Analysis of Avocado Leaf, Casmir Leaf and Morenga Leaf for the Detection of Concentration of Chlorophyll a and Chlorophyll b

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Abstract hotosynthesis can be influenced directly by the concentration of chlorophyll and hence detection of such important pigment is the central point of the work under study. The objective of the work is determination of the concentration of leaf pigments particularly chlorophyll a and chlorophyll b. UV-Visible spectrophotometer is used to determine the absorbance of leaf samples obtained from three different plant species Shiferaw, Casmir and avocado; three replicate samples has been considered in each plant species. The samples collected in each species were preserved at -20^0C and dark area. The sample preserved under such a condition is weighed i.e.; 2g of each fresh leaf sample considered and crashed in 80% acetone and the extract was filtered with what man filter paper N$\underline{o}$42. After the dilution of the extract the absorbance was determined for chlorophyll **a** and chlorophyll **b** at 663nm and 649nm respectively. From the absorbance data; the corresponding concentration determined. According to the calculation concentration of chl a in shiferaw is 0.136mg/Kg in Casmir 0.229mg/Kg and 0.1144 mg/Kg in avocado. The concentration of chlorophyll b in avocado is 0.108mg/kg in shiferaw, 0.214mg/Kg and 0.102 mg/Kg in Casmir leaf sample. The concentration of chlorophyll a is greater than chlorophyll b which coincides with the fact that the abundance of chlorophyll a is greater than the chlorophyll b in most land trees.

Keywords Vocado, Casmir, Chlorophyll, Leaf Pigments, Photosynthesis, Shiferwa

1. Introduction

The most abundant plant pigments are chlorophyll a and b; their concentration can be varied because of the cells physiological state and composition. In most land plants there are typically two types of Chlorophyll (Chl) molecules, namely, chlorophyll **a**, $C_{55}H_{72}O_5N_4Mg$ and chlorophyll **b**, $C_{55}H_{70}O_6N_4Mg$. Both of these pigments absorb photons of light in the blue and red spectral regions, but the specific wavelengths of light they absorb are different. The maximum photons absorbance for chlorophyll a is at 663 nm and that of chlorophyll b is at 649 nm. Because the absorption spectra of these two Chlorophyll molecules overlap, simultaneous equation used to solve for the amount of both pigments. The equation for this has been worked out and is known as Arnon's equation and now a day modified to be Porras equation. Over the past seventy years, the extinction coefficients for the chlorophyll pigments have been refined and are now considered exceptionally accurate [1].

The kinds of plant species selected for the current study are Moringa (Shiferaw), Casmir and Avocado. The chlorophylls of these plants are therefore one of the most critical compositions to be concerned since it maintains natural equilibrium of ecological events in animal's kingdom and plants kingdom. In addition chlorophyll has a trend of capturing the coming sun light for the purpose of photosynthesis. These processes minimize the concentration of CO_2 affecting the total heat of the atmosphere by releasing useful O_2 as by product [2].

The objective of the study is determination of the concentration of chlorophyll a and chlorophyll b and compares the concentration of each pigment with one another.

The method is focused on the study of plants characteristics on the basis of their leaf composition using Uv-visible spectroscopy and the feedback of the work become critical in controlling and advancing the relationship between chlorophyll and environmental significance.

2. Materials and Methods

2.1 Reagents, Apparatuses and Instrumentation

The common reagents used in the work were 80% acetone

solution, distilled water and ethanol.

The common apparatuses are mortar and Pestle, Cuvette, UV-visible spectrophotometer (single beam), 100mLVolumetric flasks what man filter paper (NO42) and Filtering funnel.

2.2. Sampling and Sample Collection

The sample was collected from Tepi town located at about 611Km from Addis Ababa and the samples are Morenga (Shiferaw), Avocado and Casmir leaf. In each species three replicate and hence total of nine replicate samples have been collected randomly from the site. The samples were preserved under -20℃ and at dark area to avoid direct exposure to radiation [3].

