Short Communication

Contribution of selected wild and cultivated leafy vegetables from South India to lutein and β-carotene intake

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Carotenoids, especially lutein and β-carotene, offer benefits to human health in general and to eye health in particular. However, more data on the contribution of plant foods to carotenoid intake is of importance for developing strategies for promoting eye health in regions where cataract is highly prevalent such as in South India. The most frequently consumed 5 uncultivated and 5 commercially grown South Andhra Pradesh leafy vegetables were selected based on interviews with 100 local women. The lutein and β-carotene contents of fresh and cooked samples were determined using reversed-phase high performance liquid chromatography. Lutein values ranged from 53 to 143 μg/g and 58 to 175 μg/g in fresh and cooked samples, respectively. β-carotene contents were found to range from 45 to 119 μg/g in fresh samples and from 40 to 159 μg/g in cooked samples. No significant difference was observed between the carotenoid contents of wild and commercially grown species. According to their reported frequency of consumption, the 10 species considered in this study contribute 40% of the daily recommended intake of β-carotene. This is the first report of lutein content in fresh samples of Celosia argentea L., Rumex vesicarius L., Digera maricata (L.) Mart., and Amaranthus cruentus L. as well as in cooked samples of all species included in this study.

Key Words: lutein, β-carotene, India, wild vegetables, cataract

INTRODUCTION

Benefits imparted by β-carotene in orange and dark green fruits and vegetables in preventing and treating xerophthalmia have long been established.1 Plant foods, especially green leafy vegetables (GLV), provide other carotenoids with promise in eye health.2,3 The xanthophyll lutein, found mostly in leafy vegetables, has been identified as an important protective agent in several in vitro assays, epidemiologic studies and intervention trials examining plant food consumption and prevention of age-related cataract and macular degeneration.4 Antioxidant activity and absorbance of damaging blue and UV light constitute likely mechanisms of action.5

GLV are the major sources of lutein and, in developing countries where access to animal food is restricted, contribute substantially to fighting retinol deficiencies by being rich sources of the provitamin A β-carotene, notwithstanding bioavailability issues.6 As leafy vegetables are widely available and easy to gather from the wild or in agro-ecosystems, or may be cultivated at low cost, their consumption and conservation is being promoted for increased health benefits.7 However, epidemiologic studies on eye health and nutrition tend to overlook the contribution to diet of wild or less common species.8

This work is part of a multidisciplinary project to document the importance of GLV consumption in the prevention of cataract in women living in Madanapalle, Andhra Pradesh, involving an ethnobotanical survey and hospital-based case-control study. The objective of this analysis is to quantify carotenoids in common, local cultivated and wild GLV; and to estimate the contribution of these vegetables to daily lutein intake in local women. Because of its recognized importance for eye health, β-carotene content is also examined.

In accordance with local culinary habits in which leafy vegetables are mostly consumed cooked, carotenoid values...
are reported for both fresh and cooked leafy vegetables. The effect of cooking on the retention of carotenoids is well documented and is not tested in this study.9-11

MATERIALS AND METHODS

Dietary intake

Ten local species of GLV were selected based on their frequency of consumption determined in ethnobotanical interviews and food frequency questionnaires conducted with 100 women randomly selected from 20 villages in the surroundings of the Madanapalle sub-district (Mandal) from September to December 2007. Villages were selected following an opportunistic sampling provided by the community outreach program of the Siloam Eye Center. Dietary intake was determined by administering a food frequency questionnaire among participants. Data are expressed as servings per person per week. With 78 24-hour recalls, one average portion was estimated to contain 20 g of fresh leaves, taking into account the variability between different recipes. Subject informed consent was sought before the interview and patient anonymity was respected. Ethics approval was obtained from the Human Subjects Ethics committee of McGill University and the Human Research Project Review Board of the L.V. Prasad Eye Institute, Hyderabad, Andhra Pradesh, to which the Siloam Eye Center is affiliated.

