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EXPERIMENTAL ANALYSIS ON CHLOROPHYLL, AMINO ACID AND NITRATE CONTENTS OF APOCYNACEAE AND ASCLEPIADACEAE

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Abstract

This study focused on comparative analysis on the chlorophyll, amino acid and nitrate content of selected plants in Apocynaceae and Asclepiadaceae family and checked their photosynthetic activity. Six medicinal plants, *Rauwolfia serpentina, Alstonia scholaris, Catharanthus roseus* from Apocynceae and *Calotropis gigantea, Asclepias curassavica, Hemidesmus indicus* from Asclepiadaceae were selected for the study. Generally chlorophyll content is measured in 645 and 663 nm. The chlorophyll content at 645 and 663 nm showed that more in leaves of Asclepiadaceae than in Apocynaceae. The highest chlorophyll content highest chlorophyll content was observed in *Asclepias curassavica* 0.652 mg/g at 663nm. The highest amino acid content present in *Rauwolfia serpentine is* 0.155mg/g and *Alstonia scholaris* showed lowest amino acid content about 0.002mg/g. Interesting feature is that highest and lowest amino acid content present in the same family Apocynaceae. Nitrate content checked at 410nm wavelength. Spectrophotometric comparative analysis of nitrate content of families, *Asclepias curassavica* shows highest (0.175mg/g) and *Hemidesmus indicus* shows lowest nitrate content (0.055mg/g) Asclepiadaceae family.

Key words: Apocynceae, Asclepiadaceae, chlorophyll, amino acid, nitrate content.

Introduction

Chlorophylls are dominant factor controlling leaf optical properties of healthy green vegetation and are thus an essential part of the photosynthetic process. They harness light energy from the sun to store it as chemical energy (Richardson *et al.*, 2002). The amount of chlorophyll in leaves is normally expressed in terms of either concentration (µg Chl/g tissue) or content (µg Chl/cm tissue) and can vary significantly in value among different plant kingdoms and growing stages (Taiz *et al*., 2007). The most significant plant pigments for oxygenic conversion of light energy are chlorophyll a and chlorophyll b (Jensen, 2007). When the tissue dies, chlorophyll rapidly disappears and the above mentioned pigments gain in relative importance (Matile *et al*., 1999). The scientific interest is verified by Kaufman *et al*., (2010), showing that chlorophyll content is amongest the parameters with the highest frequency within investigations of agricultural hyper spectral studies. Porra (2002) and Wright *et al*., (1997) have discussed the merits of dimethyl sulphoxide used for chlorophyll extraction and assay, and reported as efficient when pigments concentrations are low.

Today, the role of nitrogen fertilizers on growth, performance and quality of products as well as demand for available products at agriculture is increased to achieve more productivity and this leads to overuse or abuse of nitrogen fertilizers. Therefore, nitrate is considered as a chemical fertilizer and a treat to human health and environment (Dezfouli *et al*., 2009, Manavifard *et al*., 2008). According to Cadenasso *et al*., 2006, nitrate serves as an essential plant nutrient helping with tissue development and amino acids are basic unit of protein (Scot *et al*., 2006). Amino acids inûuence a variety of growth and development processes in plants (Evans *et al*., 1989, Slocum *et al*., 1991, Kakkar *et al*., 2002).

Materials and Methods

For the present investigation, six selected plant leaves of two different families, such as Apocynaceae and Asclepiadaceae were collected from home gardens and wild places. *Rauwolfia serpentine, Alstonia scholaris, Catharanthus roseus* from Apocynceae and *Calotropis gigantea, Asclepias curassavica, Hemidesmus indicus* from Asclepiadaceae (Fig. 1 & 2). The chlorophyll, amino

acid and nitrate were estimated by spectrophotometer (uv-vis spectrophotometer).

