIS spectrophotometric detector, and CR 501 chromatopac. The columns used were Particil 10 ODS-2 column (9.4 × 250 mm, 10 µm, Whatman) with precolumn (4.6 × 125 mm, Whatman) packed along with Pellicular ODS (C_{18} groups chemically bonded to 37–53 µm glass beads, Whatman). The mobile phase was 90% (v/v) HPLC-grade methanol, followed by absolute methanol. The injection volume of sample in methanol was 500 µL with a flow rate 3 mL/ min. Pure components obtained were also investigated by HPLC on an analytical column. An analytical CWSL column (4.5 × 250 mm) with Spherisorb ODS-2 (10 µm, Chromatography Sciences Company Inc. Montreal, QC, Canada) was used for this purpose. The injection volume and flow rates were 20 µL and 1.5 mL/ min, respectively. The detector was set at 280 nm for both semipreparative and analytical HPLCs. The purity of the compounds collected was also analyzed by TLC under the conditions mentioned above.

Mass spectrometric analysis

The shrimp isolates, purified by reverse-phase HPLC, were desolventized and then dissolved in HPLC-grade methanol (around 1 mg/ mL). A 20 µL aliquot of the sample was then applied to the electrospray ion (ESI) source by a continuous flow of acetonitrile/ water (70:30, v/v) using a Shimadzu LC-10 AD pump linked to the Rheodyne injector with a 20 µL loop. The ESI mass spectra were recorded with a Micromass VG-Quattro II quadrupole-hexapole-quadrupole mass spectrometer (Micromass, Cheshire, UK) in a negative-ion mode. This was equipped with an ESI source and capable of analyzing ions up to *m/z* 4000. A personal computer window equipped with Micromass MASSLYNX 3.1 Mass Spectrometry Data System software was used for data analysis. The operating voltage of the ESI capillary was 3.0 kV, and the high-voltage lens was set at 0.40 kV during the whole process, while the temperature of the ESI ionization source was kept at 75 °C. A cone voltage was set to 75 V for recording ESI mass spectra. A scanning multichannel analysis (MCA) mode with a scan time of l sec per 250 amu was used to obtain mass spectra. Product ion scans (collision-induced dissociation (CID)-MS/MS) experiments were conducted using the same instrument. Product-ion scan of mass-selected precursor ions were induced by collision with argon in the (radio frequency (RF) only) hexapole. The resulting fragment ions were analyzed by the second quadrupole. The CID-MS/MS analyses were recorded in an MCA mode and had consistently a combined average of 25 scans for each type of precursor ion. A cone voltage varying from 20 to 35 V and collision energies of 10 to 40 eV were used in all CID-MS/ MS experiments.

Statistical analysis

The data were reported as mean ± standard deviation of triplicate measurements. Analysis of variance (ANOVA) was performed, and mean values were determined using Tukey's studentized test at $p < 0.01$ or 0.05 and employing ANOVA and TUKEY'S procedures of statistical analysis system (SAS). Simple linear and multiple regression analysis were also performed using the same software.

Results and discussion

Yield, TPC, and TCC of shrimp ethanolic extracts

Among the solvents (acetone, chloroform, diethyl ether, and ethanol) used in this study to extract antioxidant components from shrimp and its by-products, ethanol was most effective (data not shown), hence used subsequently. On a wet basis, the flesh yielded the highest crude ethanolic extract (7.8%), while heads, whole shrimp, and shells yielded 6.3, 6.1, and 4.1%, respectively. However, crude extracts may contain components other than antioxidants that affect the total yield. Interestingly, the shells resulted in the highest yield of crude extract on a dry-weight basis.