Plant material

The ten plant species with highest availability, frequency of consumption and unknown carotenoid contents are listed in Table 1. Five leafy vegetables among those are uncultivated, and the remaining are cultivated in small-scale farms. For each species, approximately 200 to 500 g of plant material was collected in three different locations (different gathering areas or cultivated in fields from different regions). Each sample was analyzed separately upon arrival in the laboratory. Plants were rinsed with distilled water, dried with absorbing paper and the composite sample of leaves without stems was divided into two portions, one to be analyzed fresh and the other after boiling for 5 minutes and draining the water. Before extraction each sample was homogenized in a household blender. Voucher specimens of each species were pressed and dried and identification was confirmed, with the collaboration of Dr. T. Pullaiah (Sri Krishnadevaraya University Herbarium, Anantapur).

Chemicals and standards

HPLC grade acetone, acetonitrile, methanol and ethyl acetate plus diethyl ether, petroleum ether, sodium sulfate and sodium chloride were purchased from Merck Ltd (Mumbai, India). Acetone and triethylamine (TEA) were purchased from S.D. Fine Chemicals Ltd (Mumbai, India) and SRL (Mumbai, India), respectively. Lutein and β-carotene standards were obtained from Sigma-Aldrich (Mississauga, Canada) and zeaxanthin was obtained from Extrasynthese (Lyon, France).

Carotenoid extraction

The procedure is described in Rodriguez-Amaya et al.12-13 In brief, approximately 3 g of fresh or cooked homogenized sample was weighed and ground with mortar and pestle in Celite and acetone. Acetone was filtered through a sintered disk glass funnel mounted on a suction flask with the solid residue regrown in acetone until complete discoloration of the material (usually 2-3 repetitions). The acetone fraction was then partitioned to 50 ml of diethyl ether and petroleum ether [1:1 v:v] and washed 5 times with distilled water, sometimes with addition of 2-3 g of sodium chloride if an emulsion formed. After removing all water, the remaining diethyl ether and petroleum ether fractions were dried over a sodium sulfate bed and evaporated with a rotary evaporator (Superfit Continental Pvt Ltd, Mumbai, India). The last 1-2 ml of solvent was evaporated under nitrogen gas and the extracted sample was stored at -20°C until HPLC analysis for a maximum

Table 1. Cultivation status, botanical families, scientific and Telugu names, yearly availability and dietary intake among Madanapalle women (n=100) of the selected leafy vegetables species

<table>
<thead>
<tr>
<th>Cultivation status</th>
<th>Botanical family</th>
<th>Scientific name</th>
<th>Telugu name</th>
<th>English name</th>
<th>Availability</th>
<th>Dietary intake (serv/pers/wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncultivated</td>
<td>Amaranthaceae</td>
<td>Allmania nodiflora (L.) R. Br.</td>
<td>Errabadaku</td>
<td>NA</td>
<td>Jul – Mar</td>
<td>1.28 (1.30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alternanthera sessilis (L.) R. Br.</td>
<td>Ponnagantiaku</td>
<td>Sessile joyweed</td>
<td>Aug-Jan</td>
<td>0.64 (0.89)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amaranthus viridis L.</td>
<td>Dantu aku</td>
<td>Slender amaranth</td>
<td>All year</td>
<td>0.77 (1.28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Celosia argentea L.</td>
<td>Gurugu aku</td>
<td>Silver cock's comb</td>
<td>Jul-Dec</td>
<td>1.58 (1.27)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Digera muricata (L.) Mart.</td>
<td>Chenchaliaku</td>
<td>False amaranth</td>
<td>All year</td>
<td>0.32 (0.61)</td>
</tr>
<tr>
<td>Cultivated</td>
<td>Amaranthus cruentus L.</td>
<td>Thota aku</td>
<td>Red amaranth</td>
<td>All year</td>
<td>0.41 (0.84)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amaranthus tricolor L.</td>
<td>Sirri aku</td>
<td>Joseph's-coat</td>
<td>All year</td>
<td>0.54 (0.78)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chenopodiaceae</td>
<td>Chenopodium album L.</td>
<td>Chakrantaaku</td>
<td>Lambsquarter</td>
<td>All year</td>
<td>0.33 (0.68)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hibiscus cannabinus L.</td>
<td>Gongura</td>
<td>Brown Indian hemp</td>
<td>All year</td>
<td>0.35 (0.67)</td>
</tr>
<tr>
<td></td>
<td>Polygonaceae</td>
<td>Rumex vesicarius L.</td>
<td>Chukka aku</td>
<td>Bladder dock</td>
<td>All year</td>
<td>0.60 (0.79)</td>
</tr>
</tbody>
</table>

