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Carotenoid Content of Three Locally Available Sources in Sri Lanka: Palmyrah Fruit Pulp, Green Tiger Prawn Shells, and A Red Seaweed

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Abstract

Growing demand for sustainable, nutritious feed in aquaculture has led to the exploration of carotenoid-rich natural sources. This study examined the total carotenoid content (TCC) and pigments from three sources: the fruit of palmyrah plant, the shell of green tiger prawn, and a red seaweed. Using appropriate solvents, the crude yields were extracted, and carotenoid pigments were characterized through Liquid Chromatography Mass Spectrometry. Acetone and methanol were the most effective solvents for fruit of the palmyrah and shell of the prawn, while acetone and ether were used for a red seaweed. The green tiger prawn shells had the highest TCC (µg g^{-1}) (377.1±27.2), followed by palmyrah fruit (230.97±8.71) and red seaweed (156.51±22.14). Palmyrah fruit exhibits a high number of pigments, beta-carotene, phytoene, lutein, violaxanthin, and lycopene, suggesting its potential as a natural source for dietary supplements. Prawn Shell revealed the presence of astaxanthin, a potent antioxidant with various health benefits, along with two additional pigments, lutein and zeaxanthin. Red seaweed showed a diverse range of carotenoids, including fucoxanthin, zeaxanthin, and lutein, which support its use in functional foods. The findings emphasize the potential of the three carotenoid sources as valuable colour-producing pigments for aquaculture.

Introduction

Carotenoids are a group of natural pigments found in various sources, including fruits, vegetables, and microorganisms. These compounds are responsible for many plants' vibrant colours and are essential for animal growth, reproduction, and disease resistance (de Carvalho and Caramujo, 2017). Carotenoids have been shown to possess antioxidant properties, which help protect the cells from damage caused by harmful free radicals. Additionally, these pigments are known to enhance the immune system of aquatic organisms, making them more resistant to diseases and infections (Maoka, 2020). Some organisms particularly fish cannot synthesize carotenoid pigments, so they rely on getting them from their diet, which can be natural or synthetic. The aquaculture industry in Sri Lanka has underutilized the three selected natural sources (Fruit of palmyrah plant (*Borassus flabellifer* L.), shell of green tiger prawn (*Penaeus semisulcatus* de Haan, 1844), and a red seaweed (*Gracilaria edulis* (S.G. Gmelin) P.C. Silva 1952) for a long time. The Palmyrah tree is abundant in the North, East, South-East, and North-West of Sri Lanka. Previous studies on the carotenoid profile of palmyrah fruits collected from South-East, and North-West of Sri Lanka show that the fruit consists of a variety of

carotenoid pigments: α-carotene, β-zeacarotene, ζ-carotene, lycopene, phytofluene, and phytoene in a considerable amount (Priyadarshani and Jansz, 2014; Wijemanne et al., 2006). However, there is still a lack of research on the carotenoid profile of palmyrah fruits in other regions of Sri Lanka.

The crustacean processing industries generate significant solid waste in Sri Lanka. The discarded heads and exoskeletons comprise around 50 – 70% of the initial weight. The prawn exoskeleton is one of the important natural sources of carotenoid (Shahidi et al., 1998). Prawn exoskeletons are an important natural source of carotenoids, particularly astaxanthin, a potent antioxidant. This compound has been linked to various health benefits, including reducing inflammation and protecting against oxidative stress. The content and the types of carotenoid pigments can vary according to the region. Previous studies on the carotenoid pigments in Green tiger prawn shells were conducted in other countries (Maia et al., 2023; Hooshmand et al., 2017). Currently, there are no research studies in Sri Lanka on the carotenoid pigments of green tiger prawn shells.

Sri Lankan coastal waters are known to be rich in red seaweeds (Rhodophyta). Still, there is currently no clear record of the carotenoid pigments found in red seaweeds specifically in Sri Lanka.

The three carotenoid sources, palmyrah fruit (*Borassus flabellifer* L.), shells of green tiger prawns (*Paeneus semisulcatus*), and a red seaweed (*Gracilaria edulis*), are underutilized and easily accessible in Sri Lanka. The present study aims to promote the utilization of natural, sustainable, and locally available carotenoid sources for the benefit of both aquaculture and broader environmental and economic goals.

Material and Methods

Sampling

Ripe fruit of palmyrah plant (*Borassus flabellifer* L.*)* were collected from different locations across the Jaffna district. After removing the exocarp, the pulp was extracted by rubbing it on a sieve without adding water. The extracted pulp was kept in a sterile container and frozen at -20 ºC for further analysis.

