

Extraction and Spectrophotometric Determination of Chlorophyll Content and Carotenoids from *Cocos nucifera L.* Leaf using Various Solvents in Saurashtra Region

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ABSTRACT

Chlorophyll and carotenoids are significant photosynthetic pigments present in higher plants, algae and cyanobacteria. Plants contain three major pigments called chlorophyll a, chlorophyll b, and chlorophyll c. Chlorophyll participates in the photosynthesis process, which transforms light energy into chemical energy. Species differences in chlorophyll pigment concentration exist. Orange, yellow and vivid red colors in vegetables and fruits are due to carotenoids. The health of plants is significantly influenced by these two pigments. In addition to having excellent antioxidant qualities, chlorophylls and carotenoids show therapeutic effects on oxidative and inflammatory disorders. They also possess anti-cancer effects. Chlorophyll and carotenoids support healthy blood coagulation and help in protecting skin, hormonal balancing, and deodorization. The major objective of this study is to identify plant with a high content of carotenoids and chlorophyll since both of these bioactive compounds have several applications in herbal medicine. In the current work, carotenoids and chlorophyll were extracted from three varieties of *Cocos nucifera L.* – Dwarf, DT and tall of Veraval coast of Saurashtra region, Gujarat using DMSO, methanol, diethyl ether and 80% acetone. The amounts of chlorophyll and carotenoids were measured using a spectrophotometer. Maximum extraction of chlorophyll and carotenoids was found in methanol and DMSO from the leaves of the dwarf variety of *C. nucifera L.*

Keywords: Chlorophyll, Carotenoid *Cocos nucifera*, Pigment study, Spectrophotometric analysis, Saurashtra

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INTRODUCTION

The color of any plant (vegetable or fruit) is due to the presence of different pigments in them. These pigments are chlorophylls, carotenoids, lycopene, anthocyanins, etc. Amongst these, chlorophylls are important in the photosynthesis process (Sudhakar *et al.*, 2016). All tissues of photosynthetic plants have chloroplasts, which contain the green pigment known as chlorophyll (Beale, 2005). Chloroplast contains enzymes necessary for the manufacture of chlorophyll (Brzezowski *et al.*, 2015; Sim Choo, 2018; Tanaka and Tanaka, 2007). Chemically, chlorophyll belongs to the porphyrin group that possesses a tetrapyrrole nucleus coordinated with a magnesium ion at the center linked by methylene groups as bridges. There are five different varieties of chlorophylls; the higher plants, mosses, and ferns include chlorophyll a and b, whilst certain bacteria and algae contain chlorophyll c, d, and e.

Carotenoids give red, yellow and orange colors to vegetables, fruits and flowers (Britton and George, 1995; Maoka, 2020). These are lipid-soluble pigments, located in the chloroplast of plants, that are crucial for photosynthesis (Goodwin, 1986; JaeHwan Lee & Steven J. Schwartz, 2005; Schieber & Weber, 2016). Xanthophylls and carotenes are the two classes into which carotenoids are divided. Hydrocarbons called α -carotene, β -carotene, γ -carotene and lycopene are examples of carotenes. Xanthophylls are carotenoids containing oxidized derivatives such as hydroxy, epoxide, aldehyde, and carbonyl as in lutein, peridinin, astaxanthin, fucoxanthin and others (Eskin & E. Hoehn., 2013; George Britton *et al.*, 2004).

The spectrophotometric method (UV-visible spectrometry) is employed for the quantitative analysis of pigments present in leaves using various solvents (Vaghela *et al.*, 2022). This gives

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the results based on the color of the substance that absorbs the light, along with the electronic transitions in various portions in the UV-visible spectrum (Ritchie, 2006) The coconut palms (*Cocos nucifera L.*), which belongs to the Arecaceae family found mostly in tropical climates. *C. nucifera L.* has gained much importance due to its commercial applications commercially as well as for domestic purposes. It is known to have a presence of many phytoconstituents like alkaloids, terpenoids, saponins, phenols, tannins, etc under different extracting solvents which possess many pharmacological actions. In the present analysis, the leaves of *C. nucifera L.* were extracted using various solvents, and their contents of chlorophyll a, chl b, and carotenoids were examined using UV-visible spectrophotometry (Rajalakshmi & Banu, 2015).

MATERIALS AND METHODS

Sample collection: The *C. nucifera L.* plant was taken from the Veraval area of Gujarat, India's Saurashtra province. Three

Table 1: List of Equations used for determining concentrations of chlorophyll-a, chlorophyll-b and total carotenoids in ($\mu\text{g/ml}$) using various extracting solvents by Spectrophotometer.

