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Extraction of carotenoids and screening its antioxidant potential from marine and terrestrial sources: A comparative study

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Abstract
Carotenoids were extracted from the marine sources such as shell waste of *Aristeus alcocki*, *Solenocera indica*, *Penaeus monodon*, *Metapenaeus affinis*, *Parapenaeopsis stylifera* and from the terrestrial sources *viz. Daucus carota*, *Carica papaya*, *Psidium guajava*, *Cucurbita moschata* and *Lycopersicon esculentum* using different extraction solvents and vegetable oils. The *in vitro* antioxidant activity of the extracts was confirmed using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay and reducing power (RP) method. The highest carotenoid yield was obtained from the shell waste of *A. alcocki* (238.1 µg/g) when acetone was used as extraction solvent. The quantity of carotenoid yielded was the least when the source was *P. guajava* (0.15 µg/g) and the method employed soybean oil as the extraction medium. The antioxidant activity of the extracts correlates in DPPH scavenging assay and RP method. The relative antioxidant activity of the carotenoid extracted from diverse source materials was found following a descending pattern *A. alcocki>D. carota>L. esculentum>C. papaya>P. guajava>P. stylifera>C. moschata>S. indica>M. affinis>P. monodon*. The results obtained through present study revealed that acetone is the most appropriate solvent for the extraction of carotenoids and shell waste of *A. alcocki* is the more potent source of carotenoid with high antioxidant potential.

Keywords: antioxidant, carotenoids, extraction methods, shell waste, terrestrial source

Introduction

Carotenoids are natural pigments chiefly found in photosynthetic algae, plants and bacteria. Besides they occur in fungi, animals and some non-photosynthetic bacteria. Animals are incapable to synthesize carotenoids *de novo* and hence would rely solely on their diet for procuring this pigmental components [1]. The microalgal sources Rich in carotenoids includes *Dunaliella salina* [2, 3, 4], *Chlorella vulgaris* [5, 6], *Chlorella zofingiensis* [7], *Haematococcus pluvialis* [8, 9], *Nannochloropsis gaditana* [10], *Scenedesmus almeriensis* [11], *Spirulina platensis* [12], Red pepper [13, 14], *Moringa oleifera* Lam [15]. *Daucus carota* [16], *Cucurbita moschata* [17], *Carica papaya* [18], *Lycopersicon esculentum* [19], *Psidium guajava* [20] are some of the terrestrial sources of carotenoids. Shell waste is another category of cheapest raw material and potential sources of carotenoids. Extraction of carotenoid from shell waste has been well documented in the earlier reports [21, 22, 23, 24, 25, 26].

Due to the hydrocarbon structure, the carotenoid pigments are hydrophobic in nature; soluble only in organic solvents, oils and fats. Carotenoid molecules absorb light of wave length 400-500 nm in the electromagnetic spectrum and have long conjugated double-bonds [27]. In plants and algae, carotenoid function as the accessory light harvesting pigments for photosynthesis [28] and will also hinder the production of reactive oxygen species [29]. The oxygen scavenging property is contributed by the polyene chain of carotenoids [30]. Moreover, the pro-vitamin A and antioxidant activities of carotenoids are vital characters that provide a nutraceutical aspect to carotenoids. There are various groups of nutraceuticals being used in the aquafeed industry and carotenoid is one such among them [31]. In view of extensive usage of chemically synthesized carotenoids, only a small proportion of carotenoids extracted from natural sources are used industrially. In recent decades the global interest and consumption of products made from natural ingredients overrides their synthetic analogues which adds up to the relevance of finding new sources for the extraction of natural products [32]. This also marks the significance and future prospectus of present study. Therefore, the objectives of the present study were to optimize the extraction medium for carotenoids and to compare the extractability of carotenoids from various marine and terrestrial sources. *In vitro* antioxidant properties of the carotenoid extracts were also assessed in the investigation.

