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Carotenoid composition and retinol equivalent in plants of nutritional and medicinal importance: Efficacy of β-carotene from *Chenopodium album* in retinol-deficient rats

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ABSTRACT

This study aimed to (1) quantify carotenoids in leafy vegetables and plants of nutritional and medicinal importance, (2) evaluate retinol equivalent (RE) of provitamin-A carotenoids and (3) determine efficacy of β-carotene from *Chenopodium album* and to compare with retinol formed on feeding to retinol-deficient rats for 3 weeks. β-Carotene and lutein contents (mg/100 g dry weight) ranged from 1.5 to 120 and 11.7 to 185 (leafy greens) and 0.4 to 34.7 and 11.8 to 679 (medicinal plants) whereas, α-carotene ranged from 0.3 to 35.6 (leafy greens) and 0.1 to 15.7 (medicinal plants). RE values (mg%) ranged from 0.4 to 20 and 0.42 to 5.8 in leafy greens and medicinal plants. Efficacy of β-carotene (2400 μg/kg diet) from *C. album* in retinol-deficient rats revealed a 93.6% rise in plasma retinol levels from 0.53 to 8.4 μM. The plants analysed are a good source of retinol precursors and biologically active lutein; therefore can be exploited to meet carotenoid requirements.

1. Introduction

Leafy green vegetables and fruits have generated interest worldwide as they exhibit multiple benefits for health of human beings. Carotenoids such as β-carotene, α-carotene, γ-carotene and β-cryptoxanthin, present in agricultural and horticultural produce, have provitamin-A activity and are potent antioxidants and modulate the pathogenesis of several chronic degenerative diseases (Niizu & Rodriguez-Amaya, 2005). At least 254 million pre-school children globally suffer from clinical and sub-clinical vitamin A deficiency (WHO, 2000). Alleviation of vitamin A deficiency among vulnerable groups is thus of high priority. Plant foods account for more than 80% of dietary vitamin A in developing countries (Bhaskarachary, Sankar Rao, Deosthale, & Reddy, 1995). Ethnic groups consume certain varieties of plants as part of their diet but their active components have not yet been determined. Out of 250,000 higher plants, less than 1% have been screened pharmacologically (Grover, Yadav, & Vats, 2002). In addition, a variety of natural surroundings and climates exist in India and thus large varieties of edible leaves are available and consumed in the country (Singh, Kawatra, & Sehgal, 2001). Due to growing recognition of natural products and processes for sustenance of human health, the importance of medicinal plant sources has increased tremendously. Many of the currently available drugs have been derived directly from plants (Grover et al., 2002).

Recent research has shown that a number of herbal derivatives have excellent antioxidant action. It is believed that phytomolecules, such as carotenoids, exhibit potent antioxidant properties. β-Carotene-rich food in the daily diets of the population may be one of the successful strategies for improving vitamin A status instead of synthetic vitamin A supplementation (Gopalan, 1992). To effectively implement such a strategy, it is essential to have exhaustive information on the carotenoid composition of various plant foods consumed across the country. Moreover, carotenoid content is known to vary in plant foods due to differences in characteristics of cultivars, climate and growing conditions (Aizawa & Inakuma, 2007). Currently, only limited information is available on the carotenoids present in green leafy vegetables produced and grown (wild) from different parts of India (Bhaskarachary, Rajendran, & Thingnganing, 2008; Lakshminarayana, Raju, Krishnakantha, & Baskaran, 2005; Raju, Varakumar, Lakshminarayana, Krishnakantha, & Baskaran, 2007; Rajyalakshmi et al., 2001; Singh et al., 2001) and the information on plants used for Ayurvedic medicinal purposes is even more scarce (Raju et al., 2007; Rajyalakshmi et al., 2001). Thus, the current study was undertaken to determine the carotenoid profile and vitamin A activity (as retinol equivalents) of selected leafy green vegetables and plants of medicinal importance found in India. Further, the bioefficacy of

Abbreviations: λ_{max}, absorption maxima; APCI, atmospheric pressure chemical ionisation; HPLC, high performance liquid chromatography; LC–MS, liquid chromatography–mass spectrometry; PDA, photodiode array; RE, retinol equivalent.

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β-carotene (conversion to retinol) from *Chenopodium album* (rich source of β-carotene) in rats was evaluated.

2. Materials and methods

2.1. Materials

2.1.1. Samples

Freshly harvested green leafy vegetables (n = 19) and medicinal plants (n = 33) were collected in duplicate, from local farms and open fields (Mysore, Karnataka) and used immediately for extraction and analysis. The botanical and common name and medicinal properties of the plants used in this study are given in Table 1.

2.1.2. Chemicals

All-trans β-carotene, α-carotene, lutein, and DL-α-tocopherol were purchased from Sigma Chemical Co. (USA). Neoxanthin, violaxanthin and zeaxanthin were kindly donated by Dr. Akhiko Nagao (NFRI, Japan). HPLC grade acetonitrile, hexane, methanol and dichloromethane were purchased from Sisco Research Laboratories (Mumbai, India). All other chemicals used were of analytical grade unless otherwise mentioned.