Green tiger prawns' wastes were collected from household and processing centers. It was kept in a regifoam box and brought to the laboratory. After cleaning the wastes, the shells were obtained, it was dried in an oven at 60ºC for one hour, and then the oven's temperature was gradually increased to 100ºC for six hours. The dried shells were then ground into a fine powder using a grinder. The resulting powder was stored in a sterilized container, labelled, and kept in a refrigerator at 4 ºC until further use.

A red seaweed (*Gracilaria edulis*) (S.G. Gmelin) P.C. Silva 1952) was collected from the coastal waters of Point Pedro in the Jaffna peninsula, Sri Lanka. After cleaning, it was dehydrated in an oven at 100 ºC for six hours. The resulting dried samples were ground into a fine powder and stored in a sterilized container.

Crude Extraction

Crude extraction was carried out according to Bitwell et al. (2023). Various polar and non-polar solvents, including acetone, hexane, ether, ethanol, methanol, water, and their mixture (Table 1), were selected to determine the most suitable solvent for extracting carotenoids from different sources. The preferred solvents were mixed in various ratios. Dried shells of *P. semisulcatus* and *G. edulis* were ground into a powder and mixed well. A sample of one gram of powdered shells of *P. semisulcatus*, *G. edulis*, and fruit of *B. flabellifer* was dissolved using a vortex mixer with a ten mL solvent/solvent mixture. If needed, the amount of chemical used was increased based on the crude extraction efficiency. The solution was then filtered into labelled vials using manifold vacuum filtration (47mm diameter). After filtration, the solution evaporated in a fume hood, and the final weight of the crude was measured. Each experiment was done in triplicate, and the chemical used to extract the crude without a sample was treated as a control experiment.

Table 1. Retention factor (mean±SD) for crude of fruit of *B. flabellifer*, *P. semisulcatus*, and *G. edulis* using different solvent systems

Solvent system	RF		
	Fruit of *B. flabellifer*	*P. semisulcatus*	*G. edulis*
A	0.99^{a}	0.94^{a}±0.07	0.64^{a}±0.02
B	0.85^{c}±0.01	0.45^{c}±0.02	0.46^{d}±0.02
C	0.42^{e}±0.03	0.77^{b}±0.03	0.69^{a}±0.03
D	$0.59^{d,e}$±0.16	0.8^{b}±0.01	0.46^{d}±0.02
E	0.87^{c}±0.02	0.48^{c}±0.02	0.38^{e}±0.01
F	0.68^{d}±0.01	0.47^{c}	0.57^{b}±0.01
G	0.53^{d}±0.01	0.77^{b}±0.01	0.54^{b}±0.1
H	0.76^{d}±0.03	0.72^{b}±0.05	0.63^{a}±0.04
I	0.66^{d}±0.02	0.53^{c}±0.03	0.42^{e}
J	0.95^{b}	0.74^{b}	0.5^{c}±0.01
K	0.91^{c}±0.05	0.68^{b}±0.01	0.68^{a}±0.01
L	0.85^{c}±0.02	0.49^{c}±0.03	0.46±0.02

Different superscripts in the column represent statistical differences (P<0.05). A- Acetone, B- Acetone: Ethanol (1:1), C- Acetone: Petroleum Ether (1:1), D – Acetone: Hexane (1:1), E – Acetone: Methanol (1:1), F – Ethanol, G – Petroleum Ether, H- Hexane, I- Hexane: Methanol (1:1), J- Methanol, K-Methanol: Petroleum Ether (1:1), L-Distilled water.

Thin Layer Chromatography Experiment

It was carried out according to Sherma (2003)**.** A beaker with a watch glass was used as a Thin Layer Chromatography (TLC) chamber. A TLC plate (3 cm width x 15 cm height) was obtained, and a straight line was drawn roughly 1cm from the bottom using a pencil. A solvent or mixture of solvents (Ten mL) was added to the beaker and covered with a watch glass. A small amount of crude sample was spotted on the line, about one-inch-wide, using a capillary spotter. The TLC plate was carefully placed in the beaker using forceps, allowing the spot to dip into the solvent/ mixture. The level of the solvent/ mixture in the beaker was checked below the pencil line where the crude sample was spotted. The TLC was allowed to develop in the solvent/mixture until the solvent level reached halfway from the top of the plate. The TLC plate was then removed with forceps, and the solvent line was immediately marked with a pencil to enable retention factor (Rf) calculation, as the solvent often evaporates quickly. The following equation calculated Rf.

$$Rf = \frac{Distance\ travelled\ by\ the\ component}{Distance\ travelled\ by\ the\ solvent}$$

The appropriate solvent for separating compounds was determined after the TLC plate analysis.