Extracting Solvent	Equation used	Reference
Methanol	Chl-a = $16.72A_{665.2} - 9.16A_{652.4}$ Chl-b = $34.09A_{652.4} - 15.28A_{665.2}$ $C(x+c) = (1000A_{470} - 1.63\text{Chl-a} - 104.96\text{Chl-b}) / 221$	(Lichtenthaler and Wellburn, 1983; Porra <i>et al.</i> , 1989; Sumanta <i>et al.</i> , 2014)
(DEE) Diethyl ether	Chl-a = $10.05A_{660.6} - 0.97A_{642.2}$ Chl-b = $16.36A_{642.2} - 2.43A_{660.6}$ $C(x+c) = (1000A_{470} - 1.43\text{Chl-a} - 35.87\text{Chl-b}) / 205$	
80% Acetone	Chl-a = $12.25A_{663.2} - 2.79A_{646.8}$ Chl-b = $21.5A_{646.8} - 5.1A_{663.2}$ $C(x+c) = (1000A_{470} - 1.82\text{Chl-a} - 85.02\text{Chl-b}) / 198$	
(DMSO) Dimethyl sulphoxide	Chl-a = $12.47A_{665.1} - 3.62A_{649.1}$ Chl-b = $25.06A_{649.1} - 6.5A_{665.1}$ $C(x+c) = (1000A_{480} - 1.29\text{Chl-a} - 53.78\text{Chl-b}) / 220$	

A = Absorbance, Chl-a = Chlorophyll a, Chl-b = Chlorophyll b, C(x+c) = Carotenoids

Table 2 : Chlorophyll-a, Chlorophyll-b, and carotenoids absorbance measurements using Spectrophotometer for different varieties of *C. nucifera* L. using various extracting solvents

Sample ID	Solvent	$A_{665.2nm}$ Chl a	$A_{652.4nm}$ Chl b	A_{470nm} C (x+c)
Plant 1		1.5563	1.8237	1.9819
Plant 2	Methanol	1.0603	1.2017	1.1294
Plant 3		1.0953	1.1342	1.1348
Sample ID	Solvent	$A_{660.6}$ Chl a	$A_{642.2}$ Chl b	A_{470} C (x+c)
Plant 1		0.5573	0.7625	0.7291
Plant 2	DEE	0.4444	0.5263	0.5701
Plant 3		0.5787	0.7061	0.7852
Sample ID	Solvent	$A_{663.2}$ Chl a	$A_{646.8}$ Chl b	A_{470} C (x+c)
Plant 1		0.6512	1.134	1.5931
Plant 2	80% Acetone	0.3793	0.5597	1.2117
Plant 3		0.6928	0.7201	0.736
Sample ID	Solvent	$A_{665.1}$ Chl a	$A_{649.1}$ Chl b	A_{480} C (x+c)
Plant 1		1.6201	1.9533	2.0845
Plant 2	DMSO	1.7673	1.8117	2.0046
Plant 3		1.5049	1.4681	1.3657

plant varieties were selected for the study. Plant 1 – Dwarf variety, Plant 2 – DT hybrid variety and plant 3 – Tall variety. The leaves were properly cleaned with tap water, let to dry at room temperature in the shade, and then ground into a powder.

Extraction of Chlorophylls and Carotenoids: In 50 mg of powdered leaves of *C. nucifera* L. were taken and homogenized with 10 mL different extracting solvents like methanol, diethyl ether, 80% acetone and dimethyl sulphoxide. Centrifugation of the homogenized samples was performed at 25°C for 15 minutes at 1000 rpm. A UV spectrophotometric analysis was done to assess the green pigments chlorophyll a and b, and carotenoids in the supernatants after they had been combined with 5 mL of the extracting solvent. Chlorophyll a, b, and carotenoids were

measured at absorbance indicated in the equations as presented in Table 1 for various solvents (Lichtenthaler & Wellburn, 1983; Porra *et al.*, 1989; Sumanta *et al.*, 2014).

Quality control: The extraction technique employed AR grade (Merck) reagents. For dilution and solution formulation, milli Q water was employed. Quartz cuvette was used and pure solvent (or extractant used for the preparation of sample solutions) was taken as a reference for the spectrophotometric analysis. Table 2 displays the spectral absorbance of Chl-a (chlorophyll-a), Chl-b (chlorophyll-b), and carotenoids C(x+c) for varying solvents that are taken in triplicates. The equations used to compute the solvent-dependent concentrations of chlorophyll a, b, and carotenoids are provided in Table 1.