2.2. Methods

2.2.1. Extraction of carotenoids from plant materials

Fresh leaves were washed and dried on blotting paper. The method of Raju et al. (2007) was employed for the extraction of carotenoids. In brief, 20–30 g of edible portion were well ground, along with 2–3 g of anhydrous sodium sulphate and 2 mM α-tocopherol and pigments were extracted using ice-cold acetone. The extraction was repeated three times, or until the residue was rendered colourless, indicating complete extraction of pigments.

2.2.2. HPLC and LC–MS analysis of carotenoids

An aliquot of crude extract was evaporated under a stream of nitrogen and re-dissolved in mobile phase (acetonitrile:dichloromethane:methanol, 60:20:20, v/v/v) containing 0.1% ammonium acetate for analysis of β-carotene, α-carotene, lutein, zeaxanthin, neoxanthin, and violaxanthin and injected into the HPLC system (LC-10Avp; Shimadzu, Kyoto, Japan) equipped with a Shimadzu photodiode array (PDA) detector (SPD-M20A). All the carotenoids were separated on a Phenomenex RP-18 column (250 mm × 4.6 mm; 5 μm) isocratically eluting with (1 ml/min) mobile phase. The carotenoids were monitored at 450 nm using Shimadzu Class-VP version 6.14SP1 software. The peak identity of each carotenoid was confirmed by their UV–Vis spectra recorded with the

Table 1
Botanical and common names and health benefits of green leafy vegetables and medicinal plants.

Green leafy vegetables			Medicinal plants		
Botanical name	Common name	Medicinal/health applications[a]	Botanical name	Common name	Medicinal/health applications[a]
Allium schoenoprasum	Chives	Anti-cancer, digestive disorders	*Calotropis gigantea*	Gigantic swallow wort	Kesarayer disease
Amaranthus polygonoides	Amaranth leaves	Source of vitamins	*Cassia auriculata*	Tanner's Cassia	Antihelmenthic
Amaranthus sp. (local name: keerai)	Amaranth leaves	Source of vitamins	*Centella asiatica*	Indian pennywort	Diuretic, tonic, anti-leprosy
Amaranthus sp. (local name: yelavare)	Amaranth leaves	Source of vitamins	*Clitoria ternatea*	Butterfly pea	Cathartic, diuretic
Amaranthus spinosus	Prickly Amaranth	Sudorific, febrifuge	*Coleus aromaticus*	Indian Borage	Urinary diseases, cough, asthma
Apium graveolus var. *dulce*	Celery	Carminative, antirheumatic	*Convolvulus sepium*	Bindweed	Laxative, gall bladder problems.
Boerhavia diffusa	Hog weed	Asthma	*Curcuma domestica* (*longa*)	Turmeric leaf	Stomachic, depurative
Brassica chinensis	Chinese cabbage	Antioxidant	*Cymbopogon citratus*	Lemongrass	Carminative
Brassica oleracea var. *caulorapa*	Knol khol greens	Rich in calcium	*Cynodon dactylon*	Conch/dog grass	Diuretic, anasarca
Brassica rapa	Turnip greens	Stomachic, calcium source	*Datura stramonium*	Jimson weed	Narcotic, antispasmodic, asthma
Capsicum annuum	Chilli leaf	Carminative, antirheumatic	*Ficus glomerata*	Country fig	Bilious infections
Chenopodium album	Lamb's Quarters	Laxative, antihelminthic	*Lawsonia inermis*	Henna	Skin problems
Colocasia anti-quorum	Colocasia leaves	Astringent and styptic	*Leucas aspera*	Thumbe	Antipyretic, psoriasis
Commelina benghalensis	Jio	Laxative, anti-leprosy	*Melissa officinalis*	Lemon balm	Headache, toothache
Ipomoea pes-tigridis	Punaikkirai	Boils, pimples carbuncles	*Momordica charantia*	Bittergourd leaf	Antidiabetic[b], stomachic, cold
Lactuca sativa	Lettuce iceberg	Antioxidant	*Ocimum canum*	Hoary Basil	Diuretic
Petroselinum crispum	Parsley	Diuretic, ecbolic	*Ocimum sanctum*	Holy Basil	Antidiabetic[b], bronchitis
Portulacae oleracae	Common purslane	Antiscorbutic, diuretic	*Physalis alkekengi*	Chinese lantern	Diuretic, febrifuge, vermifuge
Tamarindus indica	Tamarind	Carminative, laxative	*Plumbago zeylanica*	Chitramula	Vesicant, diuretic
Medicinal plants			*Prunella vulgaris*	Allheal	Astringent, sore throat
			Ricinus communis	Castor	Boils, sores
Acalypha indica	Acalypha	Laxative, emetic, cough	*Salvia officinalis*	Sage	Diaphoretic, carminative
Aegle marmelos	Bel	Antidiabetic[b], digestive	*Solanum dulcamara*	Bittersweet Nightshade	Tumours, warts, rheumatism
Aloe vera	Aloe	Cathartic, eye and skin ailments	*Solanum surattense*	Wild brinjal	Carminative, dengue fever
Aristolochia indica	Indian birthwort	Cough	*Tridax procumbens*	Mexican daisy	Dysentry, diarrhoea
Azadirachta indica	Margosa, Neem	Antidiabetic[b], antiulcer	*Vitex negundo*	Indian privet	Anticancer, tranquiliser
Bacopa monnieri	Thyme leaved gratiola	Diuretic, nervous diseases	*Zingiber officianale*	Ginger leaf	Carminative, flatulence, colic