Determination of Total Carotenoid Content

The TCC of the carotenoid sources was carried out by the methods of Torrissen and Naevdal (1988). One gram of powdered sample was weighed and transferred into a labelled sample bottle, then wrapped in aluminium foil. Ten mL of acetone was added to it. The sample bottle was tightly closed with a screw cap. The sample tubes were then placed in a vortex mixer and mixed at a speed of 2000 rpm for two hours. After mixing, the mixture was refrigerated at 4 ºC for 24 h. After one day, the samples were filtered using folded filter paper into centrifuge tubes wrapped with aluminium foil. The sample residue was again dissolved with ten ml of the acetone and filtered into the same centrifuge tube. This process was repeated until the filtrate became colourless. The centrifuge tubes containing the filtrates were centrifuged at 3500 rpm for 3 minutes. The upper layer of the solution was transferred into a cleaned cuvette. The absorbance of the sample was then measured using a UV-Vis spectrophotometer at 476 nm. A zero setting (blank) was performed with the utilized chemicals. The total carotenoid content (µg/g) was calculated using the following equation;

$$= [\text{Absorption} \times \text{dilution factor}] / [A_{1cm}^{1\%} \times \text{Sample weight (g)}]$$

Where, $A_{1cm}^{1\%}$ = Extinction coefficient (0.25)
A= Absorbance

Characterization of Carotenoid Pigments

Two grams of homogenized sample were weighed, and 20 mL of selected solvent/solvent mixture was added. The mixture was continuously stirred using a vortex at 2500 rpm for 30 minutes. The remaining suspension was centrifuged, and the resulting supernatant liquid was collected. The extraction procedure was repeated twice. The solvent was evaporated inside the fume hood until the lipid fraction was obtained. The lipid fraction was then sent to Sri Lanka Institute of Nanotechnology (Pvt) Ltd, Nanotechnology & Science Park, Mahenwatta, Pitipana, Homagama. Sri Lanka for further analysis.

The carotenoid pigment of the three sources was characterized using Liquid Chromatography Mass Spectrometry (LC/MS) according to the following method of Cortés-Herrera et al. (2019).

Sample Saponification

A small portion of the fat fraction (0.3 g) was weighed and transferred to a conical centrifuge tube. A solution of KOH in ethanol (1 mmol mL^{-1}) containing pyrogallol (0.1 g $100g^{-1}$) was added (10 mL) to the tube. The mixture was saponified for 1 h at 80ºC in a heat bath. After cooling to room temperature, the mixture was transferred to a separatory funnel. The ethanol/aqueous layer was then extracted twice using hexane. The entire organic solvent layer was collected and filtered through sodium sulfate (used as a drying agent). After evaporating the solution to dryness under a nitrogen flow, it was reconstituted with Metil tert-bütil eter (MTBE) (1 mL) and 2-propanol (1 mL) used at the start of the chromatographic separation and transferred to an HPLC vial.

Stationary Phase and Selection of Chromatographic Conditions

In the present study, Methanol (MeoH) was used as a mobile phase while water was used as the stationary phase. However, the resolution was found to be insufficient. Therefore, a C18 column was selected, and only flow was adjusted (1 mL min^{-1}, Eclipse Plus C18, 4.6 mm ID × 150 mm, 3 µm). Subsequently, the column was replaced with a C30, and a less polar solvent in acetonitrile and 2-propanol was used instead of water. The solvent flow rate was reduced to 0.5 mL min^{-1}. Finally, acetonitrile and isopropanol were used instead of MTBE to retain a similar proportion of MeOH.

Chromatographic Conditions

All experiments were conducted using the LC/MS system. The gradient elution method was utilized to separate all the compounds. To optimize the solvent gradient, MeOH and MTBE were acidified with formic acid (0.1 mL/100 mL). A constant flow rate of 0.6 mL/min

was maintained, while the injection volume was held at 10 µL. The column compartment was kept at a temperature of 10ºC±0.8.

MS Detection System Conditions

To evaluate the sensitivity of compounds, the fragmentor was initially cycled through different voltages (ranging from 20 to 300 V) while ignoring column interaction. Total ion chromatographs (TIC) were then used to obtain the MS spectra for each compound. The molecular ion signal was identified from each TIC. For positive ion mode electrospray ionization (ESI+), drying gas, nebulizer pressure, drying gas temperature, and capillary voltage were set to 12.0 L min^{-1}, 50 psi, 350°C, and 4000 V. To improve sensitivity and remove interferences, selected ion monitoring was used to corroborate each compound identity.