Materials and methods

Collection of raw material

The shrimps *viz*: *A. alcoki, S. indica, M. affinis, P. monodon* and *P. stylifera* were acquired from Murikkumpadam fish landing center, Vypin, Kerala, India. The shrimps were transported in a chilled icebox from the landing center. The terrestrial sources such as raw specimen of *D. carota* (Nantes variety) and riped specimens of *C. papaya* (Pusa dwarf variety), *P. guajava* (Pear shaped *variety), C. moschata* (Ambily variety) and *L. esculentum* (Sakthi variety) were bought from the local market.

Preparation of raw material

The head, thorax and tail from the shrimp shell were stored at -20 °C for further analysis. The terrestrial sources were thoroughly

washed fruits and sliced into pieces without peeling off the outer skin. All specimens were blended in a mixer grinder prior to the recovery of carotenoid pigments.

Extraction of carotenoids

Ether: Acetone: Water extraction [33]. 10ml ether: acetone: water in the ratio of 5:75:15 were used to recover carotenoids from 1g sample until the extract become colorless. The filtered extract was evaporated at 60 ^{0}C and the remnants dissolved in 5ml hexane.

Acetone extraction [34]: 10ml acetone was used to recover carotenoids from1g sample until the extract become colorless. The extract was mixed with 12.5ml petroleum ether and 9.4ml NaCl (0.73 %) solution in a separating funnel. The epiphase recovered, evaporated at 60 ^{0}C and the remnants dissolved in 5ml hexane.

Hexane: Isopropanol extraction [35]. 10ml Hexane: Isopropanol in the ratio of 3:2 was used to recover carotenoids from 1g sample until the extract become colorless. The extract was poured to a separating funnel containing NaCl solution (1%) and mixed well. The epiphase was passed through anhydrous sodium sulfate, then evaporated at 60 ^{0}C and the remnants dissolved in 5ml hexane.

90 % Acetone extraction [8]: 10ml 90% acetone was used to recover carotenoids from 1g sample until the extract become colorless. The filtered extract was evaporated at 60 ^{0}C and the remnants dissolved in 5ml hexane.

Vegetable Oil extraction [37]. 20 ml vegetable oils *viz*. coconut oil (Kera), soybean oil (Fortune) and sunflower oil (Gold winner) was used to recover carotenoids from 1g sample. The extraction media containing 0.05% Butylated hydroxytoluene were kept in water bath at 70 ^{0}C for 150 min. Followed by 10 min centrifugation (REMI, model no. C-24, India) at 3000 rpm. The pigment containing supernatant was decanted to a separate vial.

Quantification of carotenoid

Absorbance of organic solvent extracts and vegetable oil extracts was recorded at 470 and 485 nm via UV-Visible spectrophotometer (HITACHI U-2900, model no. 2J1-0004, Japan), respectively. The values of absorbance were input to the following formula for estimating carotenoid content.

$$\text{Carotenoid concentration } (\mu g/g) = \frac{A \times V \times 10^6}{100 \times W \times D \times E^{1\%}_{1cm}}$$

Where;

A - Absorbance

V - Volume of extract in hexane

W- Sample weight in grams

10^6 - Dilution multiple

D - Cuvette width (1cm)

$E^{1\%}_{1cm}$ - extinction co-efficient

The value of $E^{1\%}_{1cm}$ is 2100 and 2155 for extraction via organic solvents and vegetable oils, respectively.

Antioxidant activity of extracted carotenoid

2, 2-diphenyl-1-picrylhydrazyl (DPPH) Free-Radical Scavenging Assay modified method [38]: 0.5ml of DPPH (0.3mM) in methanol was mixed with 0.5ml extract. The resultant mixture incubated in dark for 30 min. 0.3mM DPPH in methanol is the control. The values of absorbance recorded at 518 nm using a spectrophotometer (HITACHI U-2900, model no. 2J1-0004, Japan) were input to the following formula for estimating scavenging activity of the DPPH free radical:

$$\text{Scavenging effect } (\%) = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Reducing power method [39]: 0.5 ml sample solution was mixed by 1.2 ml phosphate buffer (0.2 M, pH 6.6) and 1.25 ml potassium ferricyanide (10 g/l). The mixture was incubated for 30 min at 50 °C ensued with the addition of 1.25 ml Trichloroacetic acid (100 g/l). The resultant solution was centrifuged for 10 min at 3000 rpm in a centrifuge (REMI, model no. C-24, India). 1.25 ml supernatant from each sample is mixed with 1.25 ml sterile water and 0.25 ml ferric chloride solution (1 g/l) taken in separate test tubes. The color change was recorded at 700nm (Hitachi, U-2900 spectrophotometer) following 10 min incubation. Increased reducing power is indicated by high optical density of reaction concoction.