A higher content of TPC was observed in shrimp shells compared to their counterparts. The TPC of shells, whole shrimp, flesh, and heads was 0.28. 0.21. 0.19 and 0.15 mg GAE/g, respectively (Table 1). Maia et al. (2023) suggested that the TPC of shrimp shell waste from *Palaemon serratus* and *Palaemon varians* from the Portuguese coast ranged between 4.7 and 10.4 mg GAE/g. The variation could be related to the shrimp species as well as geographical locations. In shrimp and lobster, enzymes (e.g., phenoloxidase) catalyze the o-hydroxylation of monophenols and dehydrogenation of o-diphenols (Perdomo-Morales et al. 2007). In crustaceans, this enzyme causes surface discoloration (melanosis) through enzymatic degradation of precursor components, which polymerize to develop insoluble pigments. The enzyme presents in shrimp provides an indication of the existence of phenolic compounds, which are

Table 1 Total phenolic content (TPC) and total carotenoid content (TCC) in shrimp and its by-products

Assay	Shells	Heads	Flesh	Whole shrimp
TPC (mg GAE/g)	0.28 ± 0.04^{a}	0.15 ± 0.0^{c}	0.19 ± 0.02^{b}	0.21 ± 0.02^{b}
TCC (µg/g)	18.0 ± 0.10^{a}	11.1 ± 0.06^{c}	7.2 ± 0.02^{d}	16.4 ± 0.12^{b}

Data represent mean values for each sample ± standard deviation ($n = 3$)

[a,b,c] Different letters for each assay indicate significant differences ($p < 0.05$) among different body parts

substrates for the enzyme. The presence of phenolics in various body parts of shrimp results in relevant activities expected of them. Phenolic compounds, along with the functional groups of amino acids, play an important role in tissue regeneration and healing (Hossain et al. 2022a). The existence of phenolics was also indicated by the appearance of a brownish-yellow color when TLC plates of the extract were applied with a ferric chloride reagent. The presence of phenolics in shrimp could occur as intermediates of a few metabolic pathways, breakdown products of other components, or as end products of specific metabolic pathways, mostly formed to protect against oxidative damage. These phenolic compounds may also have other functions related to physiological activities, which are yet to be established. Interestingly, it has been reported that marine species (e.g., sea cucumber) contain phenolic compounds, mostly phenolic acids and flavonoids, which are mainly related to their food sources, such as algal materials (Hossain et al. 2022b; Hossain et al. 2022). Similarly, the existence of phenolic compounds in shrimp could be linked to the presence of phenolic-rich materials, mainly algae and living plants, in their diet.

Carotenoids are present in crustaceans such as crab, shrimp, and lobster, as well as salmonid fish, namely salmon, char, and rainbow trout. The TCC of the different parts of shrimp was the highest in the shells (~18 µg/g sample), followed by those in the flesh, heads, and whole shrimp at 7.2, 11.1, and 16.4, µg/g, respectively (Table 1). Apart from providing desirable color, carotenoids in shrimp show antioxidant activity (Lira et al. 2017).

Antioxidant activity of the crude shrimp extract

The antioxidant activity of shrimp heads was investigated using the β-carotene/linoleate model system, as presented in Fig. 1. Absolute ethanol was found to be the most effective system among the solvents with varying degrees of polarity used in the extraction of shrimp heads. Similar trends were obtained from the other body parts, where ethanol was the best solvent (data not shown). All solvents used in this study were able to extract these compounds to varying degrees, though chloroform, acetone, and diethyl ether were less efficient than ethanol in extracting antioxidant components from shrimp. The degree of effectiveness of the extraction process in terms of antioxidant properties was in the order of ethanol> acetone> chloroform> diethyl ether. The efficient extraction of the antioxidants in ethanol could be due to the polar characteristics of these compounds. However, BHA and control samples yielded higher and lower antioxidant activity, respectively, as indicated by delayed bleaching of β-carotene in an emulsion system. The potential role of these compounds as major antioxidants of shrimp could be related to the presence of phenolic compounds in the extract. Moreover, a potential synergistic effect between carotenoids and phenolics could be responsible for the observed overall antioxidant property of shrimp. The phenolic compounds or the carotenoids may also impart