The chromatographic peak's centroid collected retention times and mass spectra. Peak areas in the samples were compared with those of standard solutions to measure the amount of compounds present. LC-ESI+-MS was used to analyze and identify targeted compounds, and the processed MS data sets were analyzed using Open Lab Chemstation C.01.07. Their retention times were measured with an accepted deviation of ±0.2 min to confirm the target analytes and the molecular ion was measured within a specific timeframe.

Statistical Analysis

Data for all measured parameters were analysed using Minitab statistical software 2021. Differences among groups were determined by one-way ANOVA or non-parametric statistics (Kruskal Wallis). Tukey significant means test was employed to identify groups that were significantly different. Statistical differences were considered when P<0.05.

Results

Crude Yield

The fruit of *B. flabellifer* source yielded more crude than the shells of *P. semisulcatus* and *G. edulis* sources. However, the solvent systems G (Petroleum Ether) and H (Hexane) only yielded a minimal quantity of crude for fruit of *B. flabellifer*. Significant results were obtained with fruit of *B. flabellifer* (17.55% ±0.11) when dissolved in a mixture of acetone and methanol (1:1) (Figure 1).

The highest crude yield of 11.60% ±0.31 was obtained from shells of *P. semisulcatus* when the dried prawn shell powder was dissolved in a mixture of acetone and ethanol (1:1). This yield was statistically significant (P<0.05) when compared to other solvents. Conversely, the lowest crude yield of 2.32 %±0.01 was extracted with petroleum ether.

When dissolved in a mixture of acetone and ethanol (1:1), *G. edulis* yielded a relatively low 3.93%. Among the systems studied, ether, water, and hexane yielded the lowest crude yields for fruit of *B. flabellifer*, shells of *P. semisulcatus*, and *G. edulis*, respectively.

Retention Factor (Rf)

The results showed that the Rf of carotenoid crude varied depending on the solvent used. The crude from fruit of *B. flabellifer* and shells of *P. semisulcatus* had a higher retention factor than *G. edulis* (Table 1). Among

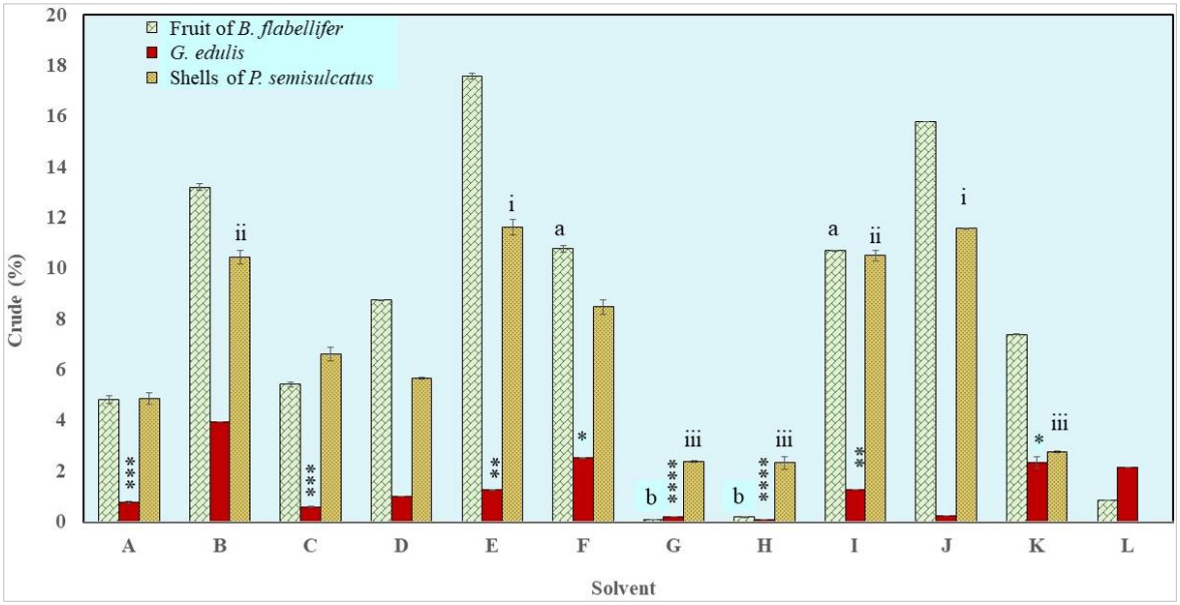

Figure 1. The mean crude yield of three carotenoid sources using different solvent systems. A- Acetone, B- Acetone: Ethanol (1:1), C- Acetone: Petroleum Ether (1:1), D – Acetone: Hexane (1:1), E – Acetone: Methanol (1:1), F – Ethanol, G – Petroleum Ether, H- Hexane, I- Hexane: Methanol (1:1), J- Methanol, K-Methanol: Petroleum Ether (1:1), L-Distilled water. Same symbols or letters not significantly (P<0.05) different. The lack of symbols or letters indicates no significant differences.

