Estimation of Chlorophyll Content of Mung Bean Plants Treated with *Amaranthus viridis*

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***Abstract*—The current research investigation was carried out with the main aim of extraction of photosynthetic pigments (chlorophyll-a and chlorophyll-b) by using acetone solvent. The study is also concern on the extraction ratio of biomolecules with respect to different concentrations. Chlorophyll a, and Chlorophyll b were extracted from the test samples of mung beans plants treated with *Amaranthus viridis* (AV) extracts of different concentrations viz. AV 100%, AV 80%, AV 60%, AV 40%, AV 20%, and 0% as control using 100% acetone. Results delineated that different trend was observed in extraction rate for chlorophylls. Highest content of chlorophylls (Ch-a & Ch-b) was noted in test sample AV 80% (mung bean plants treated with 80% concentrated *Amaranthus viridis* extract). Whereas, lowest content of chlorophylls (Ch-a & Ch-b) was present in AV 60% (AV 60% = mung bean plants treated with 60% concentrated extract of *Amaranthus viridis*) & 0% (0% = untreated mung bean plants) respectively.**

Keywords: **Chlorophyl-a, Chlorophyll-b, *Amaranthus viridis*, Solvent extraction, *Vigna radiata***

I. INTRODUCTION

- *Amaranthus viridis* Linn. Is an annual herb commonly known as slender amaranth or green amaranth.
- Amaranthus viridis is a Cosmopolitan species with an upright, light green stem that grows to about 60–80 cm in height. Numerous branches emerge from the base, and the leaves are ovate, 3–6 cm long, 2–4 cm wide, with long petioles of about 5 cm. The plant has terminal panicles with few branches, and small green flowers with 3 stamens.

Traditionally it is eaten as a leafy vegetable in South India. The leaves and seeds are highly nutritious. The nutrients present in the leaves include protein, fiber content, vitamin A, vitamin C, riboflavin (Vit B2), thiamin (Vit B1), minerals like calcium, phosphorus, iron, amino acids (arginine, histidine, lysine, methionine, cystine, phenylalanine, leucine, isoleucine, threonine, tryptophan, tyrosine, valine). The seeds possess protein and fat. Therefore, the *Amaranthus viridis* received considerable attention because of the value.[2]

The pigments which are involved in the process of photosynthesis are called photosynthetic pigments. The pigments are the colored organic compounds that have capacity to absorb certain wavelength of light and reflect to others. Chlorophyll is a green pigment found in cyanobacteria and the chloroplasts of algae and plants. Chlorophyll is an extremely important biomolecule, critical in photosynthesis, which allows plants to absorb energy from light. Chlorophyll absorbs light most strongly in the blue portion of the electromagnetic spectrum, followed by the red portion.[3] Chlorophyll was first isolated by Pierre Joseph Pelletier in 1817.[4] Several kinds of chlorophyll have been discovered in plants. The formation of chlorophyll is physiological process that occurs only in living cells. The essential conditions for chlorophyll formation are the presence of genetic factors.[5] Photosynthetic pigments are the substances with very different chemical structure; they are present in the form of porphyrin ring (chlorophyll a, b and c), carotenoids, anthocyanins and flavones.[6] Total leaf pigment includes chlorophyll-a, chlorophyll-b and carotenoids that are necessary for photosynthesis process. The content of foliar pigments varies depending on species.[7] Variation in leaf pigments (chlorophylls and carotenoids) and its relation can be due to internal factors and environmental conditions. Traditionally plants have been well exploited by man for the treatment of human diseases.[8]

II. METHODS AND MATERIALS

Extract Preparation:
200 grams of fresh and healthy leaves in 100 mL of distilled water were grinded with the help of pestle and motor and the extract of leaves were extracted.

Different concentrations of extract were prepared by following method:

- 20% concentration; 20 ml of extract of *A. viridis* was added to 80mL of water
- 40% concentration; 40 ml of extract of *A. viridis* was added to 60 mL of water
- 60% concentration; 60 ml of extract of *A. viridis* was added to 40 mL of water
- 80% concentration; 80ml extract of *A. viridis* was added to 20mL of water
- 100% concentration; 100mL extract of *A. viridis*

Procedure for the seed treatment and incubation;

- Mung bean seeds were soaked for 24 hours in a distilled water
- After 24 hours the seeds were treated in the leaf extract of different concentrations for 2 hours
- Glass Petri Plates were sterilized using 70% alcohol and blotting papers of the same size were cut and added to Petri plates
- After 2 hours of seed treatment the seeds were transferred to the Petri plates in the following manner- 2 in the centre, 8 in the 1st circle and 15 in the 2nd circle.
- The Petri plates were kept at normal day light and room temperature. Drops of water were added to the plates twice a day and the incubation was done for 7 days.