1 According to Pullaiah et al.49 and our observations.
2 Intake is expressed as mean servings/person/week with standard deviation.
duration of 1 week. The samples were not saponified as this treatment may alter the quantity of carotenoids and is not necessary in this case where the carotenoids of interest are separated from the chlorophylls. All manipulations were conducted under dim laboratory light and glass material was covered with foil.

**Analytical methodology**

Separation was performed on a Shimadzu model HPLC coupled with a tertiary pump LC-10AT, a SPD-10A UV-VIS detector and a column thermostat. The integration system was Class-VP version 7. The reversed-phase Phenomenex C18 5µ [250x4.60 mm i.d.] column was kept at 25°C. Solvent composition was modified according to Kimura and Rodriguez-Amaya for a tertiary pump system: solvent A contained acetonitrile and 0.05% TEA and solvent B methanol: ethyl acetate [1:1]. The selected flow rate was 1.0 ml/min. The initial proportion of solvent A and B was 95:5 increasing to 60:40 in 15 min following a concave gradient and the proportion was maintained until the end of the run (60 min). Re-equilibration took 15 min. Immediately before injection the sample was rediluted in 10 ml HPLC grade acetone, 1.5 ml were filtered through a 0.22 µ PTFE Millipore filter to a HPLC vial and 10 µl were injected in the system. Detection was performed at 450 nm.

Identification of lutein and β-carotene was carried out by comparison of the HPLC retention times with corresponding standards and co-chromatography with added standards. As major carotenoid patterns are highly constant in leafy vegetables these procedures are sufficient to confirm the identification of the compounds for this validated analytical method. Our method did not separate zeaxanthin from lutein, so values are reported together. However, it should be noted that GLV contain only trace amounts of zeaxanthin. Values are reported for lutein and all-trans β-carotene.

Standard curves were constructed for external quantification using lutein isolated by open-column chromatography from groundnut leaves and commercial β-carotene standard purchased from Sigma. Purity, verified with HPLC for the isolated lutein and the commercial β-carotene, was 93% and 97% respectively. The concentrations of the standards were determined spectrophotometrically, using the following absorption coefficient values A¹%λ: β-carotene, 2592 in petroleum ether; and lutein, 2550 in ethanol. Concentrations were corrected accordingly. The curves were constructed in triplicate at 3 and 4 different concentrations for β-carotene and lutein, respectively. The curves were linear, passed through the origin and their correlation coefficient were higher than 0.98.

**Statistical analysis**

The means and standard deviation in µg/g were reported for fresh or cooked samples. One-way ANOVA analyses were used to test for difference between contents in non-cultivated and in cultivated GLV. Wilcoxon rank-sum test was used to compare consumption of cultivated and non-cultivated GLV. Statistical analyses were conducted using R statistical software version 2.9.0.