all the solvents tested, acetone had the highest polarity for the carotenoid sources, leading to stronger interactions between the crude and stationary phases. The highest Rf value (0.99) was obtained in the fruit of *B. flabellifer* crude, dissolved with acetone, whereas the lowest Rf value was recorded in the crude of *G. edulis* dissolved with solvent E (Acetone: Methanol =1:1).

The fruit of *B. flabellifer* crude showed the strongest interaction with most of the solvent systems. An Rf value greater than 0.85 was observed when the fruit of *B. flabellifer* crude was dissolved in solvent systems A (Acetone), B (Acetone: Ethanol = 1:1), E (Acetone: Methanol = 1:1), J (Methanol), K (Methanol and Petroleum ether), and L (Distilled water). On the other hand, the solvent system C (Acetone: Petroleum Ether= 1:1) had the weakest affinity (0.42) for the fruit of *B. flabellifer* crude. The Rf values of the crude extract of shells of *P. semisulcatus* varied from 0.94 to 0.45 in solvent systems A (Acetone) and B (Acetone: Ethanol = 1:1) respectively. The *G. edulis* crude had a weaker interaction with all the solvent systems than other sources' crude. When dissolved in all the solvent systems, it showed an Rf value below 0.69, ranging from 0.38 in solvent E to 0.69 in solvent C.

Total Carotenoid

The results showed that the mean (± Standard Deviation) total carotenoid content in shells of *P. semisulcatus*, fruit of *B. flabellifer*, and *G. edulis* were recorded as 377.1±27.2 µg/g, 230.97±8.71 µg/g, and 156.51±22.14 µg/g respectively. The mean total carotenoid content in shells of *P. semisulcatus* was the highest, followed by fruit of *B. flabellifer*, then that of *G. edulis*. There was a significant variation in the total carotenoid content ($P<0.05$) among the three sources.

Carotenoid Profile

When using positive ion LCMS, four types of carotenoid pigments were observed. On the other hand, lycopene was detected as the base peak in the negative ion LCMS of the fruit of *B. flabellifer*.

In the ion product of $^{13}C_{10}$ β-carotene, the base peak was detected at mz^{-1} 543.16 through positive ion LCMS. During the LCMS analysis with positive ion mode, a protonated molecule of phytoene was detected with a high abundance at mz^{-1} 544.9. Water loss from the protonated molecule, characteristic of lutein, was observed at mz^{-1} 551.22, with high abundance. These observations suggest the presence of lutein in the sample. The LCMS analysis of violaxanthin's positive ion showed the most abundant fragment ions at mz^{-1} 600.87, which resulted from adding mz^{-1} 100.

The LCMS negative ion of lycopene detected the most abundant fragment ion at m/z 215.01, eliminating a terminal group (54) from the cleavage molecular ion.

The positive ion LCMS identified three types of carotenoids, while the negative ion LCMS revealed canthaxanthin as the base peak.

In the positive ion LCMS of lutein, an ion with 100% abundance was observed at mz^{-1} 551.45, corresponding to removing water from the protonated molecule. The removal of water $[MH\text{-}H2O]^+$ led to the formation of fragment ions with a lower abundance observed at mz^{-1} 579.58 in the chromatogram of astaxanthin.

Similarly, to astaxanthin, the positive ion LCMS chromatogram of zeaxanthin showed a base peak of mz^{-1} 551.4, corresponding to the loss of water. Additionally, a negative ion LCMS chromatogram of canthaxanthin revealed the presence of another ion at mz^{-1} 564.8.

Although the abundance of Fucoxanthin was low, its fragmentation was detected in the positive ion LCMS chromatogram at mz^{-1} 701.65, which corresponds to [M+42].

Despite being present in low abundance, a zeaxanthin ion with mz^{-1} 551.55 was formed by removing water. Lutein is structurally similar to zeaxanthin, differing only by the position of a carbon-carbon double bond in one of the rings. Alloxanthin was detected at mz^{-1} 152.72 in the LCMS chromatogram, with a 40% abundance of negative ions.