2.3. Sample Preparation

Plants leaf cut into pieces(to discard veins) and 2g of each plant leaf was weighed and crashed in 10mL of 80% acetone solution (prepared by mixing 80mL of acetone into 20mL of distilled water) using mortar and pestle. The extract was filtered with what man filter paper No 42 to separate from residue material. UV-Spectrophotometer was turned on before 15 minutes to carry out analysis. For the purpose of calibration, part of 80% acetone was used as blank solution and its absorbance was determined at 663nm (maximum wave length of chl a) and the possible lowest absorbance reading of the method was recorded. Sample solution of each plant leaf diluted by the ratio of 1:10 (sample: 80% acetone) was prepared and analyzed for chl **a** and the corresponding absorbance of the samples was recorded at specified wave length. The spectrophotometer was recalibrated at 649nm (maximum wave length of chl b) with blank solution prepared from 80% acetone solution and its absorbance reading nearly become 0. The sample extract solution was diluted in the ratio of 1:10 (sample: solvent) and some of it was added into cuvette to measure the absorbance at 649nm for chlorophyll b [4].

3. Results and Discussion

3.1. Absorbance Measurement

The absorbance is the response of radiation that is absorbed in the medium or sample and hence it corresponds to the amount of analyte present in the sample and related by the Beer Lambert equation $A = 2-logT\%$ *or* $A = \varepsilon lc$, where A, T%, $\varepsilon, l\&c$ .The maximum wave lengths at which chlorophyll a analyzed was 663nm and that of chl b was 649nm. The absorbance of solution was determined in the order of blank and then sample. The collected absorbance was used to determine the corresponding concentration of sample as it is described in table 1.

Table 1.   Sample absorbance

Wave length	Morenga(shiferaw)	Casmir	Avocado
663nm	0.690	0.554	0.291
	0.680	0.563	0.293
	0.600	0.576	0.294
649nm	0.429	0.405	0.246
	0.431	0.408	0.251
	0.438	0.409	0.256

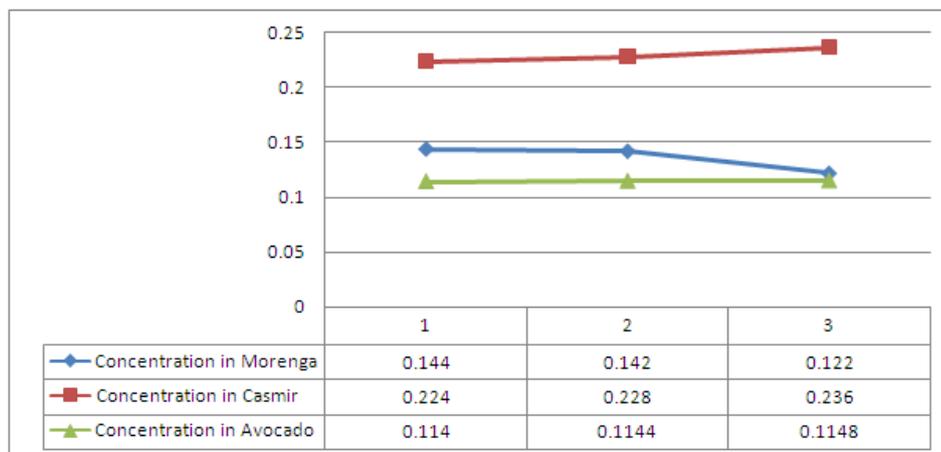|	1	2	3
Concentration in Morenga	0.144	0.142	0.122
Concentration in Casmir	0.224	0.228	0.236
Concentration in Avocado	0.114	0.1144	0.1148