[a] National Institute of Science Communication, CSIR (1986) unless otherwise mentioned.
[b] Grover et al. (2002).

PDA detector. Further, LC–MS was used to confirm the identity of carotenoids. The positive ions of the carotenoids were recorded with the HPLC system connected to a LC-Q mass spectrometer (Waters 2996 modular HPLC system, UK) equipped with an atmospheric pressure chemical ionisation (APCI) module. The APCI source was heated at 130 °C and the probe was kept at 500 °C. The corona (5 kV), HV lens (0.5 kV) and cone (30 V) voltages were optimised. Nitrogen was used as sheath and drying gas at 100 and 300 l h^{-1}, respectively. The spectrometer was calibrated in the positive ion mode and $(M+H)^+$ ion signals were recorded (Fig. 1) and confirmed with respective standards. Quantification of individual compounds was evaluated by comparing their peak area with the authentic standard.

2.2.3. Calculation of vitamin A activity

Vitamin A activities of β- and α-carotene were calculated in terms of retinol equivalents (RE) based on the *in vivo* conversion factor proposed by WHO and NRC (NRC, 1989; WHO, 1982), where 1 RE = 1 μg of retinol = 6 μg of β-carotene or 12 μg of α-carotene. All the values presented in this study are means of duplicate analyses.

2.3. Dietary study

2.3.1. Animals and diets

Animal experiments were conducted after due clearance from the Institutional Animal Ethics Committee. Weanling male albino rats (OUTB-Wistar, IND-CFT 2c), weighing 35 ± 2 g were housed in individual stainless steel cages in the institute animal house facility at room temperature (28 ± 2°C) with a 12 h light/dark cycle and had free access to food and water *ad libitum*.

2.3.2. Induction of retinol deficiency and refeeding with C. album

Retinol deficiency was induced in a group of rats ($n = 20$) by feeding a semi-synthetic diet (AIN, 1977) devoid of retinol (Sangeetha, Bhaskar, & Baskaran, 2008) for 8 weeks and was