Table 3 : Calculation of Chlorophyll-a, Chlorophyll-b and Carotenoids ($\mu\text{g}/\text{ml}$) of *C. nucifera* L. in different solvents

Sample ID	Solvent	$A_{665.2}$ Chl a	$A_{652.4}$ Chl b	Total Chlorophyll	A_{470} C (x+c)
Plant 1		20.070	9.214	29.284	17.570
Plant 2	Methanol	12.562	6.319	18.881	11.352
Plant 3		11.427	10.121	21.547	15.088
Sample ID	Solvent	$A_{660.6}$ Chl a	$A_{642.2}$ Chl b	Total Chlorophyll	A_{470} C (x+c)
Plant 1		6.594	2.886	9.481	7.609
Plant 2	DEE	4.861	3.818	8.679	6.790
Plant 3		6.408	7.203	13.611	7.507
Sample ID	Solvent	$A_{663.2}$ Chl a	$A_{646.8}$ Chl b	Total Chlorophyll	A_{470} C (x+c)
Plant 1		12.306	5.727	18.033	11.201
Plant 2	80% Acetone	7.778	4.079	11.857	8.098
Plant 3		7.422	6.768	14.190	9.724
Sample ID	Solvent	$A_{665.1}$ Chl a	$A_{649.1}$ Chl b	Total Chlorophyll	A_{480} C (x+c)
Plant 1		19.652	14.503	34.155	15.412
Plant 2	DMSO	18.362	21.455	39.817	18.444
Plant 3		13.863	19.477	33.340	14.170

Using the formulae in Table 1 to calculate the concentrations of chlorophyll a, b, and carotenoids, the average quantification of each of these compounds in different solvents is presented in Table 3.

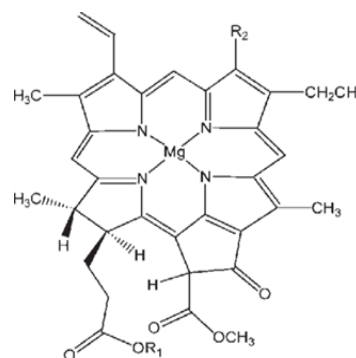
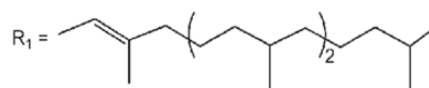
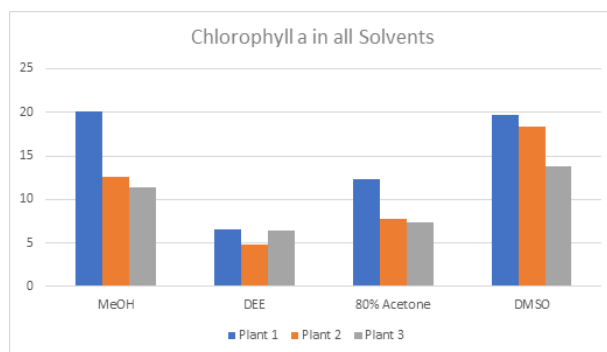
RESULTS AND DISCUSSION

Chlorophylls and Carotenoids are important bio constituents in plants that have its significance in herbal medicines. Chlorophyll a and Chlorophyll b both consist of hydrophilic and ionic Mg^{+2} ion at the centre of the porphyrin ring. They differ in one functional group attached in the side chain, where the $-\text{CH}_3$ group is present in Chlorophyll a and $-\text{CHO}$ group in Chlorophyll b as shown in Fig. 1 and Fig. 2. Carotenoids absorb light in the visible spectrum in the region 400 to 550 nm assist in the photosynthesis process in plants and also provide protection from any photodamage to chlorophyll (Butnariu, 2016; Costache *et al.*, 2012; Ghosh *et al.*, 2018; Giri *et al.*, 2013).

In the present investigation, Fig. 3A, 3B, 3C and 3D represent the concentration of chlorophyll-a, b, total chlorophyll and carotenoid concentrations in all the plant varieties of *C. nucifera* L. in different solvents. Fig. 4A, 4B, 4C and 4D show the concentration of chlorophyll-a, chl-b, total chlorophyll and carotenoids concentration of each plant variety in all four solvents.