Chlorophyll content estimation

Weighed 1g finely cut and well mixed representative sample of leaf in a clean mortar. Ground it to a fine pulp with the addition of 20ml of 80% acetone. Then centrifuged 5000 rpm for 5 minutes and transfered the supernatant to 100 ml volumetric flasks and ground the residue with 20ml of 80% acetone, Centrifuged and transfered the supernatant to the same volumetric flasks. Repeated the procedure upto the residue became colourless. Washed the mortar and pestle thoroughly with 80% acetone and collected the clear washings in the volumetric flask into 100ml with 80% acetone. Finally optical densities of the extract were read at 645,663 nm against solvent (80% acetone) blank.

From optical densities, the chlorophyll contents were calculated using the formulas

Content of chlorophyll a in 1g tissue (mg/g) = 12.7(A663) - 2.69(A645) × V/1ooo × w

Content of chlorophyll b in 1g tissue (mg/g) =22.9(A645) - 4.68(A663) × V/1000 × w

Total chlorophyll in 1g tissue (mg/g) =20.2(A645) + 8.02(A663) × V/1000 × w

Amino acid content estimation

Weighed 500mg of the plant sample and grounded it in a mortar and pestle with small quantity of acid washed sand. To this homogenate, added 5 to 10ml of 80% ethanol and filtered or centrifuged. Repeated the extraction twice with the residue and pool all the supernatants. Reduced the volume and used the extract for the quantitative estimation of total free amino acids. Tough tissues, boiled with ethanol (80%) for extraction.

To 0.1ml of extract, added 1ml of ninhydrin solution and made up the volume to 2ml with distilled water and heated by using a boiling water bath for 20 minutes. Added 5ml of diluent and mixed the contents. After 15 minutes read the intensity of purple colour against a reagent blank in a spectrophotometer at 570 nm. The colour was stable for 1 hour and also prepared the reagent blank as above by taking 0.1ml of 80% ethanol instead of the extract.

Nitrate content estimation

Preparation of reagent A (Dissolve 5 g of salicylic acid in 96% sulphuric acid, Fill up to 100 ml with 96% sulphuric acid).

Preparation of reagent B (Dissolve 40 g of NaOH in deionized water, Fill up to 500 ml with deionized water).

Preparation of standard solution (Dissolve 0.0680 g of sodium nitrate in deionized water, Fill up to 100 ml with deionized water (concentration of sodium nitrate is 8.0 mM) , Dilute the standard solution with deionized water in a range of: 0, 0.1, 0.2, 0.5, 1.0, 2.0, 4.0 and 6.0mM nitrate.

Added 0.8 ml of reagent 1 to 0.2ml standard or extract and mixed well and kept it for 20 minutes at room temperature and added 19 ml of reagent 2 slowly and mixed well. The colour became yellow and was stable for 48 hours. Finally Measured the absorption at 410nm with a spectrophotometer.

Results and Discussion

The results showed that the amount of chlorophyll, amino acid and nitrate present in different materials vary greatly. chlorophyll content of Apocynaceae plant leaves and chlorophyll content of Asclepiadaceae plant leaves

Fig. 1: Selected plants from Apocynaceae.

