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CHLOROPHYLL SURVEY IN SELECTED MEMBERS OF THE FAMILY APOCYNACEAE

NusaifaBeevi P

P G Department of Botany, Iqbal College, Peringammala, Thiruvananthapuram 695563, Kerala.

ABSTRACT

Nine plants belonging to Apocynaceae family viz;Allamandacathartica, Alstoniascholaris, Carissa carandas, Catheranthusroseus, Neriumindicum, Plumeriarubra ,Stemmadaenialittoralis,Tabarnamontanadivericataand Wrightiatinctoria were identified and collected from Iqbal College campus.Leaf colour was noted for all the selected members.Chlorophyll estimation was done in the fresh green leaf samples extracted with the acetone solvent, and the absorbance readings of chlorophyll extracts were measured in two different wavelengths 645nm and 663nm respectively. Based on the absorbance value calculations were made using Arnon's (1949) equation and the amount of chlorophyll a, chlorophyll b and total chlorophyll were estimated and tabulated. The highest value for chlorophyll a, chlorophyll b and total chlorophyll contentwas detected inCathuranthusroseuswith deep glossy green leaves. Similarly, the lowest value for chlorophyll a is detected in Alstoniascholaris and chlorophyll b in Carissa carandas.Total chlorophyll showed lowest value in Carissa carandas.

Key Words: Apocynaceae, Chlorophyll a,Chlorophyll b, Total chlorophyll, Spectrophotometer.

Introduction

The chlorophyll as an index for determination of photosynthetic ability has been reported by many workers (Starnes and Haddley,1965).Relation between higher photosynthetic activity and high yield has been reported in various crop plants (Wallace and Munger,1966; Ishar and Wallace,1967).The chlorophylls constitute about 10% of the dry weight of the chloroplast (Zelitch,1971). In green plants chlorophyll is present in several different forms but all these revert to ordinary chlorophyll a on extraction with organic solvents. Both chlorophyll a and b is very effective photoreceptors because they contain a network of alternating single and double bonds, and the orbitals can delocalize stabilizing structure. The different side groups in the two chlorophylls tune the absorption spectrum to slightly different wavelength, so that light that is not significantly absorbed by chlorophyll at 460 nm, will be instead be captured by chlorophyll b, which absorbs strongly at that wavelength. Thus these two chlorophylls complement each other in absorbing sunlight. Plants can obtain all their energy requirements from the blue and red parts of the spectrum, however, there is still a large spectral region between 500 to 600 nm where very little light is absorbed, and since it is reflected. This is the reason that plants appears in green colour. Chlorophyll was first isolated and named by Joseph Caventou and Pierre Joseph in 1817.

Apocynaceae, commonly known as the dogbane family of flowering plants, including about 415 genera and about 4,600 species of trees, shrubs, woody vines, and herbs, distributed primarily in tropical and subtropical areas of the world. According to Bentham and Hooker's System of Classification, the family belongs to the order Gentianales, under the series bicarpellatae of the subclass gamopetalae of class dicotyledons under Angiosperms of Kingdom Plantae (1862). According to APG III system of classification (Stevens, P.F, 2012), Gentianales order of flowering plants comes under Euasterids of Angiosperms, and consisting of five families with 1,118 genera and nearly 17,000 species. Nearly all members of this family are poisonous, and many species are used medicinally because of the presence of cardiac glycosides and various alkaloids. *Catharanthus* (Madagascar, or rosy, periwinkle) is a source of drugs for treatment of leukemia. *Rauvolfia* produces reserpine, which is used for hypertension and for mental illnesses. Even the commonly cultivated tropical shrub *Nerium* (oleander) is poisonous and has caused deaths in infants who ingested as little as a single leaf. Many members of Apocynaceae

are ornamental. In the dogbane group *Vinca* (periwinkle) is a common ornamental groundcover in temperate areas, and tropical ornamentals include *Allamanda*, *Carissa*, and *Plumeria*.
In photosynthesis, antenna pigments in leaf chloroplasts absorb solar radiation, and through resonance transfer the resulting excitation is channeled to the reaction centre pigments, which release electrons and set in motion the photochemical process. The chlorophylls, Chl*a* and Chl*b*, are the most important of these pigments, and are thus virtually essential for the oxygenic conversion of light energy to the stored chemical energy that powers the biosphere.