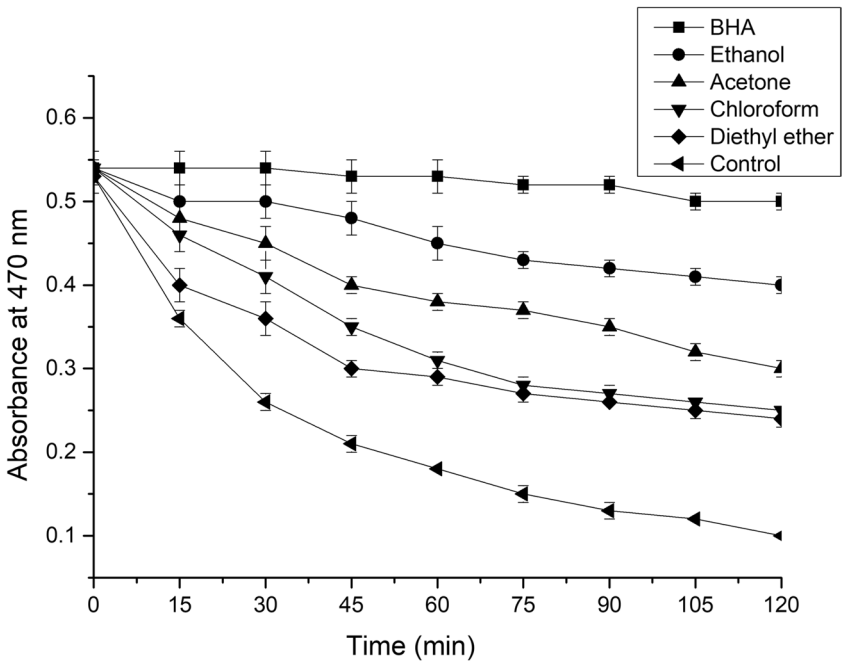

Fig. 1 Antioxidant activity of crude extracts of shrimp heads as assessed in a β-carotene-linoleate model system

their impacts independently of each other. Several studies have been conducted on the antioxidant properties of various shrimp species (e.g., *Litopenaeus Schmitti*, *Litopenaeus stylirostris*, and *Penaeus semisulcatus*), however, the actual compounds responsible for antioxidant efficacy have remained illusive (AlFaris et al. 2022; García-Romo et al. 2020; Lira et al. 2017).

Isolation of antioxidative compounds of shrimp using chromatographic techniques

A silica gel column was used to separate the crude extract of shrimp after ethanolic (95%, v/v) extraction. Two solvent systems with different polarities were used as no single solvent system was efficient in separating all compounds of the crude extract. To elute the less polar components, solvent A (n-hexane/acetone, 3:1, v/v) was used, and six fractions (fractions 1–6) were collected. The more polar compounds were eluted with n-butanol/water/ acetic acid (3: 1:1, v/v/v), which generated five fractions (fractions 7–11). The presence of phenolic compounds was indicated by the appearance of a brownish-yellow color upon spraying of the TLC plates with a ferric chloride reagent during the TLC separation of different fractions.

Fractions 3–6 and 7–11, separated by solvents A and B, were pooled together and used for analytical TLC. The polarity of solvent A was not sufficient to develop compounds from fractions 7–11. Bands 3 and 4 obtained from fractions 7–11 were more polar than bands 1 and 2. Hence, R_f values of components l and 2 were 0.30 and 0.29, respectively, while solvent A was used for developing the plates. In contrast, solvent B developed a second chromatogram, where compounds 3 and 4 had R_f values of 0.54 and 0.34, respectively. Moreover, all separated compounds delayed the bleaching of β-carotene when another set of plates was sprayed with a β-carotene-linoleate solution to determine the antioxidant properties of the spots, indicating that they were antioxidative in nature.