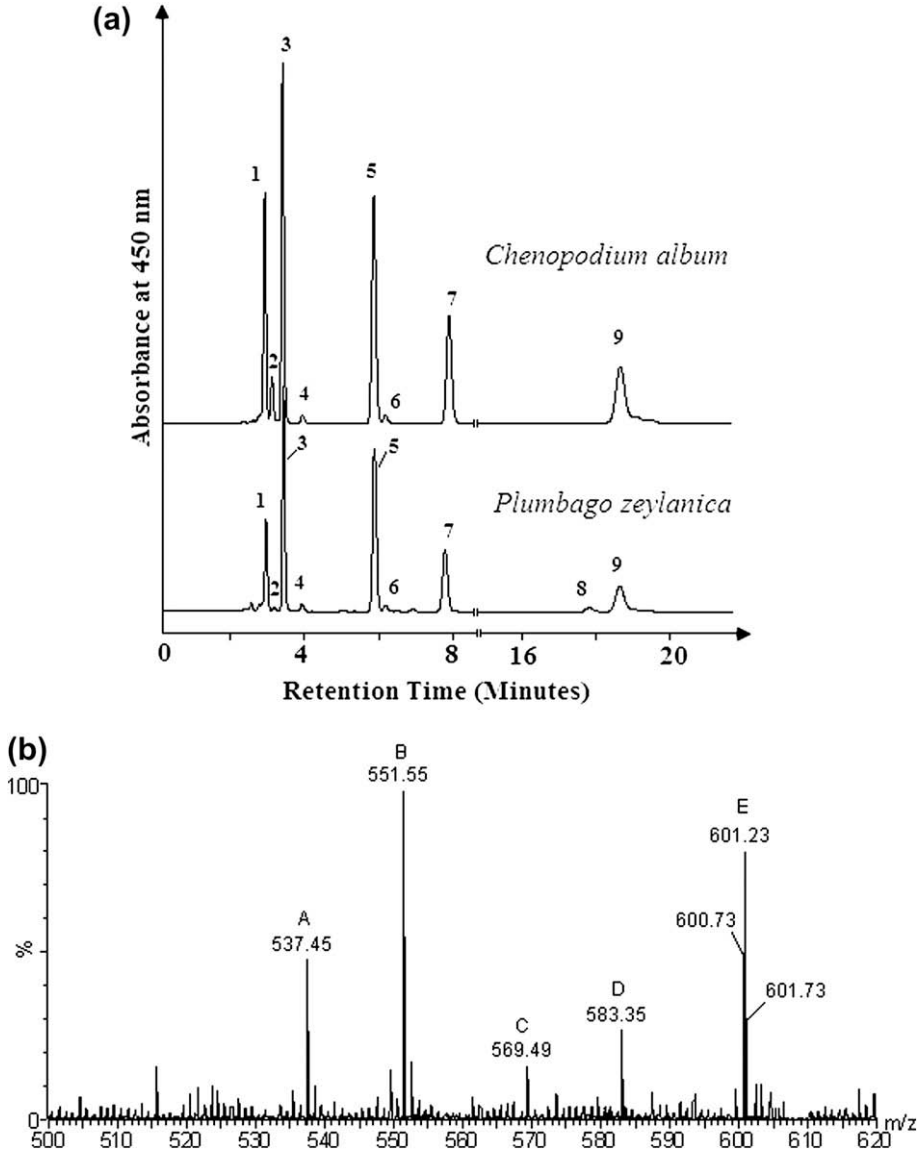

Fig. 1. (a) HPLC profile of carotenoids extracted from leafy green vegetable, *Chenopodium album* and medicinal plant, *Plumbago zeylanica*. 1 = neoxanthin, 2 = violaxanthin, 3 = lutein, 4 = zeaxanthin, 5 = chlorophyll b, 6 = chlorophyll a, 7 = α-tocopherol, 8 = α-carotene, 9 = β-carotene. (b): LC–MS (APCI) profile of extract from *C. album*. A = β-carotene (537 $[M+H]^+$), B = lutein (551 $[M+H-H_2O]^+$), C = zeaxanthin (569 $[M+H]^+$), D = neoxanthin, violaxanthin (583 $[M+H-H_2O]^+$), E = neoxanthin, violaxanthin (601 $[M+H]^+$). HPLC and LC–MS conditions are described in Section 2.2.

confirmed by blood retinol levels (0.53 μmol/l). Feed intake and gain in body weight of animals was recorded during the experimental run. Rats were divided into two groups (n = 10/group). Group one was fed diet supplemented with powdered *C. album* as β-carotene source (2400 μg/kg diet) for a period of 3 weeks while group two received diet with no added *C. album* and served as the baseline. A separate group of animals (n = 5), fed on a retinol sufficient diet (4×10^5 IU) throughout the experimental run, was considered as the control. The composition of ROH sufficient diet (g/kg) was as follows: vitamin A-free casein (200), methionine (3), cellulose (50), corn starch (325), glucose (324) mineral mix (35), vitamin A-free vitamin mix (10), choline chloride (2), ascorbic acid (1) groundnut oil (50) and ROH (400,000 IU), whereas the composition of the ROH deficient was identical, except that it had no added ROH. Animals were sacrificed using anaesthetic ether and blood and liver were sampled and processed for the analysis of β-carotene and retinol levels.

2.3.3. Extraction and analysis of β-carotene and retinol from tissue

Samples were processed under a dim yellow light, on ice to minimise isomerisation and oxidation of β-carotene and retinol. The plasma was separated from blood by centrifugation (Remi India Ltd., Mumbai) at 1000 g for 15 min at 4 °C. Liver samples (1 g) were homogenised at 4 °C in buffered saline for retinol assay. β-Carotene, retinol and retinyl palmitate were extracted from the plasma and liver samples as done by Sangeetha et al. (2008) and Raju, Lakshminarayana, Krishnakantha, and Baskaran (2006). Briefly, plasma (0.8 ml) was diluted with 3 ml of dichloromethane:methanol (2:1; v/v) containing 2 mM α-tocopherol, and mixed for 1 min using a vortex mixer, followed by the addition of 1.5 ml of hexane. The mixture was centrifuged at 5000*g* for 3 min at 4 °C and the upper hexane–dichloromethane layer was withdrawn. The extraction procedure was repeated for the lower phase, twice, using dichloromethane:hexane (1:1.5, v/v). The pooled extract was evaporated to dryness under a stream of nitrogen, re-dissolved in dichloromethane:methanol (2:1; v/v) and used for HPLC analysis. Liver was homogenised in nine parts of ice-cold isotonic saline and 0.8 ml of the homogenate was used for the extraction of β-carotene and retinol metabolites following the procedure described for plasma.

Retinol and β-carotene in plasma and liver were quantified by HPLC (conditions described earlier for analysis of carotenoids from plant samples). Retinol and retinyl palmitate were monitored at 325 nm, while β-carotene was monitored at 450 nm. The peak identity of each component was confirmed by their characteristic spectrum and they were quantified by comparing their peak areas with authentic standards.