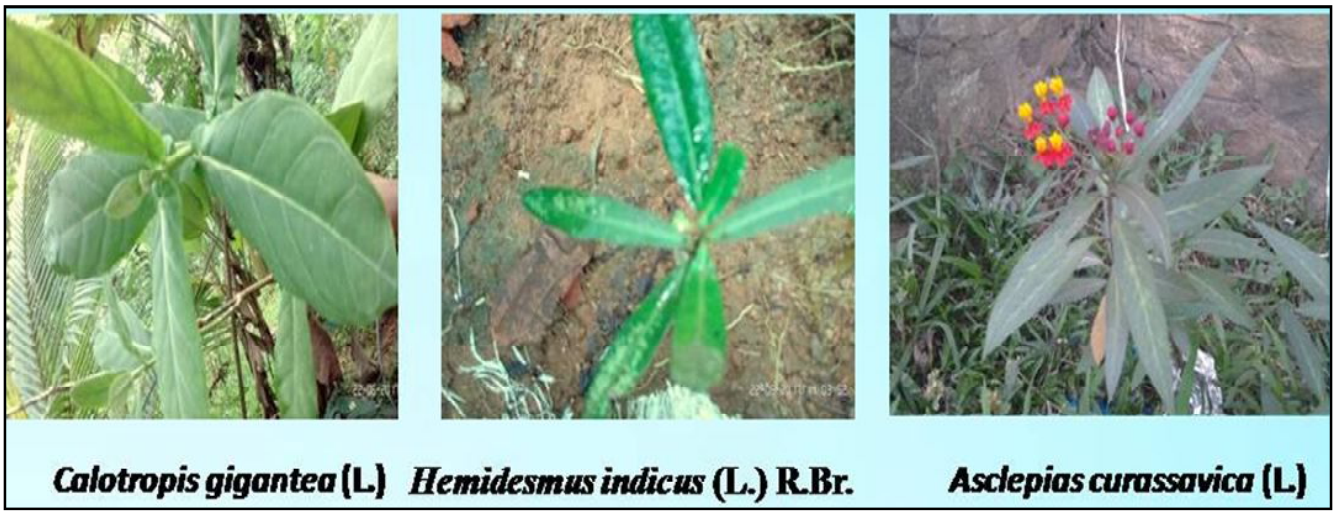

Fig. 2: Selected plants from Asclepiadaceae.

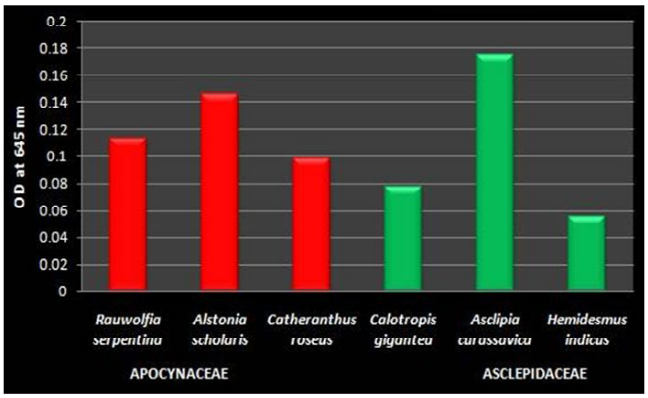

Fig. 3: Comparative analysis of chlorophyll content of Apocynaceae and Asclepiadaceae (645nm).

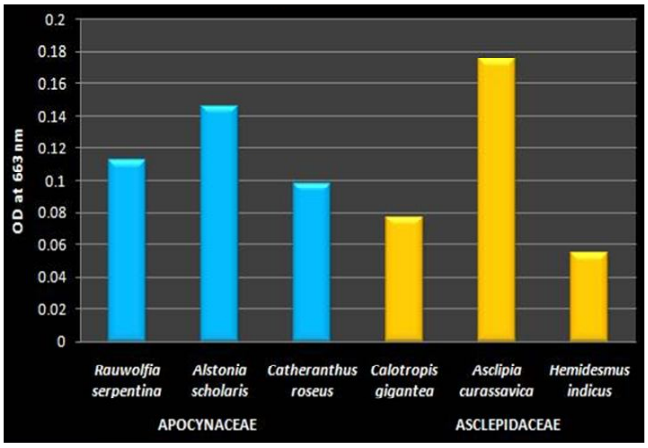

Fig. 4: Comparative analysis of chlorophyll content of Apocynaceae and Asclepiadaceae (663 nm).

showed in Fig. 3 and 4. With the help of formulas, calculated value of chlorophyll content of Apocynaceae and Asclepiadaceae showed in Fig. 7 and 8. Amino acid content of Apocynaceae and Asclepiadaceae plant leaves showed in Fig. 5 and also nitrate content in leaves of these two families showed in Fig. 6.