Isolation of individual compounds by reversed-phase HPLC

Various compounds were not eluted from the column when concentrations of 20 to 90% (v/v) methanol were used; however, only l00% methanol was efficient as the elution solvent. In the semi-preparative reversed-phase HPLC, the retention times of these peaks were 13, 47, 21, and 16 min for compounds 1, 2, 3, and 4, respectively (Supporting information, Fig. S1). Nevertheless, when analytical reversed-phase HPLC was employed, the corresponding retention times of the compounds were 4, 14, 8, and 3 min, respectively (Supporting information, Fig. S2). The purity of the isolated components was confirmed by using analytical TLC. The isolated components dissolved in methanol were examined by UV-visible spectroscopy. The absorption maxima were 270, 280, and 274 nm for compounds 1, 3, and 4, respectively. Nevertheless, compound 4 had multi-peak absorptions at 262, 272, 282, and 294 nm. This could be due to the presence of impurities.

Structure elucidation of antioxidative compounds of shrimp using electrospray ionization- mass spectrometry (ESI-MS) and low-energy CID-MS/MS

The electrospray ionization mass spectrum (negative ion mode) of compound 2, $C_{18}H_{22}N_2O$ (MW 282), was elucidated with a cone-voltage of 75 V (Supporting information, Fig. S3). The low-energy collision induced dissociation tandem mass spectrum of the precursor ion deprotonated molecule $[M\text{-}H]^-$ at *m/z* 281 afforded a series of diagnostic product ions, which was tentatively identified and summarized in Fig. 2. The deprotonated molecule $[M\text{-}H]^-$ at *m/z* 281 may lose a fragment of acetylene (C_2H_2, 26 Da) to produce the $[M\text{-}H\text{-}CH{=}CH]^-$ the product ion at *m/z* 255. This may also lose a molecule of methylene amine ($CH_2{=}NH$) to provide the product-ion $[M\text{-}H\text{-}CH_2NH_2]^-$ at *m/z* 253. Moreover, the deprotonated product ion $[M\text{-}H\text{-}CH{=}CH]^-$ at *m/z* 255 loses consecutively, molecules of acetylene (C_2H_2, 26 Da) and ketene (CH_2CO, 42 Da) to afford either of the two possible product-ion $[M\text{-}H\text{-}2(C_2H_2)\text{-}CH_2CO]^-$ or $[M\text{-}H\text{-}C_2H_2\text{-}CH_2CO\text{-}C_2H_2]^-$ at *m/z* 187. The product ion at *m/z* 255 can simultaneously lose a molecule of propylene amine ($CH_2{=}CH\text{-}CH{=}NH$) and 2 molecules of hydrogen (or vice versa) to afford the product-ion $[M\text{-}H\text{-}C_2H_2\text{-}CH_2{=}CH\text{-}CH{=}NH\text{-}2(H_2)]^-$ at *m/z* 196. This latter product-ion undergoes a retro-Diels Alder rearrangement and its additional aromatization by the loss of 2 hydrogen atoms to afford the deprotonated fragment ion at *m/z* 154. Therefore, compound 2 which possesses a molecular formula of $C_{18}H_{22}N_2O$ contains 9 unsaturation (Klemm 1995) was identified as 7-(3-butenyl)-2-hydroxy-6-(1,2,5,6-tetrahydropyridin-2-yl)-7,8-dihydroquinoline.

The ESI-MS (negative ion mode) of compound 4, $C_{18}H_{20}N_{20}$ (MW 280), was measured with a cone voltage of 75 V (Supporting information, Fig. S4). The product ion scan of the deprotonated molecule $[M\text{-}H]^-$ at *m/z* 279 was tentatively characterized as summarized in Fig. 3. The deprotonated molecule $[M\text{-}H]^-$ at *m/z* 279 consecutively loses a molecule of $CH_2{=}CH\text{-}OH$ (44 Da) and a molecule of $CH_2{=}CH\text{-}C{=}CH$ (52 Da) to afford the product-ion $[M\text{-}H\text{-}C_2H_4O\text{-}C_2H_4O\text{-}C_4H_4]^-$ at *m/z* 183. This latter product-ion consecutively loses the side chain molecule of $CH_2{=}CH\text{-}CH{=}CH_2$ (154 Da) and 2