2.4. Statistical analysis

The data obtained from the animal experiment were subjected to analysis of variance (ANOVA). In cases of significant difference, mean separation was accomplished by Tukey's highest significant difference test using STATISTICA software (Statsoft, 1999). The level of significance was set to $p < 0.05$ for all the tests. Mean values have been reported for the carotenoid composition of the plants as the data were from duplicate analyses.

3. Results and discussion

3.1. HPLC analysis of carotenoids in plants

A representation of the carotenoid profile of leafy vegetables and medicinal plants is shown in Fig. 1. Under the HPLC conditions adopted, carotenoids were well separated and the peaks eluted in the following order: neoxanthin (peak 1), violaxanthin (peak 2), lutein (peak 3), zeaxanthin (peak 4), chlorophylls a and b (peaks 5 and 6), α-tocopherol (peak 7), α-carotene (peak 8) and β-carotene (peak 9). α-Carotene was detected in eight greens and 25 medicinal plants. Since *cis*-β-carotene did not separate well in all samples, β-carotene was calculated as the sum of *cis*-β-carotene and β-carotene. Xanthophylls were eluted first (within 4.5 min), followed by the chlorophylls (6.5–7.5 min) and then the carotenes (17–20 min). Kimura and Rodriguez-Amaya (2003) reported separation of carotenoids from lettuce by HPLC under gradient elution, with a runtime of 50 min, whereas, Bhaskarachary et al. (2008) achieved separation of carotenoids from leafy vegetables in 25 min with acetonitrile:dichloromethane:methanol (70:10:20 v/v/v). In contrast, carotenoids in this study were well separated within 20 min. The absorption maxima (λ_{max}) of the carotenoids eluted (β-carotene: 426, 454, and 480; α-carotene: 421, 448, and 475; lutein: 421, 447, and 475; neoxanthin: 415, 440, and 468; violaxanthin: 425, 449, and 476) were comparable with reported (Eitenmiller & Lander, 1999; Khachik et al., 1992) values. Reported values of λ_{max} were: β-carotene (452–454, 453 nm), α-carotene (448–450, 444 nm), lutein (446, 445), neoxanthin (438–440, 439) and violaxanthin (440–442, 443). Eitenmiller and Lander (1999) have reported the λ_{max} of zeaxanthin as 452 nm and it was 454 nm in this study.

As can be seen from the HPLC profiles of the green leafy vegetables and medicinal plants (Fig. 1), the profiles of the carotenoids were very similar, except for α-carotene. The differences were in their concentrations (Tables 2 and 3). For example, the chromatogram from the extract of *C. album* shows the presence of both hydrocarbon and xanthophyll carotenoids; however, their relative concentrations were found to be different from those of other greens studied (Table 2). The greatest values for β-carotene were for *C. album*, *Amaranthus spinosus*, *Commelina benghalensis*, *Colocasia anti-quorum*, and *Amaranth* sp. (keerai). The highest values for lutein content were found in *C. album*, *C. benghalensis*, *Capsicum annuum*, *Ipomoea pes-tigridis*, *A. spinosus* and *C. anti-quorum*. Therefore, *C. album*, *A. benghalensis* and *A. spinosus* are rich sources of both lutein and β-carotene. This accords well with a previous study (Raju et al., 2007) that reported the levels (mg% dry weight) of β-carotene and lutein as 115 and 92.8, 188 and 181 in *C. album* and *C. benghalensis*, respectively. The difference in the concentrations of carotenoids in greens is most likely related to difference in species and variety. Differences in carotenoid levels have been reported, even within species, and these can be attributed to cultivar, climate, growing conditions, seasonal changes (Aizawa & Inakuma, 2007), variety and stage of maturity of the samples used for analysis (Kimura & Rodriguez-Amaya, 2003).

In this study, concentration (mg/100 g dry weight) of β-carotene in green leafy vegetables and medicinal plants ranged from 1.5 to 120 and 0.4 to 34.7, respectively, and lutein ranged from 11.7 to 185 and 11.8 to 679, respectively. Neoxanthin levels ranged from 0.03 to 54.1 in greens and 1.8 to 18.5 in medicinal plants while violaxanthin levels were 0.03 to 141 and 0.1 to 6.4, respectively (Tables 2 and 3). α-Carotene was detected in eight leafy greens (0.3–35.6) and 25 medicinal plants (0.1–15.7) and was highest in *C. benghalensis* (35.6) and *Coleus aromaticus* (15.7). Carotenoid composition of 50% of the plants screened in this study has been reported for the first time. Aizawa and Inakuma (2007) have previously reported the β-carotene content (3.64 mg/100 g fresh weight) of *A. tuberosum*. In this study, higher contents of lutein and β-carotene were recorded in *Allium schoenoprasum* (8.2 and 5.4 mg/100 g fresh weight). β-Carotene content of amaranth leaves ranged from 2.3 to 14.7 mg/100 g fresh weight in the present study and was similar to those in reports of Rajyalakshmi et al. (2001) and Singh et al. (2001) who reported 10.1 and 5.4 mg/100 g fresh weight, respectively. β-Carotene contents of *C.*