From a physiological perspective, leaf Chlorophyll content varies both between and within species and is therefore a parameter of significant interest in its own right. However, from an applied perspective, leaf pigmentation is important to both land managers and eco physiologists because, the amount of solar radiation absorbed by a leaf is largely a function of the foliar concentrations of photosynthetic pigments (Curran et al, 1990; Filella *et al.*, 1995). According to Moran,et al (2000), much of leaf nitrogen is incorporated in chlorophyll, so quantifying chlorophyll content gives an indirect measure of nutrient status. In addition, pigmentation can be directly related to stress physiology, as concentrations of carotenoids increase and chlorophylls generally decrease under stress and during senescence (Peñuelas&Filella, 1998). Similarly the relative concentrations of pigments are known to change with abiotic factors such as light, for e.g. sun leaves have a higher Chl*a* : Chl*b* ratio (Larcher, 1995) and so quantifying these proportions can provide important information about relationships between plants and their environment.

The objective of the present study is to cconduct a thorough campus survey,identification and collection of members belonging to Apocynaceae and exraction and estimation of chlorophyll content.

Materials and Methods

Collection of materials

A campus field survey was conducted for the taxonomic study during August to January. The plants belonging to the family Apocynoceae were identified. Nine plants were selected for the present study from the campus and from nearby houses and based on their more availability. No

priority has been made for the selection of plants, but is only its availability in the campus. Morphlogical study was carried out for all the selected members and photographed. The leaves were collected from each plant. The collected leaves were washed thoroughly in tap water and then in distilled water to remove all dust particles.

Extraction of chlorophyll (Arnon, 1949)

Leaves were sliced into small pieces.One gram of finely cut fresh leaves were taken and ground in a clean mortar and pistil. 20 ml of 80% chilled acetone was the grinding medium. The slurry was centrifuged at 3000 rpm for 3 minutes. The resultant supernatant was collected and the volume was noted. The supernatant was transferred and the procedure was repeated till the residue becomes colorless. 1 ml of supernatant was taken and made up to 5 ml using 80% acetone. The absorbance of the solution was red at 645nm and 663nm against the solvent (acetone) blank. Since the blue region cannot be used because of carotenoids in the crude extracts, value at 490 nm is not utilized for the present study

Estimation of Chlorophyll content

The concentrations of chlorophyll a, chlorophyll b and total chlorophyll (in μg/ml) were calculated using the following equation:

Chlorophyll a: 12.7(A663) – 2.69(A645)

Chlorophyll b: 22.9(A645) – 4.68(A663)

Total Chlorophyll: 20.2(A645) + 8.02(A663)

Each experiment was carried out in triplicate and results averaged expessed as mean ± standard deviation (SD).

Results

Chlorophyll estimation was done in the fresh green leaf samples extracted with the acetone solvent (Fig: 10), and the absorbency readings of chlorophyll extracts were measured in two different wavelengths 645nm and 663nm respectively. Based on the absorbancy value calculations were made using Arnon's (1949) equation and the amount of chlorophyll a,

chlorophyll b and total chlorophyll were estimated and tabulated (Table 1). The study revealed that *Allamandacatharitica,* Linn.isa woody climber commonly called as Golden trumpet with milky latex in stem. It is commonly planted as an ornamental in gardens. It is also used in traditional medicines. Leaves and roots are used as purgative.Chlorophyll estimation revealed that Chlorophyll a 1.165 ± 0.11 (µg/ml), Chlorophyll b 0.938 ± 0.14 (µg/ml), with a total chlorophyll content 1.896± 23 (µg/ml) (Table 1).