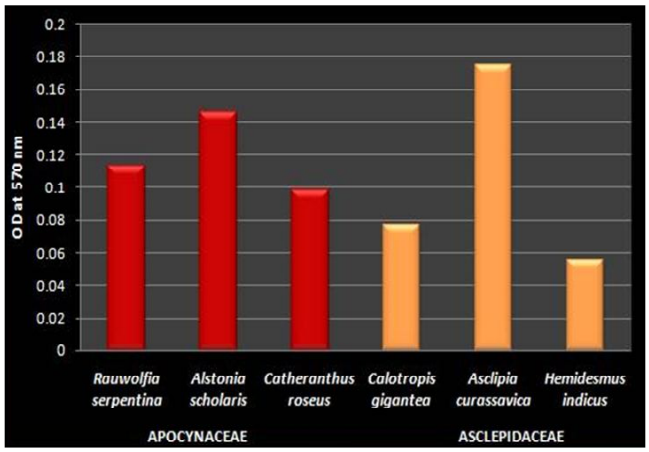

Fig. 5: Comparative analysis of amino acid content of Apocynaceae and Asclepiadaceae (570 nm).

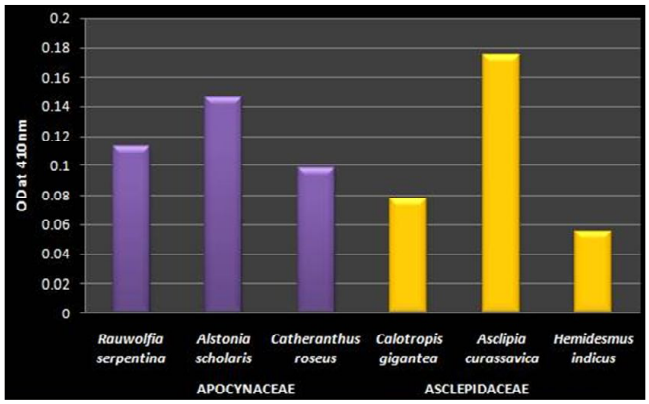

Fig. 6: Comparative analysis of Nitrate content of Apocynaceae and Asclepiadaceae (410 nm).

The studies shows that the spectrophotometric analysis of chlorophyll content of studied plant leaves of these two families vary greatly. Generally chlorophyll content is measured in 645 and 663 nm. Here, chlorophyll content of Asclepiadaceae plant leaves greater than Apocynaceae plant leaves. Comparative analysis of spectrophotometric values between two family's shows

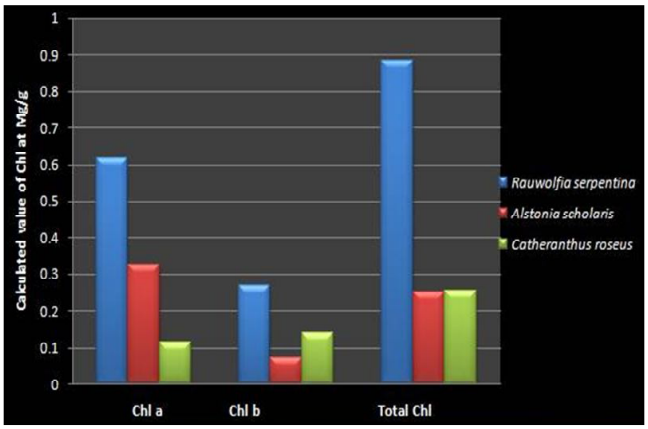

Fig. 7: Analysis of calculated values of chlorophyll a, b and total chlorophyll content of Apocynaceae.

