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Estimation of pigments from *Gnidia glauca* (Fresen.) Gilg

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Abstract

Chlorophyll a and chlorophyll b are pivotal pigments within plant photosystems, with chlorophyll A serving as the primary photosynthetic pigment responsible for energy production. Notably, chlorophyll concentrations vary among different plant species. This investigation centers on *Gnidia glauca*, a medicinal plant. The current study offers a comprehensive analysis of *Gnidia glauca*'s pigment profile, encompassing the quantification of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content in both fresh leaves and bark material. To our knowledge, this represents the initial exploration of pigment investigations conducted on *Gnidia glauca*.

Key words : Chlorophyll, photosystems, *Gnidia glauca*.

Green plants exhibit distinct spectral features attributable to the presence of diverse pigments, notably chlorophyll, carotenoids, various other pigments and water content. Chlorophyll, the pigment responsible for the characteristic green coloration in plants, fulfills a critical and exclusive role in the physiological, productive, and economic facets of green flora. It serves as the principal photosensitive molecule in the intricate process of photosynthesis, wherein carbon dioxide is enzymatically converted into carbohydrates and molecular oxygen[12]. Chlorophyll is predominantly sequestered within the chloroplasts of angiosperms and gymnosperms, with its primary localization occurring in the chlorophyll-rich regions of leaves, stems, flowers, and roots. The biosynthesis of chlorophyll is contingent upon the absorption of solar radiation, which functions as the primary energy source facilitating the vital processes of plant life. The concentration of chlorophyll is subject to variation among distinct plant species and is intricately influenced by factors such as nutrient availability and environmental stressors, encompassing drought, salinity, cold and heat[18]. The precise quantification of chlorophyll concentration holds paramount importance in the realm of plant physiology research[11] and holds promising applications in the field of medicine, given its substantial involvement in antioxidant, anti-inflammatory, and anticancer properties[15], as well as its potential to promote optimal human visual health[10]. Moreover, chlorophyll and its derivatives have shown promise as photodynamic agents in the context of tumor and cancer therapy[3]. As such, the present investigation has been undertaken to elucidate the pigmental profile of *Gnidia glauca*.

Gnidia glauca exhibits a wide distribution spanning East Africa, peninsular India, and Sri Lanka[9]. This plant species is notably esteemed for its substantial medicinal efficacy, finding application in the treatment of diverse maladies, including cancers, burns, wounds, abdominal pain, snake bites, and sore throat. Furthermore, the leaves of *Gnidia glauca* are employed for the mitigation of joint pain, back pain, as well as the reduction of swellings and contusions[8].

Plant collection :

Plant material was sourced from Palakkad, Kerala, and subjected to a thorough washing with tap water. Subsequently, the leaves and bark were separated. A voucher specimen was duly deposited within the Department of Botany, Karyavattom, and fresh plant material was exclusively employed for the pigment analysis.

Estimation of Chlorophyll[2] :

Approximately one gram of finely minced samples was placed in a clean mortar and subsequently ground into a fine pulp while adding 10 ml of chilled acetone. The resulting mixture was then subjected to centrifugation at 5000-10000 rpm for a duration of 5 minutes, after which the supernatant was carefully transferred into a dry volumetric flask and obtain the known volume. The absorbance of the resultant solution was measured at wavelengths of 645 nm, 663 nm, and 480 nm with reference to the solvent (acetone) blank. Formula for calculation[20,22]

Chlorophyll ‘a’ (mg/g fresh weight) = 12.7 (A_{663})- 2.69(A_{645}) x V /1000 x W

Chlorophyll ‘b’ (mg/g fresh weight) = 22.9 (A_{645}) - 4.68(A_{663}) x V /1000 x W

Total Chlorophyll (mg/g fresh weight) = 20.2 (A_{645}) + 8.02(A_{663}) x V /1000 x W

Carotenoid (mg/g fresh weight) =A_{480}+(0.114 x A_{663}) - (0.638 x A_{645}) x V /1000 x W

Here, A = Absorption at specific wavelength, V = Final volume of extract with acetone and W = Fresh weight of tissue taken for extraction

Statistical analysis :

Each assay was performed in triplicate, and the outcomes were presented as the mean of the replicated measurements, accompanied by the standard error.