Alstoniascholaris, R.Br.is a medium sized evergreen tree commonly called as devil's tree with milky juice in stem. The tree is often planted as an avenue plant and as ornamental in gardens. The bark is used for treating asthma and heart ailments, fever and diarrhea.Chlorophyll estimation revealed that Chlorophyll a 0.089 ± 0.12 (µg/ml), Chlorophyll b 1.969 ± 0.11 (µg/ml), with a total chlorophyll content 2.005± 0.14 (µg/ml) (Table 1).*Carissa carandas,* Linn.is a spiny shrub grown throughout India for its sour edible fruits. The fruits are eaten both raw, and also used to make pickle.The plant makes a good hedge.Chlorophyllestimation revealed that Chlorophyll a 0.107 ± 0.15 (µg/ml), Chlorophyll b 0.357 ± 0.24 (µg/ml), with a total chlorophyll content 0.439±0.27 (µg/ml) (Table 1).*Catharanthusroseus,* (Linn.)G.Donis an evergreen herbaceous plant growing 1 m tall. Commonly called as periwinkle, or rosy periwinkle. The species has long been cultivated for herbal medicine and as an ornamental plant. In Ayurveda (Indian traditional medicine) the extracts of its roots and shoots, though poisonous, are used against several diseases. In traditional Chinese medicine, extracts from it have been used against numerous diseases, including diabetes, malaria, and Hodgkin's lymphoma. Many of the vinca alkaloids were first isolated from *Catharanthusroseus*. The substances vinblastine and vincristine extracted from the plant are used in the treatment of leukemia and Hodgkin's lymphoma.Chlorophyll estimation revealed that Chlorophylla 8.669± 0.23 (µg/ml), Chlorophyll b 6.858 ± 0.22 (µg/ml), with a total chlorophyll content 13.993± 0.15 (µg/ml) (Table 1).*Neriumindicum,* Mill.is a shrubaceous plant with erect stems that splay outward as they mature; first-year stems have a glaucous bloom, while mature stems have a grayish bark. Chlorophyll estimation revealed that Chlorophyll a 1.323 ± 0.31 (µg/ml), Chlorophyll b 1.284 ± 0.20 (µg/ml), with a total chlorophyll content 2.368± 0.28 (µg/ml) (Table 1).*Plumeriarubra,* Linn. grows as a spreading shrub or small tree. The plants are grown as an ornamental, Red pulp around seed is used as a dye,wood is refrigerant.Chlorophyll estimation revealed that Chlorophyll a 1.901 ± 0.45 (µg/ml), Chlorophyll b 2.055 ± 0.23 (µg/ml), with a

total chlorophyll content 3.90± 0.33 (µg/ml) (Table 1).*Stemmadaenialittoralis*commonly known as the Milky Way Tree. Latex obtained from the plant is used as a masticatory. Medicinal and other uses are unknown.Chlorophyll estimation revealed that Chlorophyll a 6.589 ± 0.18 (µg/ml), Chlorophyll b 5.688± 0.15 (µg/ml), with a total chlorophyll content 11.102± 0.08 (µg/ml) (Table 1).*Tabernaemontana divaricate* (Linn.)R.Br.*Tabernaemontana* is either a tree or a shrub growing to 1-15m tall. Some members of the genus are used as additives to some versions of the psychedelic drink. These plants are commonly used as ornamental.Chlorophyll estimation revealed that Chlorophyll a 1.356 ± 0.12 (µg/ml), Chlorophyll b 2.059 ±0.23 (µg/ml), with a total chlorophyll content 3.30± 0.10 (µg/ml) (Table 1).*Wrightiatinctoria*, R.Br. commonly known as 'danthapala', is a deciduous shrub or tree. The tree is harvested from the wild as a medicine and source of a dye and wood. Leaves are extracted as fodder for livestock. The leaves, flowers, fruits and roots are sources of indigo-yielding glucoside, which produces a blue dye or indigo- like dye. It is occasionally planted as an ornamental in the tropics. It is recommended as a good agroforestry species as it intercrops well. The wood of *Wrightiatinctoria* is used extensively in India for carving and lacquer work for toys. The timber is high in quality and valuable. The white wood, which is very fine, is used for turnery, carving, toy making, matchboxes, small boxes and furniture.Chlorophyll estimation revealed that Chlorophylla 0.869± 0.31 (µg/ml), Chlorophyll b 1.894± 0.21 (µg/ml), with a total chlorophyll content 2.584± 0.11 (µg/ml) (Table 1).Morphological study revealed that all members under study showed typical Apocynous characteristics. Leaves are dark green for all the members, but *Cathuranthusroseus* and *Stemmadaenalittoralis* showed deep glossy green leaves.