Statistical analysis

The statistical analysis was executed via Statistical Package for the Social Science (SPSS) version 16.0. The significant difference between different treatments was examined using ANOVA at the significance level of 5%. Experimental results were represented as average of triplicate determinations.

Results

Extraction and quantification of carotenoids

Extraction media

Carotenoids are extracted from various marine and terrestrial sources via organic solvents, vegetable oils and organic solvent mixtures. The mean carotenoid content from various sources using different extraction media were represented in Table 1. Acetone provided maximum extractable carotenoid, which was significantly different ($P \leq 0.05$) when related to other extraction media. Extraction with vegetable oil provided only lesser extractable carotenoids. However, coconut oil yielded the maximum carotenoid content among the three vegetable oil and was significantly different ($P \leq 0.05$). Water-miscible organic solvents with polar nature for the revival of carotenoid pigments from tissue containing water [40]. Reports [35] on carotenoid extraction from shell waste of *P. indicus* showed that hexane: isopropyl alcohol provided utmost carotenoid yield than acetone. Nevertheless supreme extractability of carotenoid via acetone was proclaimed [41] when related to ethanol, methanol and isopropyl alcohol from *P. monodon* waste. Similarly, acetone extraction was affirmed to give the most prominent carotenoid quantity from *A. alcocki* shell waste [42]. Correspondingly, in the present work also acetone extraction yielded maximum carotenoid associated with other extraction media used. Higher recovery of carotenoids using soybean oil of the waste of crawfish when related to herring oil and menhaden oil extraction was reported [43]. The concentration of carotenoid was comparatively greater with the usage of sunflower oil over vegetable oils of coconut, gingelly, groundnut, mustard, rice bran and soya [37]. The recovery of carotenoid from *C. moschata* illustrated a higher extraction yield from virgin coconut oil when compared by acetic acid, ethanol and ethyl acetate [44]. Similarly, in the existing study, amongst the three vegetable oil extraction

media used, coconut oil extraction yielded maximum carotenoid compared to sunflower oil and soybean oil.

Sources

The carotenoid extracted from shell wastes using various extraction media ranged from 6.05 to 238.1 µg/gin *A. alcocki*, 0.76 to 74.72 µg/g in *S. indica*, 1.1 to 85.31 µg/g in *P. monodon*, 3.75 to 44.65 µg/g in *M. affinis* and 3.02 to 75.41 µg/g in *P. stylifera*. Whereas carotenoid quantity in terrestrial sources ranged from 22.86 to 184.54 µg/g in *D. carota*, 7.71 to 113.33 µg/g in *C. papaya*, 0.03 to 11.75 µg/g in *P. guajava*, 5.4 to 152.54 µg/g in *C. moschata* and 20.10 to 127.00 µg/g in *L. esculentum*. The maximum carotenoid content (238.1 µg/g) was achieved from the *A. Alcocki* shell waste via acetone extraction which was significantly different ($P \leq 0.05$) from other raw materials and extraction media. The least carotenoid yield (0.87 µg/g) was attained from *P. guajava* employing soybean oil extraction, which does not differ significantly ($P \geq 0.05$) from extracted carotenoid via sunflower oil (0.16 µg/g), coconut oil (1.83 µg/g) and 90% acetone (2.83 µg/g) from *P. guajava*. The carotenoid yield from crustaceans has been affirmed to alter among species [45]. The body color of each species also varied with carotenoid content [35]. Various studies have elucidated carotenoid recovery from *A. Alcocki* shell waste [46, 42, 47], *S. indica* [46], *P. monodon* [48] and *P. stylifera* [36] which ranged from 43 µg/g to 185 µg/g, 67.7 to 116 µg/g, 84.02 µg/g and 129 µg/g, respectively. These reports corroborate with the existing results except the carotenoid recovery from *P. stylifera*, which was 1.7 times less in the existing study. The quantity of extractable carotenoids from varied terrestrial sources as detected in this study has well accorded including the pioneer reports [49, 15, 50].