Discussion

Crude Yield and Rf

The acetone and methanol (1:1) mixture was the most effective among the different solvent systems used in the crude extraction from the fruit of *B. flabellifer*. These findings are consistent with previous studies presented in Table 2. The previous studies (Priyadarshani et al., 2009a, Uluwaduge et al., 2009, Wijemanne et al., 2006) have commonly used acetone, methanol, and petroleum ether to extract the crude from the fruit of *B. flabellifer*. In contrast, hexane was rarely used to extract the crude as reported by Samarasinghe and Jansz (2001). Therefore, acetone and methanol are the best solvent systems for extracting the crude from the fruit of *B. flabellifer*.

The Rf value of the crude fruit of *B. flabellifer* obtained in the present study was from 0.42 to 0.99 using the solvent systems of acetone and acetone + ether (1:1) mixture, respectively (Table 1), which agrees with the findings reported by Priyadarshani et al. (2009a) (Table 2).

On the other hand, other researchers have reported different solvent systems for extracting crude from prawn shells of *Peneaus* species (Table 3). Acetone, hexane, and isopropanol were used to extract crude from the shells of the *Penaeus* sp (Hooshmand, et al., 2017, Mezzomo et al., 2011, Su et al., 2018). In the present study, conversely, the solvent systems were acetone and methanol (1:1). It might be the reason for the different sampling locations.

The TLC plate of carotenoid extracts from the shells of *P. semisulcatus* yielded different bands, ranging from 0.45±0.02 to 0.94±0.07 for the solvent systems of acetone + ethanol (1:1) and acetone respectively (Table 1). In this study, an Rf of 0.78 was obtained in the present study when it was subjected to the solvents of acetone + ether (1:1). This Rf value represented the carotenoid pigment of astaxanthin diester and was consistent with the results reported by Sindhu and Sherief (2011) and Sánchez-Camargo et al. (2011) for the Arabian red shrimp *Aristeus alcocki* and Red-spotted shrimp waste (*Farfantepenaeus pailensis*). Additionally, the Rf value of 0.53 (±0.03) was recorded in the present study for the solvent system of the Hexane and Methanol mixture corresponded to the astaxanthin monoester (Sánchez-Camargo et al. 2011). The present

Table 2. Studies on carotenoids and used solvent systems in *fruit of B. flabellifer*

Solvent system	Rf Value	Total carotenoid	Carotenoid pigments		References
Hexane, Dichlorornethane	0.50, 0.53, 0.57, 0.64	----	Alpha- carotene, zeta-carotene Lycopene Beta-zeacarotene	8.1 8.1 13.3 11	Samarasinghe and Jansz (2001)
Methanol, Ether	0.43 - 0.82	2-253 mg $100g^{-1}$	---	---	Ariyasena et al. (2000)
---	---	---	β-Carotene	818.62 to 2111.59 µg $100g^{-1}$	Wijewardana et al. (2016)
Acetone, Ether	----	3.3-9.3 mg $100g^{-1}$ (Fresh weight)	Violaxanthin Neoxanthin Crocetin Beta-carotene, Zeta-carotene Beta-zeacarotene Phtofluene Phytoene Alpha-carotene	----	Wijemanne et al. (2006)
Acetone	0.98	----	Phytoene Phytofluene Beta-carotene Alpha Carotene	7.7 ± 1.5 mg $100g^{-1}$ DW 3.8 ± 0.9 Trace amounts Trace amounts	Priyadarshani et al. (2009a)
----	----	15.5 to 35.4 mg $100g^{-1}$ DW	Phytoene Phytofluene	5.7 to 10.3 mg $100g^{-1}$ DW 2.8 to 4.8 mg $100g^{-1}$ DW	Priyadarshani et al. (2009b)
Methanol	----	----	Phytofluene	----	Uluwaduge et al. (2009)
----	----	0.0043 ± 0.043 g Kg^{-1}	----	----	Ariyasena (2000)