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*Figure 1. HPLC chromatograms of Allmania nodiflora (L.) R. Br. fresh (A) and cooked (B). Chromatographic conditions are described in the text. Peak identification: 1: neoxanthin, 2: violaxanthin, 3: lutein, 4: chlorophyll b, 5: chlorophyll a, 6: β-carotene.*
RESULTS

Dietary intake

Celosia argentea and Allmania nodiflora were the two species most frequently consumed. In total the weekly average number of GLV servings per person was 6.81±3.91, comprised of 4.59±3.00 servings/person/week of uncultivated species and 2.22±2.14 of cultivated ones. There was a significant difference between the number of servings of cultivated and uncultivated GLV (p < 0.01) (Table 1).

Qualitative analysis

According to the standard co-chromatography and retention times, lutein (β,ε-carotene-3,3'-diol; tR = 9.6 min) and β-carotene (β,β-carotene; tR = 37.5 min) were identified. A typical GLV carotenoid chromatogram is presented in Figures 1A and 1B (fresh and cooked A. nodiflora). The elution patterns of all GLV were very similar, with the exception of Hibiscus cannabinus and Rumex vesicarius, which showed different patterns when fresh and cooked.

Fresh leafy vegetables

The concentrations obtained for A. nodiflora, Alternanthera sessilis, Amaranthus cruentus, Amaranthus tricolor, Amaranthus viridis, C. argentea, Chenopodium album, Digera muricata, H. cannabinus and R. vesicarius ranged from 53 to 143 μg/g for lutein and from 45 to 119 μg/g for β-carotene (Table 2).

Our results for A. viridis compare well with that of Kobori and Rodriguez-Amaya as well as Liu and col-

![Table 2. Lutein and β-carotene contents (μg/g) of fresh and cooked leafy vegetables compared with values obtained from other studies reported in μg/g fresh weight](image)

1Values are reported as the mean of samples from three different locations with standard deviation. 2Lutein only. 3Fresh weight basis.
leagues who report 119 and 147 µg/g respectively.\textsuperscript{11,17} For the same species, Tee and Lim report 42 µg/g after saponification and both Raju et al. and Lakshminarayana et al., from the same laboratory, reported 904 µg/g on a dry weight basis which does not allow comparison.\textsuperscript{18-20} Kumar et al. reported 1850 µg/g for C. album, also on a dry weight basis.\textsuperscript{21} Wills and Rangga and Kidmose et al. reported 29 and 23 µg/g for A. tricolor (syn. A. mangostanus and A. gangeticus).\textsuperscript{22,23}

Previous HPLC analyses yielded values for β-carotene contents of 57, 86, 11 and 12 µg/g for A. sessilis, A. tricolor, A. viridis and C. argentea.\textsuperscript{24} Our results also compare well with Kobori and Rodriguez-Amaya who reported 114 µg/g in A. viridis, and Rajyalakshmi et al. with values ranging from 56 to 90 µg/g (table 2).\textsuperscript{17,19} Wills and Rangga found a lower value of 20 µg/g in A. tricolor.\textsuperscript{25} A number of factors influence the carotenoid concentration among species, including timing of collection, seasonality, climate, growing conditions, geographic location, varieties (genetic variation) and cultivars, and may explain the variation among results.\textsuperscript{10-13}

**Cooked GLV**

The means obtained for A. nodiflora, A. sessilis, A. cruentus, A. tricolor, A. viridis, C. argentea, C. album, D. micricta, H. cannabinus and R. vesicarius range from 58 to 175 µg/g for lutein and 40 to 159 µg/g of β-carotene in the cooked samples (Table 2).