Table 2
Carotenoid composition (mg/100 g dry weight) and retinol equivalents (RE) of green leafy vegetables.[a]

Name	Xanthophylls				Total[c] xanthophylls	Provitamin-A carotenoids		Total provitamin-A[d] carotenoids	RE[e]
	Neoxanthin	Violaxanthin	Lutein	Zeaxanthin		α-Carotene	β-Carotene		
A. schoenoprasum	23.5	3.15	73.4	44.4	145	ND[b]	48.1	48.1	8.02
A. polygonoides	12.5	2.41	25.7	1.19	41.8	ND	20.6	20.6	3.43
Amaranthus sp. (keerai)	38.1	4.92	71.9	1.30	116	1.03	66.4	67.4	11.2
Amaranthus sp. (yelavare)	16.4	2.74	29.2	0.95	49.3	1.38	23.2	24.6	3.98
A. spinosus	54.1	3.27	117	2.78	177	ND	97.9	97.9	16.1
A. graveolus	30.8	2.87	56.5	1.45	91.6	ND	49.9	49.9	8.32
B. diffusa	1.64	0.16	54.1	0.32	56.2	0.31	2.43	2.80	0.43
B. chinensis	0.69	3.07	22.4	0.63	26.2	ND	1.50	1.50	0.25
B. oleracea	18.5	4.73	43.0	1.57	67.8	0.67	27.1	27.8	4.57
B. rapa	0.54	5.41	11.7	1.54	19.2	ND	8.98	8.98	1.50
C. annuum	2.73	0.27	136	0.39	140	0.31	3.48	3.79	0.61
C. album	0.03	141	185	5.00	331	ND	120	120	20.0
C. anti-quorum	44.1	1.33	105	2.43	153	6.70	57.3	64.0	10.1
C. benghalensis	0.03	100	176	2.06	278	35.6	95.7	131	18.9
I. pes-tigridis	2.71	0.13	135	0.31	138	ND	4.31	4.31	0.72
L. sativa	2.53	0.15	26.5	0.04	29.2	ND	2.87	2.87	0.48
P. crispum	10.4	2.56	29.7	0.81	43.5	ND	18.4	18.4	3.07
P. oleracae	2.37	0.09	36.2	0.20	38.8	ND	4.20	4.20	0.70
T. indica	2.43	0.03	16.4	0.55	19.4	0.76	4.77	5.53	0.86

[a] Values are means of duplicate analyses.
[b] ND: not detected.
[c] Total xanthophylls = neoxanthin + violaxanthin + lutein + zeaxanthin.
[d] Total provitamin-A carotenoids = α-carotene + β-carotene.
[e] 1 RE = 6 mg β-carotene or 12 mg α-carotene.

Table 3
Carotenoid composition (mg/100 g dry weight) and retinol equivalents (RE) of medicinal plants.[a]

Name	Xanthophylls				Total[c] xanthophylls	Provitamin-A carotenoids		Total provitamin-A[d] carotenoids	RE[e]
	Neoxanthin	Violaxanthin	Lutein	Zeaxanthin		α-Carotene	β-Carotene		
Acalypha indica	7.57	1.28	420	0.75	429	0.33	14.3	14.6	2.41
A. marmelos	2.89	0.42	193	0.27	197	0.60	5.28	5.88	0.93
A. vera	2.27	0.15	14.9	0.39	17.7	0.87	6.59	7.46	1.17
Aristolochia indica	11.1	1.19	392	0.79	405	0.69	14.2	14.9	2.43
Azadirachta indica	3.33	0.40	11.8	0.48	16.0	0.27	6.93	7.19	1.18
B. monnieri	18.5	6.37	54.7	1.52	81.1	ND[b]	34.7	34.7	5.78
C. gigantea	4.47	1.75	135	0.48	141	0.57	11.2	11.7	1.91
C. auriculata	3.54	0.63	196	0.19	200	0.05	6.96	7.01	1.17
C. asiatica	4.83	1.22	235	0.55	242	ND	8.88	8.88	1.48
C. ternatea	15.3	2.39	679	1.44	698	1.50	31.2	32.7	5.32
C. aromaticus	4.85	1.08	28.6	1.01	35.6	15.7	0.35	16.1	1.37
C. sepium	11.8	0.92	395	1.07	409	0.86	20.2	21.1	3.44
C. domestica	5.29	0.96	293	0.77	300	3.06	7.64	10.7	1.53
C. citratus	4.55	0.76	292	0.26	298	ND	7.85	7.85	1.31
C. dactylon	8.79	0.69	484	0.70	494	0.22	16.5	16.7	2.77
D. stramonium	2.83	9.70	307	0.62	320	0.16	13.8	14.0	2.31
F. glomerata	3.85	0.21	185	0.25	189	4.42	3.27	7.69	0.91
L. inermis	2.52	0.11	361	0.38	364	0.67	8.93	9.60	1.54
L. aspera	4.31	0.68	134	0.12	140	0.07	8.11	8.17	1.36
M. officinalis	6.81	0.46	225	0.64	233	3.32	9.44	12.8	1.85
M. charantia	2.15	0.10	109	0.23	111	0.09	3.03	3.13	0.51
O. canum	4.66	1.29	458	0.92	465	0.87	12.7	13.6	2.19
O. sanctum	11.4	2.75	48.2	1.58	63.9	0.72	31.2	31.9	5.27
P. alkekengi	3.49	0.68	185	0.48	190	ND	4.47	4.47	0.74
P. zeylanica	4.46	0.14	245	0.46	250	0.79	5.38	6.17	0.96
P. vulgaris	5.61	0.92	349	0.72	357	0.19	8.67	8.86	1.46
R. communis	7.84	2.46	432	0.61	443	ND	20.8	20.8	3.47
S. officinalis	3.18	1.34	301	0.35	305	0.43	7.85	8.28	1.35
S. dulcamara	6.27	0.90	325	0.59	333	3.11	7.18	10.3	1.46
S. surattense	5.58	0.87	261	0.27	267	ND	11.6	11.6	1.93
T. procumbens	5.48	0.26	285	0.26	291	ND	8.72	8.72	1.45
V. negundo	3.00	0.27	101	0.16	104	ND	9.33	9.33	1.56
Z. officianale	1.84	0.75	101	0.22	104	2.59	1.21	3.80	0.42