(----) Data is not available

Table 3. Studies on carotenoid extractions of prawn / shrimp shells

Source of carotenoid	Solvent system	Rf	Total carotenoid	Organic solvent for extraction	Carotenoid pigments	References
Green Tiger Prawn (*Peneaus semisulcatus*)	Acetone	----	61.321 ± 2.176 µg g^{-1} for Acetone	Acetone, hexane, Isopropyl alcohol, and Petroleum ether	Astaxanthin	Hooshmand, et al. (2017) Sharayei, et al. (2021)
Indian White prawn (*Penaeus indicus)*	Acetone, Isopropyl alcohol, and Hexane	----	43.9 µg g^{-1}	Acetone, Isopropyl alcohol, and Hexane	----------	Sachindra et al. (2003)
Shrimp	Acetone, Ethyl acetate, Methanol, and Hexane	----	----	----	Astaxanthin	Weeratunge and Perera (2016)
Giant Tiger Prawn (*Penaeus monodon*)	Acetone and hexane	----	124.14 ± 10.77 mg g^{-1}	Acetone and hexane	Astaxanthin, Zeaxanthin, Astacene, Canthaxanthin, β-carotene	Su et al. (2018)
Pink shrimp (*Peneaus brasiliensis*)	Acetone , Hexane, and Isopropanol	----	252 µg g^{-1}	Acetone, Hexane, and Isopropanol	Astaxanthin	Mezzomo et al. (2011)

(----) Data is not available

study predicted that using TLC plate bands, astaxanthin is one of the carotenoid pigments found in the shells of *P. semisulcatus*.

In the present study, acetone and ethanol (1:1) were the most effective solvent systems for crudely extracting carotenoids from *G. edulis*. These findings are consistent with previous studies (Table 4).

Table 1 shows the Rf values of the crude *G. edulis*, ranging from 0.38±0.01 to 0.69±0.03 for the solvent systems of acetone+ methanol (1:1) and acetone+ petroleum ether (1:1), respectively. The Rf value of 0.63 (±0.04) obtained in the present study agreed with the study done by Koizumi et al. (2018); however, it was obtained when crude *Pyropia yezoensis* was run with the solvent system petroleum ether and acetone (1:1). This value (0.42) also observed in the present study, but it was recorded for the solvent system of acetone and methanol (1:1). The Rf value of 0.5 (±0.01) was similar to the previously reported by Pedra et al. (2017) for *Kappaphycus striatum*. On the other hand, we recorded the Rf value as less than 0.69; previous studies reported higher values for the *Gracilaria edulis* and *Gracilaria corticata*, namely 0.84 and 0.95 (Rosemary et al., 2019).

Total Carotenoid and Carotenoid Profiles

Although the pigment composition in the three carotenoid sources varied, a few carotenoid pigments were present in all three sources. Lutein pigment was identified in fruit of *B. flabellifer* and *G. edulis*, whereas zeaxanthin pigment was found in shells of *P. semisulcatus* and *G. edulis*.

The fruit of *B. flabellifer* analyzed in the present study presented distinct values when compared to their counterparts reported throughout the literature (Table 2), while lower values were found in previous studies (Ariyasena, 2000; Wijemanne et al., 2006).

In the present study, the carotenoid compounds in fruit of *B. flabellifer* are β-carotene, phytoene, lutein, violaxanthin, and lycopene. The phytoene and β-carotene were higher than the other pigments in the tested palmyrah fruit. This agreed with the study by Wijewardana et al. (2016) and Priyadarshani et al. (2009a, b). Wijewardana et al. (2016) reported that β-carotene of palmyrah fruit pulp ranges from 818.62 to 2111.59 µg/100. Priyadarshani et al. (2009a, b) reported that the phytoene of the palmyrah fruit pulp ranges from 7.7 to 10.3 mg $100g^{-1}$ DW.

Findings of the pigment compositions in the fruit of *B. flabellifer* show that the fruit is rich in β-carotene and phytoene pigments, whereas a considerable amount of lutein, violaxanthin, and lycopene were observed. These pigments are utilized for various purposes because of their beneficial characteristics for the animals. β-carotene is a mixture of trans and cis isomers, which are preferred by the market of natural products due to their potential anticancer activity (Chan and Matanjun, 2017). It is also used as a dietary supplement in salmonid diets to give the fish their typical pink flesh colour (Păpuc et al., 2024). Diets enriched with β-carotene and some zeaxanthin can be converted into astaxanthin and absorbed in the crustaceans (Matsuno, 2001). Therefore, it ensures that using the palmyrah fruit in the ornamental fish feed will produce skin colouration.

Screening of carotenoid pigments has been studied on prawn and shrimp shells of various species (Table 3). Pure astaxanthin and its esters have been isolated as primary pigments from *Penaeus monodon*, *Penaeus semisulcatus*, *Parapenaeopsis hardwickii, Aristeus alcocki, Farfantepenaeus pailensis*, *Peneaus brasiliensis, Palaemon serratus,* and *Palaemon varians,* constituting 4.25 - 124 mg/g of the total carotenoids in these animals.

