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Chlorophyll and carotenoid contents of some green leafy vegetables

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Abstract

In the present study, the chlorophyll and carotenoid contents of *Alternanthera sessilis, Moringa oleifera, Sesbania grandiflora, Piper betle, Trigonella foenum-graecum* and *Spinacia oleracea* were estimated. It is found that *Sesbania grandiflora* has the highest chlorophyll-a, chlorophyll-b and total chlorophyll contents among the six green leafy vegetables followed by *Piper betle*. Among the six green leafy vegetables, the highest contents of carotenoid (38.765mg/100g f.w) were recorded in the leaves of *Moringa oleifera*.

Keywords: Carotenoid; Chlorophyll-a; Chlorophyll-b; Green leafy vegetables; Total chlorophyll

1. Introduction

According to the Food and Agriculture Organization's (FAO) report, about 70-80% of the world's population, especially in developing countries, relies on herbal medicine to prevent and cure diseases, and about 25% of the synthesized drugs are manufactured from medicinal plants [1].

Consumption of leafy vegetables is considered to be associated with several positive effects on health. It has been shown that low consumption of fruit and vegetables is related to more cardiovascular disease and cancer [2]. Diversified and highly nutritive vegetables are of great importance in alleviating malnutrition. The presence of phytochemicals, in addition to vitamins and pro-vitamins, in fruits and vegetables has been considered of crucial nutritional importance in the prevention of chronic diseases, such as cancer, cardiovascular disease and diabetes [3]. Green leaves have been used as medicine since ancient times and have been playing a very important role in our diet and nutrition. Green leaves are a rich source of vitamins such as beta carotene, ascorbic acid, folic acid and riboflavin as well as minerals such as iron, calcium and phosphorous. Many leafy vegetables especially, amaranth, fenugreek, palak and spinach have attained commercial status and their cultivation is widespread in India. Because of their low production cost and high yield, green leaves are considered to be one of the cheapest vegetables on the market and they could be rightly described as 'poor man's vegetables [4]. They are the most readily available sources of carbohydrates, fats, important proteins, vitamins, minerals, essential amino acids, and fibers [5]. Being a photosynthetic tissue, leafy vegetables have higher levels of vitamin K when compared with other fruits and vegetables due to the direct involvement of vitamin K (phylloquinone) in the photosynthesis process [6]. Leafy vegetables are a natural source of antioxidants and are rich in phytochemicals [7].

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Limited information is available on the medicinal properties associated with green leafy vegetable consumption in South India. Moreover, most botanical studies on green leafy vegetables concentrate on wild and weedy species and do not take cultivated and managed species into account [8]. By documenting the traditional knowledge of South Indian green leafy foods and by describing their importance in terms of consumption, we highlight health and secondary metabolites with potential impacts. In this study, the green leafy vegetables like *Alternanthera sessilis*, *Moringa oleifera*, *Sesbania grandiflora*, *Piper betle*, *Trigonella foenum-graecum* and *Spinacia oleracea* were collected from Mappillaiyurani village, Tuticorin district, Tamil Nadu, India to assess the phytoneutraceutical.

Chlorophylls are light-harvesting pigments essential to the photosynthetic process. Photosynthetic pigments are present in the form of porphyrin pigments (chlorophyll a, b and c), carotenoids, anthocyanins and flavones. Chlorophyll is an antioxidant compound which is present and stored in the chloroplast of green leaf plants and mainly it is present in the green area of leaves, stems, flowers and fruits [9]. The chlorophyll can act as nutrition in declining blood sugar conditions, detoxification, digestion, excretion and decreasing allergens [10]. Reports on traditional medicinal uses of chlorophyll in alternative forms of medicine are known for ages. Nowadays chlorophyll has been used in the field of medicine as a remedy and diagnostics. Chlorophyll molecules are used in pharmacy as a photosensitizer for cancer therapy. Their roles as modifiers of genotoxic effects are becoming increasingly important, besides it being known to have multiple medicinal uses. Research and developments of medicinal uses of chlorophyll were reviewed along with challenges of potential applications of chlorophyll and its derivatives as chemotherapeutic agents [11]. Because of the therapeutic potential of chlorophylls, in the present study, the chlorophyll contents of the selected green leaves were assessed.