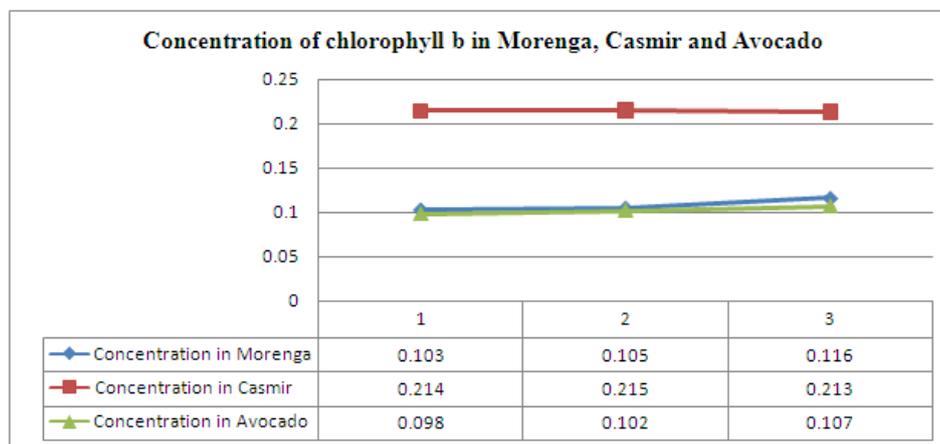**Figure 1**.   The concentration of chlorophyll a in Morenga, Casmir and Avocado leaf sample

Figure 2. The concentration of chlorophyll b in Morenga, Casmir and Avocado leaf sample

From table1 the absorbance reading are shown and in all the cases it is less than 0.7 and greater than 0.1. The absorbance in case of Morenga at 663nm and 649nm is higher than any of the plants studied **[6].**

Another fact that can be explained from the table is the relationship between the absorbance at 663nm and 649nm. At 663nm the absorbance become higher in all the samples than at 649nm, in other words chlorophyll **a** absorbed at smaller energy than chlorophyll **b** which may be explained in terms of arrangement of atoms in the molecules**[7].**

3.2. Concentration of Chlorophyll in Leaf Sample

The concentration of pigments was determined from the absorbance reading using the Beer-Lambert equation A = αlc relationship in coordination with Lichtenthaler and Welburn who reported accurate Beer-Lambert absorption coefficients for the major pigments of photosynthesis, allowing their concentrations to be easily determined from absorbance reading displayed by spectrophotometer[8].

- [Chlorophyll a](mgL^{-1}) = 12.21 (A663) - 2.81 (A649)
- [Chlorophyll b] (mgL^{-1}) = 20.13 (A649) - 5.03 (A663)

A663 and A649 are absorbance of chlorophyll "a" at 663nm and chlorophyll "b" at 649nm respectively. The concentration obtained by this method was multiplied by the reciprocal of dilution ratio i.e. by 10 since the extract was diluted by 1:10 for Morenga using the formula $C_1V_1 = C_2V_2$ where C_1V_1 is amount before dilution and C_2V_2 is amount after dilution. The actual concentration of the extract was not detected in concentrated solution sample. Hence dilution become way of handling the problem and it was carried out using the formula $C_1V_1 = C_2V_2$. Where (C_1) is more concentrated and corresponding volume is V_1; whereas C_2 is less concentrated and corresponding volume is V_2. Therefore for the calculation of concentration in original leaf extract; the appropriate formula become $C_1 = {C2V2}/{V1}$ V2 is 10mL which is the reciprocal of dilution factor. Finally the concentration, C_1 was multiplied by 0.002,

$(\frac{C1 \times 0.002}{Kg})$ to express in terms of total weight of the leaf used for obtaining the extract and the results described in table 2.

Unlike extract of Morenga, Casmir and avocado was multiplied by the dilution factor of 20 since the extract was diluted in the proportion of 1:20. The results explained in table 2

$$C_1 = {C2V2}/{V1}, \text{ V2 is 20.}$$

Table 2. Sample Concentration (mg/Kg), of the three plant species

Wave length	Morenga	Casmir	Avocado
663nm	0.136±0.012	0.229±0.006	0.1144±0.0004
649nm	0.108±0.007	0.214±0.001	0.102±0.0045

From table2 the concentration of chlorophyll a is higher than that of chlorophyll b in all sample studied.

3. Conclusions

The maximum absorbance for chlorophyll **a** and chlorophyll **b** were determined at 663nm and 649nm respectively. The concentration of pigments determined simultaneously using Porra's 2002 and the results obtained from three different leaf samples showed that, concentration of chlorophyll a is higher than that of chlorophyll b in each sample and concentration of pigments varied among the leaf samples that is the content of chlorophyll a and chlorophyll b in Morenga is lower than Casmir and higher than in avocado sample. More over concentration of each leaf sample was related directly to absorbance.

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