This research discovered that the Shells of *P. semisulcatus* contain a total carotenoid content of 377.1±27.2 µg/g. Although controversial in the literature, Hooshmand et al. (2017) recorded the lowest total carotenoid value (61.321±2.17 µg/g) for the same species (Table 3).

Several previous studies, summarized in Table 3, described the colour pigment compositions in the shells of the different prawns and shrimps. The giant tiger prawn (*Penaeus monodon*) had the highest carotenoid content, with a value of 124.14±10.77 mg/g (Su et al., 2018), while Lira et al. (2017) obtained the lowest value for the white shrimp (*Litopenaeus schmitti*).

Our research on the pigment composition of Green tiger prawn shells confirms the findings of Hooshmand et al. (2017) and Sharayei et al. (2021). The study observed that the primary carotenoid in giant tiger prawns is astaxanthin, as recorded in Table 3. Our study identified two additional pigments beyond astaxanthin and canthaxanthin: lutein and zeaxanthin. Interestingly, these two pigments were not previously reported in the literature.

Table 4 compiles data on the carotenoid content of various red seaweeds. According to this table, *Grateloupia filicina*, a red seaweed, has the highest total carotenoid content of 146.82 µg/g, as recorded by Bhat et al. (2021). However, the present study found that *G. edulis* contained a higher value (156.51±22.14 µg/g) of total carotenoids. A different study by Rosemary et al., (2019) reported the lowest total carotenoid content for the same *G. edulis* at 2.99±0.56 µg g^{-1}. This variation in values could possibly attributed to environmental factors.

Table 4 shows previous studies of seaweed carotenoid profiles, which revealed a distinct variation of carotenoid pigments. Among the listed pigments in Table 4, *G. edulis* contains 11 different types of carotenoid pigments such as zeaxanthin, fucoxanthin, beta-carotene, violaxanthin, antheraxanthin, lutein, and alloxanthin. However, the present study has only identified three pigments in the same species: fucoxanthin, zeaxanthin, and lutein. Various factors might influence the lowest pigments recorded in the present study.

Table 4. Studies on carotenoid extraction in red seaweed

Red seaweed species	Solvent systems	Rf	Total carotenoid content	Carotenoid pigments	References
Gracilaria birdiae, Gracilaria domingensis	Methanol:Acetone (1:1, v:v)	---	---	Zeaxanthin β - carotene Alloxanthin Violaxanthin Antheraxanthin	Guaratini et al. (2012)
Gracilaria tikvahiae *Eucheuma denticulatum*	Ethyl acetate, Chloroform	---	25 – 33 μg g^{-1} DW	---	Othman et al. (2018)
Gracilaria vermiculophylla *Grateloupia livida and Grateloupia asiatica*	Methanol, acetone	----	----	Zeaxanthin Zeaxanthin Fucoxanthin Lutein	Terasaki et al.(2012)
Gracilaria changii	Hexane:Acetone:Ethanol (2:1:1, v/v)	---	74.22±0.48 (μg g^{-1} DW)	---	Chan and Matanjun (2017)
Gracilaria edulis and *Gracilaria corticata*	Methanol	0.84, 0.95	2.99±0.56 and 12.82±0.50 μg g^{-1}	---	Rosemary et al. (2019)
Ceramium sp	Acetone, Hexane, (Acetonitrile/Methanol/Dichloromethane =3:1:1, v/v/v)	---	8.77 μg g^{-1}	Fucoxanthin, Luetin, Zeaxanthin	Bhat et al. (2021)
Gracilaria corticata		---	11.10 μg g^{-1}		
Grateloupia sp		---	202.91 μg g^{-1}	Luetin, Zeaxanthin	
Grateloupia filicina		---	146.82 μg g^{-1}	Neoxanthin, Violaxanthin	

(----) Data is not available

Conclusion

This study discovered significant TCC and pigment compositions. Although the three carotenoid sources had different pigment compositions, a few carotenoid pigments were present in all three sources. Lutein pigment was found in fruit of *B. flabellifer* and *G. edulis*, while zeaxanthin pigment was present in shells of *P. semisulcatus* and *G. edulis*. However, further research is needed to determine the effect of feed enriched with the three carotenoid sources on the aquaculture industry.

Ethical Statement

The research work was conducted following the ethical rules and regulations of the society.

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Author Contribution

Sutharshiny Sathyaruban: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Visualization, Funding Acquisition, Project Administration and Writing -original draft; Shivatharsiny Yohi: Data Curation, Formal Analysis, Visualization, and Writing -review and editing; Deepthi Inoka Uluwaduge: Supervision, Writing -review and editing; and Sivashanthini Kuganathan: Resources, Supervision, and Writing - review and editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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