Fiedor and Burda [12] highlighted the beneficial (protective) effects of dietary carotenoid intake in exemplary widespread modern civilization diseases, i.e., cancer, cardiovascular or photosensitivity disorders, in the context of carotenoids' unique antioxidative properties. Hence, in the present investigation, the carotenoid contents of the selected green leaves were evaluated.

2. Material and methods

Healthy fresh green leaves of *Alternanthera sessilis* (L.) R.Br. ex DC., *Moringa oleifera* Lam., *Sesbania grandiflora* (L.) Poir., *Piper betle* L., *Trigonella foenum-graecum* L. and *Spinacia oleracea* L. were collected from the Mappillaiyurani village, Tuticorin district, Tamil Nadu, India and were used for the present study.

2.1. Estimation of Chlorophyll

Healthy fresh green leaves of *Alternanthera sessilis*, *Moringa oleifera*, *Sesbania grandiflora*, *Piper betle*, *Trigonella foenum-graecum* and *Spinacia oleracea* were collected and 1g of each was weighed and finely cut. For the extraction of chlorophyll, 1g of finely cut fresh leaves were ground to a fine pulp with the addition of 20ml of 80% acetone with a mortar and pestle. This paste was then centrifuged for 5min at 5000rpm. The supernatant was transferred to a 100ml beaker. The residue was then ground with 20ml of 80% acetone, centrifuged for 5min at 5000rpm and the supernatant was transferred to the same beaker. This process was repeated four times till the residues became almost colourless. The inside of the mortar and pestle were also washed with 80% acetone and the clear washings were also collected in the beaker. The volume was made up to 100ml with 80% acetone. The absorbance of the extract solutions was read at 645 and 663nm against the solvent (80% acetone) blank. The average value of triplicate determination was expressed as mg of chlorophyll per gram of the leaf tissue. The amount of chlorophyll present in the extract *i.e.*, mg of chlorophyll present per gram of tissue was calculated using the following equations [13].

- mg of chlorophyll-a per gram of tissue = $[12.7\ (A_{663}) - 2.69\ (A_{645})] \times V \div (1000 \times W)$
- mg of chlorophyll-b per gram of tissue = $[22.9\ (A_{645}) - 4.68\ (A_{663})] \times V \div (1000 \times W)$
- mg of total chlorophyll per gram of tissue = $[20.2\ (A_{645}) + 8.02\ (A_{663})] \times V \div (1000 \times W)$

Here, A= absorbance at specific wavelengths, V= final volume of chlorophyll extract in 80% acetone which in this case is 100ml and W= fresh weight of tissue extracted which is 1g.

2.2. Estimation of Carotenoids

Absorbance values of the 80% acetone pigment extract at 480, 647 and 666nm were used to find the corrected optical density (OD) for carotenoids which was used for further calculations [14].

$$\text{Corrected OD} = A_{480} + [(0.114 \times A_{666}) - (0.638 \times A_{647})]$$

$$\text{Carotenoid (mg g}^{-1}\text{ f.w)} = \text{Corrected OD x } \frac{1}{100} \text{ x } \frac{V}{W}$$

Where, A_{480} = Absorbance at 480nm, A_{647} = Absorbance at 647nm, A_{666} = Absorbance at 666nm, V = volume of the extract in ml and W = fresh weight of the sample in g.

3. Results and discussion

In the present study, six locally available green leafy vegetables such as *Alternanthera sessilis*, *Moringa oleifera*, *Sesbania grandiflora*, *Piper betle*, *Trigonella foenum-graecum* and *Spinacia oleracea* were selected for phytonutraceutical evaluation. The amount of chlorophyll-a, chlorophyll-b and total chlorophyll were estimated and the data were shown in Table 1.