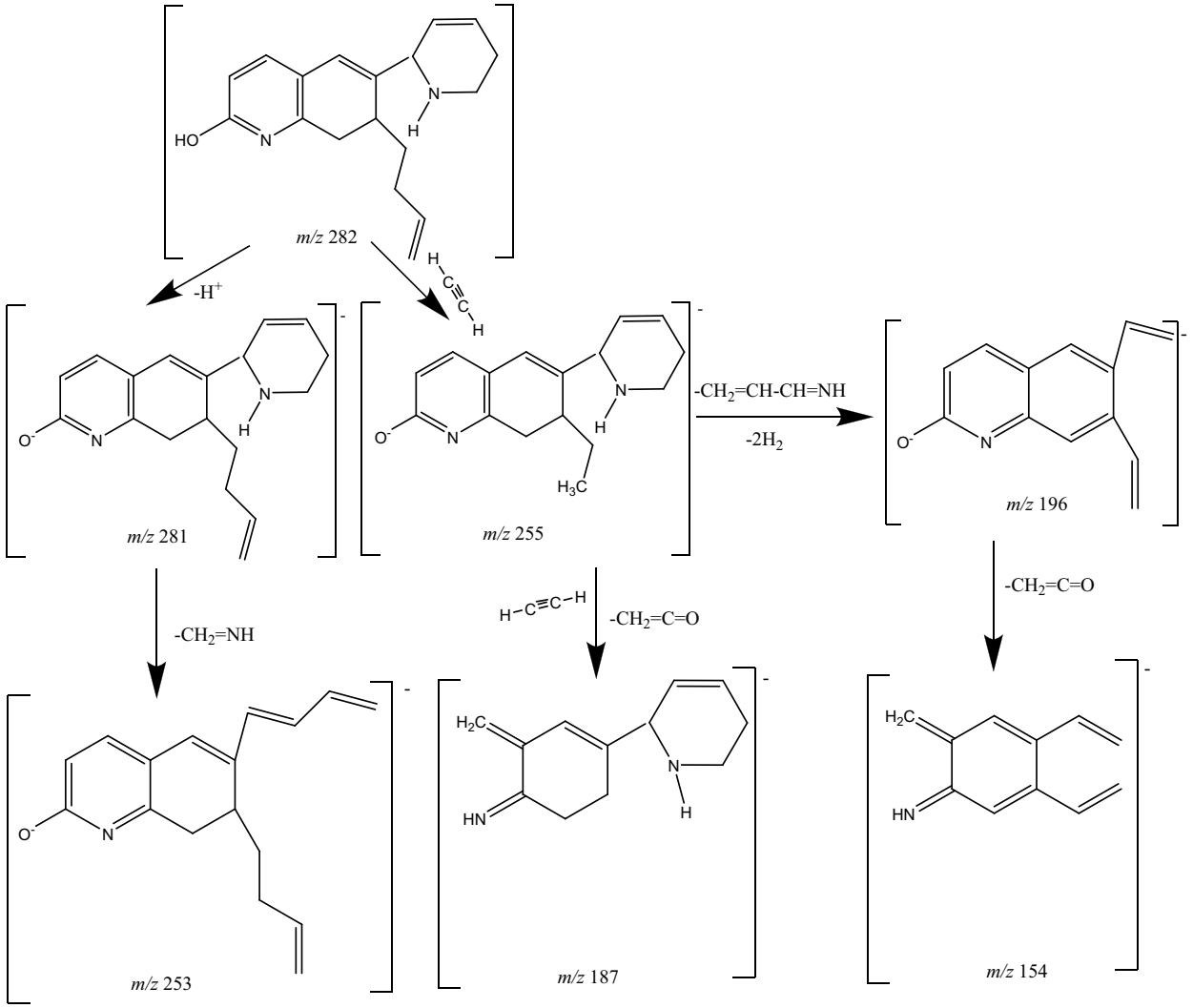

Fig. 2 Proposed CID-MS/MS fragmentation scheme of the precursor ion 2 at *m/z* 282 identified as 7-(3-butenyl)-2-hydroxy-6-(1,2,5,6-tetrahydropyridin-2-yl)-7,8-dihydroquinoline