Table 1: Chlorophyll content in studied members

SL.no	Binomial	Chl a µg/ml	Chl b µg/ml	Total chlµg/ml
1	*Allamandacathartica*	1.165 ± 0.11	0.938 ± 0.14	1.896± 23
2	*Alstoniascholaris*	0.089 ± 0.12	1.969 ±0.11	2.005± 0.14
3	*Carissa carandas*	0.107 ± 0.15	0.357 ± 0.24	0.439± 0.27
4	*Catharanthusroseus*	8.669 ± 0.23	6.858 ± 0.22	13.993± 0.15
5	*Neriumindicum*	1.323 ± 0.31	1.284 ± 0.20	2.368± 0.28
6	*Plumeriarubra*	1.901 ± 0.45	2.055 ± 0.23	3.90± 0.33

7	*Stemmadaenialittoralis*	6.589 ± 0.18	5.688± 0.15	11.102± 0.08
8	*Tabarnamontanadivaricata*	1.356 ± 0.12	2.059 ±0.23	3.30± 0.10
9	*Wrightiatinctoria*	0.867± 0.31	1.894± 0.21	2.584± 0.11

Discussion

The highest value for chlorophyll *a* was detected in *Cathuranthusroseus*(8.669 µg/ml), followed by *Stemmadaenalittoralis*(6.589µg/ml).The highest value for chlorophyll *b* was also detected in *Cathuranthusroseus*(6.858 µg/ml), and*Stemmadaenalittoralis*(5.688µg/ml).The highest value for total chlorophyll content (*a* + *b*) was detected in *Cathuranthusroseus*(13.993 µg/ml), followed by *Stemmadaenalittoralis*(11.102µg/ml). The deep green coloured and glossy leaves of *Cathuranthusroseus*indicate that chlorophyll content is highest in the taxa as is revealed in the present study. The highest value for chlorophyll *a,* chlorophyll *b and* total chlorophyll contentwas detected in*Cathuranthusroseus*. Similarly, the lowest value for chlorophyll *a* is detected in *Alstoniascholaris*(0.089 µg/ml) and chlorophyll b in *Carissa carandas* (0.357 µg/ml) According to Arnon (1949), the ratio of chlorophyll *a* to chlorophyll *b* in higher plants is approximately 3:1. The chlorophylls, Chl*a* and Chl*b*, are the most important of these pigments, and are thus virtually essential for the oxygenic conversion of light energy to the stored chemical energy that powers the biosphere.

Reduction in the concentration of chlorophyll content in leaves of polluted area was observed in various plant species. Similar changes in concentration of pigments were also observed in leaves of six tree species expose to air pollution due to vehicle emission (Joshi and Swami, 2009). Leaves from polluted area had significantly lower chlorophyll content than control (Tripati and Prajapathi, 2008; Stevvovic et al., 2010). Chlorophyll estimation has been made in different members of family Apocynaceae by different workers. Chlorophyll estimation in *Tabarnamontanadivericata* (Amulya et al.,2015; Tripati and Prajapathi,2008), Chlorophyll estimation in *Catheranthusroseus* (Choudhary and Gupta,1998) etc. Amulya*et al* (2015),observed a reduction in chlorophyll content in the leaves of *Tabarnamontanadivericata*frompolluted area. According to Choudhury and Gupta (1998), chlorophyll content reduced significantly after treatment with sodium dikegulacin *Cathuranthusroseus*.

Conclusion

A campus field survey was conducted for the taxonomic study during August to January. The plants belonging to the family Apocynoceae were identified. Nine plants were selected for the present study from the campus and from nearby houses and based on their more availability.Chlorophyll estimation was done in the fresh green leaf samples of collected members extracted with the acetone solvent, and the absorbency readings of chlorophyll extracts were measured in two different wavelengths 645nm and 663nm respectively. Based on the absorbancy value calculations were made using Arnon's (1949) equation and the amount of chlorophyll a, chlorophyll b and total chlorophyll were estimated and tabulated. The study revealed thatThe highest value for total chlorophyll content (*a* + *b*) was detected in *Cathuranthusroseus*(13.993 µg/ml), followed by *Stemmadaenalittoralis*(11.102µg/ml). The deep green coloured and glossy leaves of *Cathuranthusroseus*indicate that chlorophyll content is highest in the taxa as is revealed in the present study. The highest value for chlorophyll *a,* chlorophyll *b and* total chlorophyll contentwas detected in*Cathuranthusroseus*. Similarly, the lowest value for chlorophyll *a* is detected in *Alstoniascholaris*(0.089 µg/ml) and chlorophyll b in *Carissa carandas* (0.357 µg/ml).

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