Although no lutein values for cooked samples have been reported for the species of interest in this study, our results compare well with the USDA nutrient database for boiled kale (Brassica oleracea var. acephala DC.), spinach (Spinacia oleracea L.), turnip (Brassica rapa subsp. rapa L.), collards (Brassica oleracea var. viridis L.), mustard (Brassica juncea (L.) Czern.) and dandelion (Taraxacum officinale G.H. Weber ex Wiggers) greens, with reported lutein values of 182, 113, 84, 77, 60 and 47 µg/g (cooked weight basis).\textsuperscript{26} During the cooking process, at least two mechanisms contribute to the decrease, increase or lack of modification in the concentration of carotenoids, namely the disruption of the food matrix and the consequent release of water and/or carotenoids and the degradation of the heat-labile carotenoids.\textsuperscript{10}

Values ranging from 27 to 67 µg/g for β-carotene reported for cooked species by Rajyalakshmi et al. are different from ours because they are expressed on fresh weight instead of cooked weight basis.\textsuperscript{9}

**Contribution to total β-carotene and lutein intake**

Daily intakes of β-carotene and lutein were calculated on the basis of 20 g fresh leaves portions and reported frequencies (Table 1). The selected species contribute to 1489 µg/day of β-carotene (980 µg from non-cultivated GLV) and 1788 µg/day of lutein (1186 µg from non-cultivated GLV). One-way ANOVA analyses of the results obtained between cultivated and non-cultivated GLV showed no significant differences.

**DISCUSSION**

Cataract is a condition affecting the transparency of the crystalline lens. It is the main cause of blindness in India and affects particularly women from rural areas.\textsuperscript{26} In mammalian systems carotenoids originate exclusively from the diet. Lutein and zeaxanthin are the only carotenoids found in the human lens where their concentrations range between 15.1 to 44.1 ng/g of wet weight.\textsuperscript{27-28} In both the lens and the macula lutea the two xanthophylls are responsible for blue light absorption and antioxidant protection which are the proposed mechanisms for their protective role against cataract.\textsuperscript{5}

A number of studies in Western countries have demonstrated an inverse relationship between leafy vegetables consumption, especially lutein-rich species like spinach and kale,\textsuperscript{15,25} and the risk of developing cataract.\textsuperscript{29-34} However, no such studies have previously been conducted in India. To demonstrate the preventive effect of leafy vegetables consumption in countries where cataract is of high prevalence and occurs early in life, more data is needed on the carotenoid content of local foods. Increasing intake of leafy vegetables and associated carotenoids may constitute efficient strategies for promoting eye health and reducing the burden of cataract. In this study, we have determined the lutein and β-carotene concentrations of commonly consumed leafy vegetables of South Andhra Pradesh, thus providing useful data to further evaluate the contribution of wild and cultivated plant foods to cataract prevention.

Nutritional surveys tend to overlook the contribution of wild foods, GLV and varieties within species,\textsuperscript{35-36} which may represent important sources of nutrients, including lutein and β-carotene. In our study, we found the carotenoid profile and contents of uncultivated leaves to be similar to the cultivated ones, confirming their important contribution to health and nutrition, and supporting their inclusion in nutritional and epidemiological surveys. Wild A. viridis and commercially available H. cannabinus had similar contents of lutein and β-carotene when fresh and cooked, and both compare well with globally available raw spinach (122 and 56 µg/g lutein and β-carotene, respectively).\textsuperscript{22} In addition, within the selected species the uncultivated leaves were more frequently consumed on a weekly basis than the commercially grown ones, thus contributing further to carotenoid intake.