Table-1 and Figures 1 a, b and c depict the pigment profile of *Gnidia glauca*. The highest concentrations of chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll were observed in the leaves of *Gnidia glauca*. Specifically, the chlorophyll a content in fresh leaves and bark was 0.141±0.006 mg/g and 0.014±0.001mg/g respectively. The carotenoid content ranged from 0.076±0.004 mg/g in leaves to 0.012±0.002 mg/g in bark. Moreover, the total chlorophyll content in leaves measured 0.192±0.004 mg/g whereas in bark, it was

Table-1. Pigment profile of *Gnidia glauca*

Plant part	Chlorophyll a (mg/g)	Chlorophyll b(mg/g)	Total Chlorophyll (mg/g)	Carotenoid (mg/g)
Fresh Leaf	0.141±0006	0.081±004	0.192±004	0.076±0004
Fresh Bark	0.014±0001	0.012±0002	0.028±0004	0.012±0002

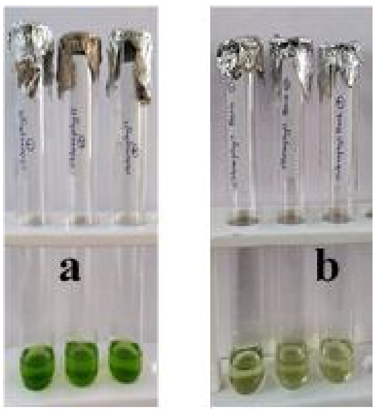

Fig. 1 a and b: Chlorophyll extracts of *Gnidia glauca* leaf and bark with triplets

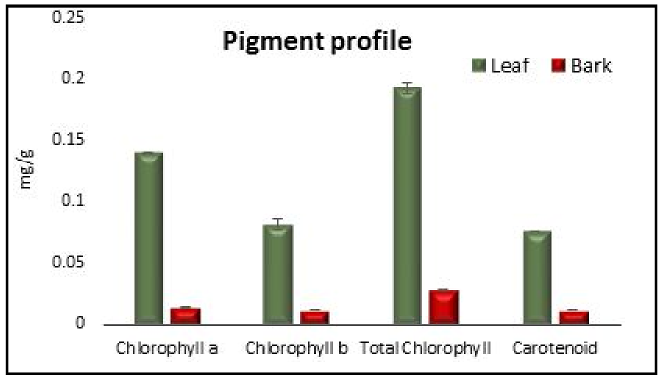

Fig c: Chlorophyll a, b, total chlorophyll and carotenoid content in fresh samples of *Gnidia glauca*

0.028±0.004 mg/g. As reported by Porra in 2002[19], elevated chlorophyll concentrations within leaves serve as a reliable gauge of plant health. The synthesis of chlorophyll is predominantly contingent on the absorption of sunlight, which serves as the primary energy source for plants[24]. Chlorophyll, an antioxidant compound, is primarily localized and stored within the chloroplasts as well as the green regions of leaves and stems[15,24]. Extensive research has validated its effectiveness against various conditions, including skin ailments, cancer, the aging process, arthritis and fibromyalgia, while also demonstrating blood-regulating, wound-healing, and antioxidant properties[1,4,25]. According to Srichaikul and colleagues[24], the concentration of chlorophyll a in plants is typically 2-3 times higher than that of the secondary chlorophyll b. Chlorophyll a and chlorophyll b are vital pigments within plant photosystems[21]. These two types of chlorophyll display distinctive light absorption characteristics, with chlorophyll a primarily absorbing light at wavelengths of 430 nm and 662 nm, while chlorophyll b predominantly absorbs light at 453 nm and 642 nm[17]. Mishra *et al.*,[18] have reported significant antioxidant and radioprotective properties of chlorophyll and its derivatives, including pheophytins, both *in vivo* and *in vitro*. These compounds effectively inhibit lipid peroxidation, protein oxidation, and provide protection against DNA and membrane damage. Numerous scientific investigations and empirical observations have highlighted the significance of the chlorophyll a:b ratio in enabling higher plants to efficiently adapt to varying light conditions, optimizing their utilization of ambient light intensities and quantities[7]. In contrast, carotenoids, as emphasized by Viera *et al.*,[27], play a pivotal role as pigments associated with immune functions and act as antioxidants, thereby reducing susceptibility to chronic diseases. As per the research findings of Meléndez-Martínez and colleagues[14], optimal carotenoid consumption may be associated with a reduced risk of specific types of cancers (such as cervical, ovarian, colorectal and breast), cardiovascular diseases, bone disorders, skin conditions, and ocular disorders. Furthermore, diets abundant in carotenoid-rich foods have been linked to a reduced risk of Parkinson's disease and have shown potential as protective agents against conditions such as head and neck cancers and prostate cancer[13,23,26]. Carotenoids are pigment compounds found in plants that give rise to vibrant red, yellow, and orange colors in various fruits and vegetables. In the context of leaves, carotenoids serve important functions related to photosynthesis and photoprotection[5]. Additionally, they are integral to the synthesis of abscisic acid, which plays a key role in initiating the stomatal closure mechanism[6].

The current study's results clearly demonstrate that the levels of chlorophyll a, chlorophyll b, and total chlorophyll content in *Gnidia glauca* were notably higher in the leaves compared to the bark. Additionally, the findings indicate that carotenoid content was more concentrated in the leaves than in the fresh bark extracts. This research offers the initial comprehensive analysis of pigment estimation in fresh leaves and bark from *Gnidia glauca*.

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