The extraction of chl-a, chl-b, carotenoids and total chlorophyll in four different solvents for three plant varieties, plant 1-dwarf variety, plant 2-DT variety and Plant 3-Tall variety are found as,

Plant 1-Dwarf variety of C. nucifera L. shows chl-a and carotenoids extraction as: Methanol > DMSO > 80% Acetone > DEE and Chl-b and total chlorophyll shows as: DMSO > Methanol > 80%Acetone > DEE

**Fig. 1:** Structure of Chlorophyll-a and Chlorophyll-b**Fig. 2:** Structure of Carotenoids**Fig. 3A:** Chlorophyll-a concentration ($\mu\text{g}/\text{ml}$) in plant varieties of *C. nucifera* L. in different solvents

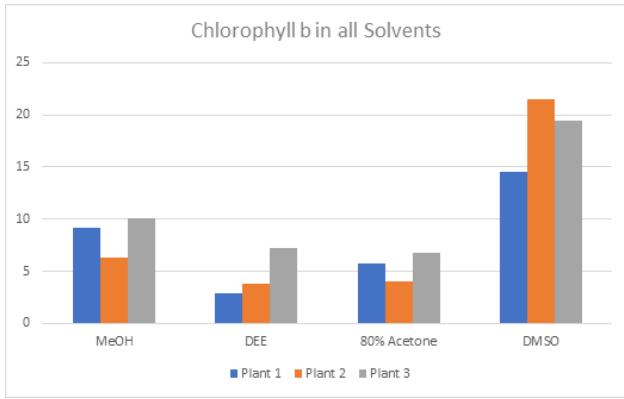


Fig. 3B: Chlorophyll-b concentration ($\mu\text{g/ml}$) in plant varieties of *C. nucifera* L. in different solvents

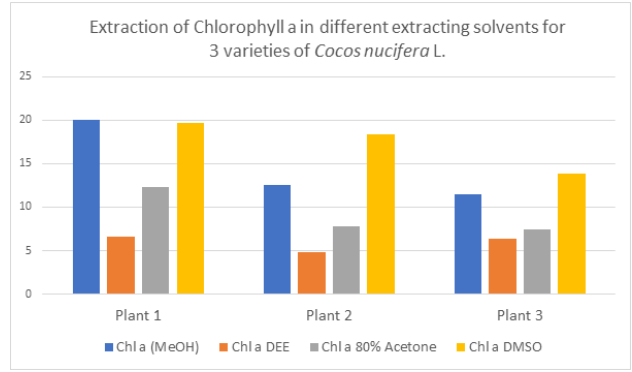


Fig. 4A: Chlorophyll a concentration ($\mu\text{g/ml}$) in all solvents for different plant varieties of *C. nucifera* L.

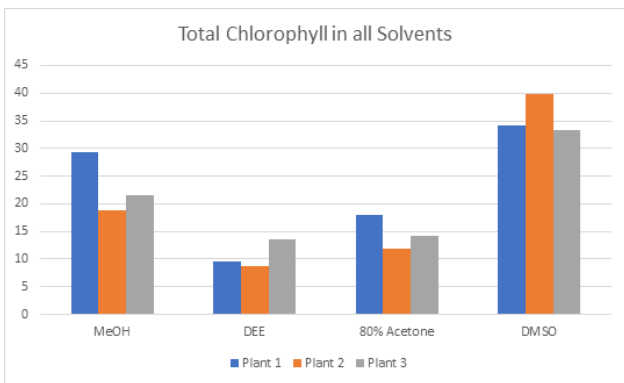


Fig. 3C: Total Chlorophyll concentration ($\mu\text{g/ml}$) in plant varieties of *C. nucifera* L. in different solvents

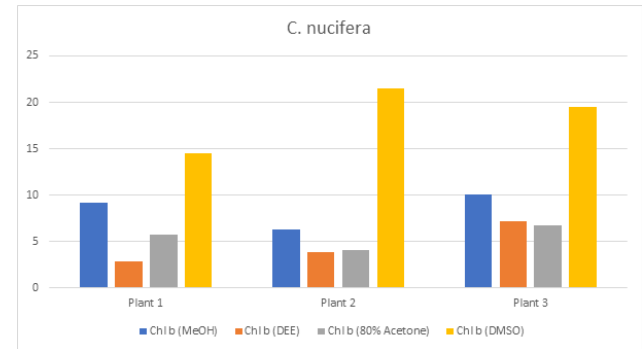


Fig. 4B: Chlorophyll b concentration ($\mu\text{g/ml}$) in all solvents for different plant varieties of *C. nucifera* L.