On a daily basis, the reported consumption of the 10 selected GLV species provides 1489 µg of β-carotene, contributing over 40% of the daily intake recommended by WHO/FAO.\textsuperscript{37} To date, there is no recommendation for lutein intake. The reported intake attributable solely to the selected species in this study is 1788 µg/day. In the United States, various studies with women populations reported daily lutein intakes of 1832, 1300, 1860, 4404 and 1232 µg respectively.\textsuperscript{38-42} Johnson-Down et al. estimated that Canadian women (18-65 years old) consumed 1382 µg/day of lutein.\textsuperscript{43} Lutein intake in Europe was found to be 3250, 2500, 1590,1560 and 2010 µg/day in Spain, France, United Kingdom, Ireland and the Netherlands, respectively (male and female intakes not statistically different and reported together).\textsuperscript{44} Recently, Hamula et al. found a population of Polish women who consume 2160 µg/day of lutein.\textsuperscript{45} In Asia, Zhang et al. estimated the lutein consumption of a Chinese women population to be 1810 µg/day.\textsuperscript{46} To our knowledge, there is no published data on the lutein intake of Indian populations. However when compared with reported daily in-
takes in various countries, the 10 species of interest in this study contribute an important portion of the lutein consumed by the women of Madanapalle. Olmedilla et al. reported improved visual acuity and glare sensitivity in patients with cataract with approximately 7 mg of lutein supplementation/day.47 A portion of 100 g of either fresh *A. viridis* and *H. cannabinus* provides 14 mg of lutein. Moreover, 100 g of cooked *C. album or A. tricolor* will each contribute 18 mg of lutein with substantially increased bioavailability due to the disruption of the food matrix resulting from the cooking process.31,48

In conclusion, we have determined high contents of lutein and β-carotene in cultivated and wild leafy vegetable species commonly consumed by the women in the Madanapalle mandal. To our knowledge, this study is the first to report lutein values for fresh *C. argentea, R. vesicarius, D. muricata* and *A. cruentus* and for all cooked species, and β-carotene values for cooked *A. cruentus, C. album and R. vesicarius*. The reported concentrations can be used directly to estimate the contribution of either fresh or cooked species to lutein and β-carotene intake. One drawback of this work is the single time collection during the peak availability period. Based on these results, the selected cultivated and wild leafy vegetables are equally important sources of β-carotene and lutein. In a country such as India where cataract incidence is very high and occurs early in life, identifying strategies to help reduce or delay the burden of cataract is of primary importance. Increasing consumption and use of local GLV might be a valuable strategy. These data will be used in a population-based study to further evaluate the potential of leafy vegetables to contribute to cataract prevention in women from Madanapalle. This work providing evidence on the importance of local plant foods to health contributes to the implementation of Decision VIII/23A of the Convention on Biological Diversity (COP8, Curitiba, 2006), an initiative linking nutrition and food with global biodiversity conservation policy.8

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AUTHOR DISCLOSURES

Julie Bélanger, Mungara Balakrishna, Putta Latha, Shoba Katumalla and Timothy Johns, no conflicts of interest.

REFERENCES


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印度南部野生及栽種的多葉蔬菜對葉黃素及β-胡蘿蔔素攝取的貢獻

蘋胡蘿蔔素，尤其是葉黃素及β-胡蘿蔔素，對人體健康有益，並特別有助於眼睛健康。然而，在白內障高盛行率的地區，例如印度南部，為了促進眼睛健康而發展的策略中，有關植物性食物對類胡蘿蔔素攝取的貢獻之資料是非常重要的。在印度南安得拉邦(South Andhra Pradesh)地區，透過與 100 名當地婦女的面談，選出最常攝取的 5 種野生及 5 種商業栽種的多葉蔬菜。使用逆相高效液相層析儀定量新鮮及烹煮蔬菜中的葉黃素及β-胡蘿蔔素。在新鮮及烹煮後的樣本之葉黃素含量，範圍分別是 53-143 μg/g 及 58-175 μg/g。β-胡蘿蔔素含量在新鮮樣本是 45-119 μg/g，在烹煮樣本是 40-159 μg/g。比較野生與商業栽種的品種，胡蘿蔔素含量沒有顯著的差異。依據攝取頻率的報告，本研究選出的這 10 種蔬菜佔每日β-胡蘿蔔素建議攝取量的 40%。本文是第一個報告Celosia argentea L.、Rumex vesicarius L.、Digera muricata (L.) Mart. 和 Amaranthus cruentus L.之新鮮樣本的葉黃素含量，也是首篇報告所有 10 種蔬菜的烹煮樣本之葉黃素含量。

關鍵字：葉黃素、β-胡蘿蔔素、印度、野生蔬菜、白內障