Antioxidant activity of extracted carotenoids

The *in vitro* antioxidant potential of carotenoid extracted from various sources are represented in Table 2. The highest scavenging activity of DPPH radical was showed by carotenoid from *A. alcocki* (97.01%) which does not differ significantly ($P \geq 0.05$) from *D. carota* (96.84%) and *L. esculentum* (96.82%). There was a significant difference ($P \leq 0.05$) in the scavenging activity of DPPH radical in carotenoid from *C. papaya*. (96.45%), *P. guajava* (96.01%), *P. stylifera* (95.82%), *C. moschata* (93.44%), *S. indica* (87.34%), *M. affinis* (82.56%) and *P. monodon* (76.31%). *A. alcocki* carotenoid extract showed the highest reducing power with an absorbance of 4.91, which is significantly different ($P \leq 0.05$) from *L. esculentum* (4.627), *D. carota* (4.626), *P. guajava* (4.604), *C. papaya* (4.601), *P. stylifera* (4.59), *S. indica* (4.493), *C. moschata* (4.36), *M. affinis* (2.697), *P. monodon* (0.981). Carotenoids possess antioxidant property with which they defend the cells from the damaging effect of singlet oxygen and free radicals [51]. The *in vitro* antioxidant action of the extracted carotenoid was screened with reference to their scavenging capacity of DPPH and reducing power assay. The DPPH radical-scavenging assays provide unstable violet DPPH free radical. This free radical will be stabilized into stable yellow DPPH compound via antioxidant by donating a hydrogen ion [52]. Thus the optical density of DPPH is reduced from 517 nm. The reduced absorbance measures the degree of radical scavenging potential [53]. The reducing power of a compound has been accounted to be concomitant with antioxidant action [54]. Thus reducing power method is simple and effective for analyzing the antioxidant action. The substance having reduction potential will form a ferric-ferrous complex with maximum optical density at 700nm on reaction with potassium ferricyanide (Fe^{2+}) and subsequent reaction with ferric chloride [55]. Increased reducing power is indicated by high optical density of the reaction mixture [56]. The significant antioxidant power of carotenoids entails applicability in antioxidant therapy.

Tables and Figures

Table 1: Extraction yield of carotenoid (mean ± SD) from various raw materials using different extraction media.