[a] Values are means of duplicate analyses.
[b] ND: not detected.
[c] Total xanthophylls = neoxanthin + violaxanthin + lutein + zeaxanthin.
[d] Total provitamin-A carotenoids = α-carotene + β-carotene.
[e] 1 RE = 6 mg β-carotene or 12 mg α-carotene.

album (120 mg) and *C. benghalensis* (95.7) were comparable with those reported by Raju et al. (2007). Lutein contents of *C. album* and *C. benghalensis* recorded in this study were comparable with those reported by Raju et al. (2007) whereas it was higher (54.1 mg/100 g dry weight) in *Boerhavia diffusa*. Lutein value (7.4 mg/100 g fresh weight) for *Petroselinum crispum* was higher than the β-carotene value (4.6 mg/100 g fresh weight). Total provitamin-A carotenoids and total xanthophylls (Table 2) were higher than those reported by Chanwitheesuk, Teerawutgulrag, and Rakariyatham (2005) for *A. graveolens* and *T. indicus*.

There is very limited literature on the carotenoid composition of plants used in Ayurvedic medicine. The β-carotene value for *C. asiatica* accords well with Raju et al. (2007), while the lutein value reported by them was lower (15.9 mg/100 g dry weight) than the present result. β-Carotene levels for *Leucas aspera* (2.3 mg/100 g fresh weight) of the present study are in agreement with values (2.3 mg/100 g fresh weight) reported by Rajyalakshmi et al. (2001). β-Carotene level for *Ocimum sanctum* (10 mg/100 g fresh weight) was similar to those reported by Aizawa and Inakuma (2007) and Bhaskarachary et al. (1995). Lutein level of *O. sanctum* (15.4 mg/100 g fresh weight) was almost twice the amount reported by Aizawa and Inakuma (2007). The difference in carotenoid levels may be due to the different solvent system (hexane:ethanol:acetone:toluene, 10:7:6:7, v/v/v) used for extraction. Chanwitheesuk et al. (2005) reported total xanthophyll (4.24 mg%) and total provitamin-A carotenoid (2.54 mg%) levels in *Coleus amboinicus*, whereas, these were higher (35.6, 16.1 mg%) in *C. aromaticus*.

3.2. Vitamin A activity of carotenoids

Provitamin-A activity, measured as RE in mg (where 1 RE = 6 mg β-carotene or 12 mg α-carotene), was found in the range of 0.4–20 in leafy greens (Table 2) and 0.42–5.8 in medicinal plants (Table 3). Of the 19 greens analysed, seven had RE values greater than 5 mg (*A. schoenoprasum*, *Apium graveolus*, *C. anti-quorum*, *Amaranthus* sp. (keerai), *A. spinosus*, *C. album* and *C. benghalensis*) while, of the 33 medicinal plants studied, three had RE values greater than 5 mg (*O. sanctum*, *Clitoria ternatea* and *Bacopa monnieri*). Rajyalakshmi et al. (2001) have reported lower RE values for *A. spinosus* (1.68 mg%), *C. anti-quorum* (0.58 mg%), *C. benghalensis* (0.53 mg%), *L. aspera* (0.39 mg%) and *P. oleraceae* (0.07 mg%) than those in the present study (Tables 2 and 3). These differences may have arisen due to geographical differences and the different extraction procedure adopted.