molecules of hydrogen), to produce the product-ion at *m/z* 126, which eventually loses a molecule of HCN to afford the product-ion at *m/z* 98. Therefore, compound 4 which possess a molecular formula of $C_{18}H_{20}N_2O$ contains 10 unsaturation (Klemm 1995), was identified as 7-(3-butenyl)-2-hydroxy-6-(1,2,5,6-tetrahydropyridin-2-yl) quinoline.

The ESI-MS (negative ion mode) of compounds 1 and 3 (Supporting information, Figs. S5 and S6) suggested that they were isomers of compounds 2 and 4, respectively. In fact, the ESI-MS spectra obtained from compounds l and 3 were similar to those of compounds 2 and 4, indicating the presence of potential geometrical isomers. These isomers could vary in their steric geometry across the double bonds, and the orientation of groups existing in the molecules. Although structural evidence is obtained from these ESI-MS/MS spectra, it is challenging to confirm the genealogy of the product ions produced. Specifically, the MS/MS spectra were complex by ions developing from multiple fragmentations, which resulted in intermediate ions of very low abundance. We have not endeavored to explain the different fragmentation patterns of these compounds by acquiring precursor ion spectra of several intermediate ions. Thus, the assignment of product ions structures was mostly based on the tentative interpretation of selected precursor ions and further confirmation, and quantification studies need to follow up.