Extraction media Raw Material.	Acetone (µg g^{-1})	Hexane: isopropane (3:2, v/v) (µg g^{-1})	90%acetone (µg g^{-1})	Ether: acetone: water (15:75:10 v/v) (µg g^{-1})	Coconut oil (µg g^{-1})	Soybean oil (µg g^{-1})	Sunflower oil (µg g^{-1})
A. alcocki	238.1 ± 0.09^{a1}	27.45 ± 2.69^{b1}	67.03 ± 1.19^{c1}	49.76 ± 2.66^{d1}	6.76 ± 0.75^{e1}	6.94 ± 0.59^{e1}	6.05 ± 0.87^{e12}
S. indica	74.72 ± 3.76^{a2}	0.76 ± 0.622^{b2}	61.74 ± 0.60^{c2}	74.38 ± 3.23^{d2}	6.08 ± 0.53^{bd12}	5.52 ± 0.33^{bd1}	6.95 ± 0.65^{d12}
P. monodon	85.31 ± 1.78^{a3}	1.11 ± 0.59^{b2}	93.56 ± 1.14^{c3}	79.09 ± 2.08^{d2}	6.06 ± 1.15^{e12}	7.07 ± 1.76^{e1}	7.98 ± 1.14^{e1}
M. affinis	44.65 ± 0.78^{a4}	3.75 ± 1.48^{b2}	38.36 ± 1.83^{c4}	44.51 ± 0.39^{a1}	4.65 ± 0.62^{b2}	6.62 ± 0.71^{b1}	5.56 ± 0.46^{b2}
P. stylifera	75.41 ± 2.35^{a2}	3.02 ± 2.49^{b3}	63.37 ± 2.24^{c12}	62.52 ± 2.88^{c3}	6.22 ± 0.40^{b12}	5.79 ± 1.63^{b1}	5.71 ± 0.96^{b12}
D. carota	184.54 ± 2.97^{a5}	176.29 ± 2.59^{b4}	73.64 ± 2.28^{c5}	184.54 ± 2.15^{a4}	57.52 ± 0.76^{d3}	22.86 ± 0.41^{e2}	30.59 ± 0.71^{f3}
C. papaya	113.33 ± 3.14^{a6}	38.5 ± 1.59^{b5}	24.42 ± 0.31^{c6}	51.76 ± 3.92^{d1}	21.06 ± 1.03^{c4}	7.71 ± 1.46^{e1}	11.53 ± 0.93^{e4}
P. guajava	11.75 ± 3.67^{a7}	4.8 ± 0.55^{cb2}	2.83 ± 0.46^{bcd7}	6.27 ± 1.11^{c5}	1.83 ± 0.10^{bd5}	0.03 ± 0.03^{d3}	0.16 ± 0.10^{d5}
C. moschata	152.54 ± 3.20^{a8}	20.25 ± 3.29^{b6}	30.83 ± 0.14^{c8}	73.49 ± 0.99^{d2}	25.45 ± 0.58^{e6}	5.44 ± 0.55^{f1}	12.63 ± 0.13^{g4}
L. esculentum	127.00 ± 2.27^{a9}	53.61 ± 1.93^{b7}	98.69 ± 0.94^{c9}	104.45 ± 3.75^{d6}	55.28 ± 0.72^{b7}	20.10 ± 1.35^{e2}	24.35 ± 0.33^{e6}

Values in the same column with different numbers (1-9) as superscript are significantly different ($P<0.05$) according to Tukey's test.
Values in the same row with different alphabets (a-g) as superscript are significantly different ($P<0.05$) according to Tukey's t

Table 2: *In vitro* antioxidant activity (mean ± SD) of carotenoid extracts from various raw materials.

Carotenoid Extract	% DPPH scavenging activity	Reducing power absorbance at 700nm
A. alcocki	97.01 ± 0.18^{a}	4.905 ± 0.01^{a}
S. indica	87.34 ± 0.16^{b}	4.493 ± 0.00^{b}
P. monodon	76.31 ± 0.02^{c}	0.981 ± 0.01^{c}
M. affinis	82.56 ± 0.08^{d}	2.697 ± 0.04^{d}
P. stylifera	95.82 ± 0.06^{e}	4.59 ± 0.01^{e}
D. carota	96.84 ± 0.07^{a}	4.626 ± 0.01^{e}

C. papaya.	96.01 ± 0.03^{f}	4.601 ± 0.01^{e}
P. guajava	96.45 ± 0.07^{e}	4.604 ± 0.01^{e}
C. moschata	93.44 ± 0.04^{g}	4.36 ± 0.03^{f}
L. esculentum	96.82 ± 0.11^{a}	4.627 ± 0.02^{e}

Values in the same column with different letters (a-g) as superscript are significantly different ($P < 0.05$) according to Tukey's test.

Conclusion

Analysis using various marine and terrestrial sources for extraction of carotenoid proved shell waste of *A. alcocki* as the formidable source of carotenoids. The recovery of carotenoids via various organic solvents, vegetable oils, and organic solvent mixtures validated that acetone produces the utmost extraction yield among other extraction media. Strong antioxidant power of the extracted carotenoid implies it may replace many toxic synthetic antioxidants food additives. Carotenoid extracts when integrated to aquaculture found enhancing the stress tolerance and survivorship in finfish and shellfish. Potential antioxidant activity of carotenoid extract hence holds prospective applications in aquaculture industry.

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