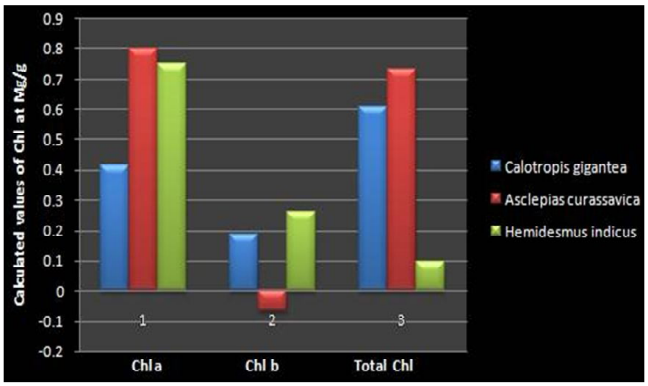

Fig. 8: Analysis of calculated values of chlorophyll a, b and total chlorophyll content of Asclepiadaceae.

that highest chlorophyll content present in *Asclepias curassavica*. It is about 0.652 mg/g at 663nm. While it shows lowest amount of chlorophyll in Asclepiadaceae family at 645nm. Second highest value 0.643 mg/g present in *Hemidesmus indicus* of Asclepiadaceae family at 663nm. From the analysis of two families, lower most value 0.021 shows by *Alstonia scholaris* of apocynaceae family at 645nm. In both families highest chlorophyll content present in 663nm.Spectrophotometric comparative analysis of selected plant leaves of two families reveals that photosynthetic capacity is higher in Asclepiadaceae family than Apocynaceae family.

The spectrophotometric analysis of Amino acid is estimated in 570nm. Unlike chlorophyll, highest amino acid content present in Apocynaceae family. It is about 0.155mg/g that shows by *Rauwolfia serpentina*. When comparing the values of both families, *Alstonia scholaris* of Apocynaceae family shows lowest amino acid content about 0.002mg/g. Interesting feature is that most highest and most lowest amino acid content present in the same family. 0.012mg/g of amino acid content present in *Hemidesmus indicus*. It is the highest value of Asclepiadaceae family. While *Asclepias curassavica* shows lowest value (0.008 mg/g) of this family. It reveals *Rauwolfia serpentina* and *Hemidemus indicus* shows highest amino acid content of Apocynaceae and Asclepiadaceae family respectively. Due this property they are highly used as medicine.

When using the formula, highest calculated value of chlorophyll is present in *Rauwolfia serpentina* of Apocynaceae family. It is about 0.88 Mg/g. Lowest amount of chlorophyll a present in *Catheranthus roseus*, chlorophyll b present in *Alstonia scholaris* and also lowest amount of total chlorophyll present in *Alstonia scholaris* of Apocynaceae family. In Asclepiadaceae, highest calculated value of chlorophyll a and total chlorophyll present in *Asclepias curassavica*, chlorophyll b is present in *Hemidesmus indicus* and also lowest amount of chlorophyll a and total chlorophyll present in *Calotropis gigantea* and chlorophyll b is present in *Asclepias curassavica* and shows - 0.064 Mg/g.

Apocynaceae family shows highest nitrate content than Asclepiadaceae family at 410nm wavelength in spectrophotometer. In Apocynaceae family, *Alstonia scholaris* shows 0.146mg/g, *Rauwolfia serpentina* shows 0.113mg/g and *Catherathus roseus* shows 0.098mg/g. While In Asclepiadaceae family, *Calotropis gigantea* shows 0.007mg/g, *Asclepias curassavica* shows 0.175mg/g and *Hemidesmus indicus* shows 0.055mg/g. According to the spectrophotometric comparative analysis of nitrate content of families, *Asclepias curassavica* shows highest and *Hemidesmus indicus* shows lowest nitrate content. Both are present in Asclepiadaceae family. As usual, nitrate content is varying among these plants. But nitrate is considered as essential nutrient of these plants.

Conclusion

Chlorophyll is important to carry the photosynthesis in nature. In the absence of Photosynthesis, life on earth does not exist. In addition of photosynthesis, chlorophyll play important role in medicinal applications. Nitrate content will help the plants to acquire good growth. Also, plants which show high rate of amino acid content that will show resistance against high temperature, low humidity and pest attack and also is involved in the chlorophyll production. Thereby it involved in photosynthesis.

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