Chlorophyll a and Chlorophyll b were extracted from the test samples of mung bean plants treated with *Amaranthus viridis* (AV) leaf extract of different concentrations viz. AV 100%, AV 80%, AV 60%, AV 40%, AV 20%, and 0% as control using 100% acetone. Test samples (~1gm each) were taken and macerated in mortar and pestle with 20 mL Acetone. Acetone extract was filtered through Whatmann No. 1 filter paper and filtrate was collected in 50 mL Falcon tubes. Optical densities were recorded at 662 nm and 645 nm. The amount of Chl. a, and Chl. b were calculated by (according to the formula) Lichtentaler and Wellburn method.[15]

Formula for Calculation

1. Chlorophyll a (µg/mL) = 11.75 x Absorbance 662nm – 2.350 x Absorbance 645nm
2. Chlorophyll b = 18.61 x Absorbance 645 – 3.960 x Absorbance 662

III. RESULTS AND DISCUSSION

The chlorophyll a, content was found to be high in test sample AV 80% (19.91 µg/mL) followed by AV 20% (16.08 µg/mL), AV 100% (15.42 µg/mL), AV 40% (12.65 µg/mL), and AV 60% (11.40 µg/mL). Similarly, the chlorophyll b, content was high in test sample AV 80% (10.67 µg/mL), followed by AV 20% (9.67 µg/mL), AV 100% (7.28 µg/mL), AV 40% (6.33 µg/ml), and AV 60% (5.17 µg/mL) respectively (Table 1).

Table 1: Content of pigment in test samples

Test Sample	Chlorophyll a (µg/mL)	Chlorophyll b (µg/mL)
Control (0%)	12.64	6.02
AV 20%	16.08	9.67
AV 40%	12.65	6.33
AV 60%	11.40	5.17
AV 80%	19.91	10.67
AV 100%	15.42	7.28

Present study revealed the inhibition of Amaranthus viridis on Seed germination of Vigna radiata but did not have any effect on the chrophyll content of the plant.

Chlorophyll is the green pigment present in plant plays a vital role in photosynthesis which absorbs light from sun and uses its energy to synthesize carbohydrates from CO_2 and water. Carotene function as accessory pigments in plants, helping to fuel photosynthesis by gathering wavelengths of light not readily absorb by chlorophyll. They have been shown to act as antioxidants and to promote healthy eye sight in humans. Chlorophyll-a is recognized as the main pigments which convert light energy into chemical energy. Chlorophyll-b as accessory pigments acts indirectly in photosynthesis by transferring the light it absorbs to chlorophyll-a.[16] The chlorophyll molecule has Mg^{2+} at its center which makes it ionic and hydrophilic, and a ring that is hydrophobic in nature with a carbonyl group at its tail which makes it polar. It is held in place in the plant cell within a water-soluble chlorophyll-binding protein (WSCP). Chlorophyll-b differ from

chlorophyll-a only in one functional group (i.e., -CHO) bounded to the porphyrin ring, and is more soluble than chlorophyll-a in polar solvents because of its carbonyl group.[15] Chlorophyll extraction capabilities of solvents are very much time dependent. The observation reveals rapid extraction of chlorophylls by acetone showing sharp peaks for chlorophyll-a and chlorophyll-b.

V. CONCLUSION

In conclusion, among six different concentrations of *Amaranthus viridis* extract treated samples chlorophyll a was very low in AV 60% (10.55 µg/mL) (AV 60% = mung bean plants treated with 60% concentrated extract of *Amaranthus viridis*) and chlorophyll a was high in AV 80% (19.91 µg/mL) (AV 80% = mung bean plants treated with 80% concentrated extract of *Amaranthus viridis)*. Chlorophyll b was found to be very low in 0% (6.02 µg/mL) (0% = untreated mung bean plants) and chlorophyll b was high in AV 80% (10.67 µg/mL) (AV 80% = mung bean plants treated with 80% concentrated extract of *Amaranthus viridis)*.

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