The present study reports individual values for different carotenoids present in leafy greens and medicinal plants. Previously, only a few studies have reported values of both xanthophyll and provitamin-A carotenoids (Aizawa & Inakuma, 2007; Raju et al., 2007). In the southeast Asian sub-continent, only Raju et al. (2007) have reported levels of individual carotenoids in leafy greens. Information on medicinal plants and other greens is thus still very limited. Moreover, no reports are available for nearly 50% of the plants analysed in this study, many of which are used by the local people as food or medicine. *C. album*, *A. spinosus*, *C. benghalensis*, *C. anti-quorum* and *Amaranth* sp. (keerai) were rich in β-carotene while *C. album*, *C. benghalensis*, *C. annuum*, *I. pes-tigridis*, *A. spinosus* and *C. anti-quorum* were found to have considerable amounts of lutein. Interestingly, *C. album*, *A. benghalensis* and *A. spinosus* are rich sources of both lutein and β-carotene. *C. ternatea*, a medicinal plant, had the highest lutein content. Lutein is predominant in the macula of the retina where it acts as an antioxidant against photo-oxidation. Thus, the lutein-rich plants can be exploited for the management of age-related macular degeneration (Klein, Rowlad, & Hartis, 1995). Other plants, e.g., *Cynodon dactylon*, *Ocimum canum*, *Acalypha indica* and *Ricinus communis*, also had very high levels of lutein. Medicinal plants, such as *B. monnieri*, *O. sanctum* and *C. ternatea*, were rich sources of β-carotene. *C. ternatea* is thus a good source of both lutein and β-carotene. From the results, it is seen that RE levels of medicinal plants are lower than those of leafy greens; however, the total carotenoid values are significantly higher (1.5-fold). This could be one of the reasons for the medicinal properties of these plants and their use in Ayurvedic medicine.

3.3. Dietary study

This study determined the intestinal uptake of dietary β-carotene from *C. album* and its efficacy (conversion of β-carotene to retinol) in retinol-deficient rats. Although the food intake was not different between rats fed on a diet, with or devoid of retinol, the gain in body weight of rats fed on a diet devoid of retinol was 25% lower ($p < 0.05$). Deprivation of retinol for 8 weeks did not affect liver weight, while the retinol stores were significantly depleted in liver and plasma compared to the control group. The retinol-deficient rats did not exhibit any abnormal morphological or behavioural signs during the experimental run.

β-Carotene, in its native form was not detected in the plasma or liver of the retinol-deficient group (baseline) or the retinol-sufficient group (control). A significant decrease ($p < 0.05$) was observed in plasma and liver retinol levels (0.53 μmol/l, 1 μmol/g) after induction of retinol deficiency as compared to the control (59.3 μmol/l, 306 μmol/g). A 3-week dietary feeding of *C. album* to retinol-deficient rats resulted in a rise in plasma retinol levels (retinol + retinyl palmitate) by 93.6% to 8.4 μmol/l. The plasma and liver β-carotene response showed significant elevation to 42.6 nmol/l and 189 pmol/g compared with the baseline and control (Fig. 2). The results show that *C. album* is a good source of β-carotene, and consequently retinol, and its consumption could be a feasible strategy for alleviating vitamin A deficiency, as observed in the present study. Studies have shown that feeding drumstick leaves to rats significantly improved the blood response of β-carotene and retinol (Nambiar & Seshadri, 2001).

In conclusion, many of the plants analysed were rich in carotenoids, such as lutein and β-carotene. The results could be useful to health workers and persons practising Ayurveda in the selection of plants for their antioxidative properties for alleviation of diseases. However, before recommending these plants for consumption, investigation on the antinutritional factors and toxic substances present is needed. Moreover, more than 50% of the plants screened in this study are unexplored and have been studied for the first time. Feeding *C. album* to retinol-deficient rats improved their plasma retinol levels significantly. Awareness and consequently increased use of, these plant materials may go long a way toward

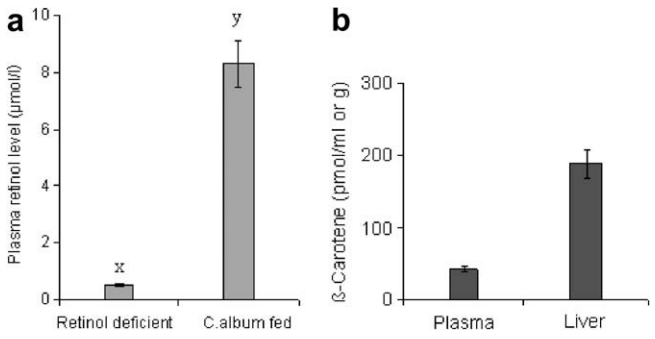

Fig. 2. Plasma retinol response (a) and plasma, liver β-carotene response (b) on feeding *Chenopodium album* to retinol-deficient rats for 3 weeks. No β-carotene was detected in plasma or liver of retinol-deficient group (baseline). Data represent the mean ± SD, $n = 5$. Groups not sharing a common letter are significantly different ($p < 0.05$), as determined by one-way ANOVA, followed by Tukey's test.

preventing, not only vitamin A deficiency and age-related macular degeneration-related disorders, but also may protect against chronic degenerative diseases, such as cancer and cardiovascular disorders and hence will be highly beneficial to the rural community.

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