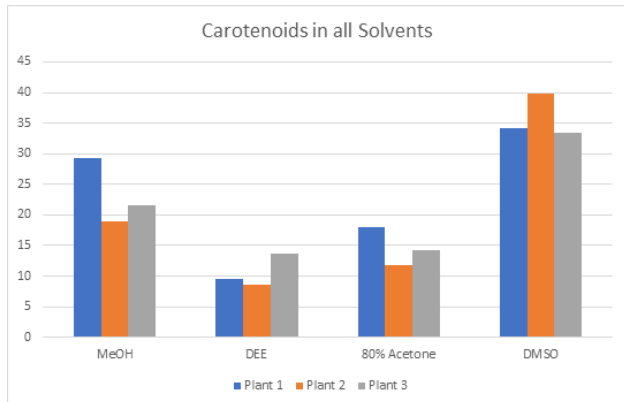


Fig. 3D: Carotenoids concentration ($\mu\text{g/ml}$) in plant varieties of *C. nucifera* L. in different solvents

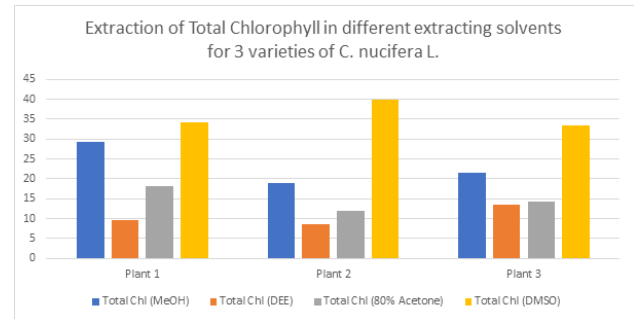


Fig. 4C: Total Chlorophyll b concentration ($\mu\text{g/ml}$) in all solvents for different plant varieties of *C. nucifera* L.

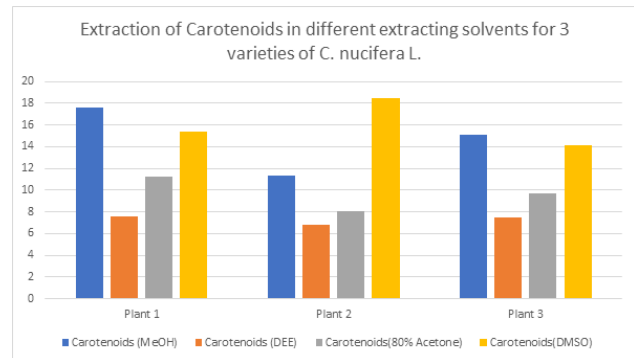


Fig. 4D: Carotenoids concentration ($\mu\text{g/ml}$) in all solvents for different plant varieties of *C. nucifera* L.

Plant 2-DT variety of *C. nucifera* L. exhibited maximum extraction in DMSO for chl-a, chl-b, carotenoids and total chlorophyll followed by methanol and 80% acetone and least extraction in DEE.

Plant 3-Tall variety of *C. nucifera* L. revealed extraction of chlorophyll-a, Chl-b and total chlorophyll as DMSO > Methanol > 80% Acetone > DEE whereas carotenoids showed Methanol > DMSO > 80% acetone > DEE.

The results show that maximum extraction was obtained in methanol and DMSO for chlorophylls and carotenoids both. Extraction of chlorophyll-a with methanol (20.070 µg/mL) shows high values in the dwarf variety compared to DT and tall variety. Chlorophyll-b content was found to be maximum in DMSO (14.503 µg/mL). Carotenoids showed maximum extraction value in DMSO (18.444 µg/mL) for DT variety. When using DEE, the extraction of carotenoids decreased for all the varieties, whereas the dwarf variety indicates the maximum content of carotenoids in methanol (17.570 µg/ mL).

CONCLUSION

Different extracting solvents are utilized for maximum extraction depending on the chemical makeup of the pigments (chlorophyll a and b, and carotenoids) found in plants. All three varieties of *C. nucifera* L. have the maximum amounts of chlorophylls and carotenoids in DMSO and methanol. The variations in pigment concentrations among plant species may be attributed to the physiological characteristics of various species, seasonal fluctuations, and geological environments. The current study demonstrates that all the types of *C. nucifera* L. have more chlorophyll a than chlorophyll b. For thorough studies in this area, more research is recommended.

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AUTHOR CONTRIBUTION

Analysis, Interpretation of data, and manuscript writing done by Jalpa Kotecha. Overall supervision, critical revision and review of paper approved by Vijay Ram.

CONFLICT OF INTEREST

Authors have no conflict of interest.

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