Table 1 Data on chlorophyll contents of the plant samples

Plant name	Chlorophyll-a (mg/g f.w)	Chlorophyll-b (mg/g f.w)	Total chlorophyll (mg/g f.w)
Alternanthera sessilis	0.3879 ± 0.3	0.1535 ± 0.8	0.5414 ± 0.5
Moringa oleifera	0.5864 ± 0.2	0.3031 ± 0.5	0.8893 ± 0.4
Sesbania grandiflora	0.9029 ± 0.8	0.3705 ± 0.1	1.2731 ± 0.5
Piper betle	0.8501 ± 0.3	0.2290 ± 0.1	1.0796 ± 0.1
Trigonella foenum-graecum	0.2481 ± 0.2	0.1894 ± 0.5	0.4374 ± 0.5
Spinacia oleracea	0.6820 ± 0.3	0.2328 ± 0.2	0.9148 ± 0.6

a – values of mean ± standard error of the mean of three determinations f.w - fresh weight

The highest amount of chlorophyll-a, chlorophyll-b and total chlorophyll were recorded in *Sesbania grandiflora* followed by *Piper betle*. Thus, they can be used as a relatively cheap and very easily available source of chlorophyll instead of chlorophyll supplements for availing the health benefits of chlorophyll compounds. Green leaves are an integral part of Indian cuisine. Thus, incorporating it into our diets will aid in the growth and repair of tissues and also possess anti-mutagenic and anti-carcinogenic properties to a certain extent as it is a very good chelating agent due to its physical and chemical structure. Thus, we don't have to rely on external chemicals or supplements for these benefits in keeping our body healthy and for increasing our immunity. Also, they stimulate red blood cells in the uptake of oxygen and efficiently deliver magnesium ions to our bodies. All these health benefits can easily be availed while utilizing the medicinal values also. However, the presently investigated green leafy vegetables are found to contain a lower amount of chlorophyll-a chlorophyll-b and total chlorophyll than that of *Ocimum tenuiflorum*, *Azadirachta indica*, *Murraya koenigii* and *Mentha arvensis* [15].

Table 2 Data on carotenoids, carotenes and β carotenes contents of the plant samples

Plant name	Carotenoids (mg/100g f.w)
Alternanthera sessilis	24.206
Moringa oleifera	38.765
Sesbania grandiflora	36.087
Piper betle	13.735
Trigonella foenum-graecum	12.755
Spinacia oleracea	9.553

a – values of mean ± standard error of the mean of three determinations f.w. – fresh weight

Because of the health benefits of the addition of carotenoids in the diet, in the present study, six green leafy vegetables like *Alternanthera sessilis*, *Moringa oleifera*, *Sesbania grandiflora*, *Piper betle*, *Trigonella foenum-graecum* and *Spinacia*

oleracea were collected from the Mappillaiyurani village, Tuticorin district and were investigated for carotenoids and their data were shown in Table 2. Among the six green leafy vegetables, the highest contents of carotenoids (38.765mg/100g f.w) were recorded in the leaves of *Moringa oleifera*.

4. Conclusion

After estimating the chlorophyll content of *Alternanthera sessilis*, *Moringa oleifera*, *Sesbania grandiflora*, *Piper betle*, *Trigonella foenum-graecum* and *Spinacia oleracea* by using spectrophotometric methods using acetone as the solvent, it is found that *Sesbania grandiflora* has the highest chlorophyll-a, chlorophyll-b and total chlorophyll contents among the six green leafy vegetables followed by *Piper betle* and thus these leaves can be utilized to avail the health benefits of chlorophyll compound along with their other medicinal benefits. They are also easily available and inexpensive. Among the six green leafy vegetables, the highest contents of carotenoid (38.765mg/100g f.w) were recorded in the leaves of *Moringa oleifera*.

Compliance with ethical standards

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Disclosure of conflict of interest

All the authors hereby disclose no conflict of interest.

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