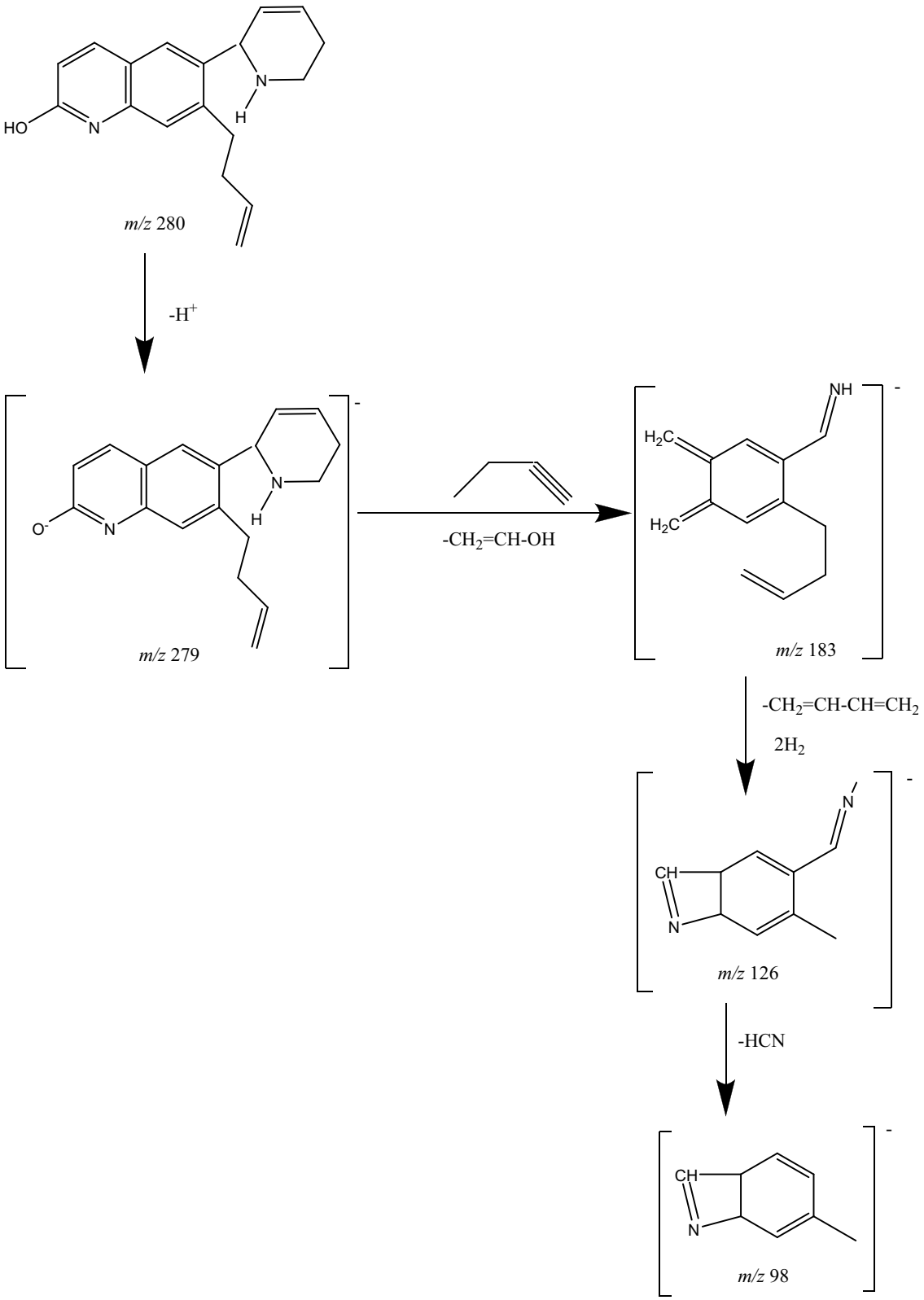

Fig. 3 Proposed CID-MS/MS fragmentation scheme of the precursor ion 4 at *m/z* 280, identified as 7-(3-butenyl)-2-hydroxy-6-(1,2.5,6-tetrahydropyr idin-2-yl) quinoline

The presence of antioxidant compounds in shrimp and its by-products was established. These components were isolated and characterized using TLC, HPLC, and ESI-MS. The compounds were identified as 7-(3-butenyl)-2-hydroxy-6-(1,2,5,6-terahydropyridin-2-yl) quinoline and 7-(3-butenyl)-2-hydroxy-6-(1,2,5,6-tetrahydropyridin-2-yl)-7,8-dihydroquinoline and their isomers. The proposed structures of these compounds in shrimp may lead to a better understanding of the antioxidant effect exhibited by shrimp components. Therefore, the successful isolation and structural elucidation of the antioxidant compounds in shrimp, for the first time, is a major contribution to the existing knowledge in the field. Nevertheless, further studies should be conducted using spectroscopic methods (e.g., HPLC-MS/MS and NMR) to confirm the proposed structures and provide additional information about their structural identities.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43014-023-00215-3.

Additional file 1: Figure S1. Semi-preparative reversed-phase HPLC chromatograms. **Figure S2.** Analytical reversed-phase HPLC chromatograms. **Figure S3.** ESI-MS (negative ion mode) of compound 2, *m/z* 282. **Figure S4.** ESI-MS (negative ion mode) of compound 4, *m/z* 280.**Figure S5.** ESI-MS (negative ion mode) of compound 1. **Figure S6.** ESI-MS (negative ion mode) of compound 3.

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Authors' contributions
Conceptualization, A.C.O., and F.S.; methodology, A.C.O., J.B., F.S., and A.H.; software, A.C.O., and J.B.; formal analysis, A.C.O., and J.B.; investigation, A.C.O., J.B., and F.S., resources, A.C.O., A.H., J.B., and F.S.; data curation, A.C.O., and J.B.; writing—original draft preparation, A.C.O., and A.H.; writing—review and editing, A.H., and F.S.; visualization, A.C.O., and A.H.; supervision, F.S.; funding acquisition, F.S. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
Not applicable.

Declarations

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
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