International Journal of Basic and Applied Biology
p-ISSN: 2394-5820, e-ISSN: 2349-5839, Volume 10, Issue 1; January-March, 2023, pp. 1-3

http://www.krishisanskriti.org/Publication.html

Fluorescence Spectroscopy as a Potential Tool in Determining the Leaf Age of *Mangifera indica*

V. Kavini[1] and Sujatha Narayanan Unni[2]

[1]PhD Scholar, Biophotonics Lab, Department of Applied Mechanics and Biomedical Engineering
Indian Institute of Technology, Madras
[2]Faculty Guide, Biophotonics Lab, Department of Applied Mechanics and Biomedical Engineering
Indian Institute of Technology, Madras
E-mail: [1]am21d200@smail.iitm.ac.in, [2]nsujatha@iitm.ac.in

Abstract—*Leaves are the major photosynthetic organs of a plant. The photosynthetic capacity of the plant depends on the age of the leaf, biotic and abiotic stress factors. Plants have their own protective mechanism against various stress factors. The phytochemical content in healthy leaves varies depending on the leaf age of Mangifera indica. Mangiferin, a phytochemical with enormous medicinal properties varies based on leaf age. In this paper, we mainly focus on changes in pigment content as a good indicator of age in the leaves of* Mangifera indica*. The results obtained from the optical absorbance and fluorescence analysis provide information about the photosynthetic capacity across the leaves of varied age groups. We propose fluorescence spectroscopy and associated optical probes to be used as a potential tool in assessing the leaf age to help identify the right category of leaves for various medical applications.*

Keywords: *leaf age, phytochemicals, fluorescence, photosynthetic capacity, Mangifera indica.*

INTRODUCTION

Plants produce food on their own through the process of photosynthesis. This depends on the photosynthetic capacity of the leaves which are the principal organs of photosynthesis [1]. The photosynthetic pigments chlorophyll *a* and chlorophyll *b* that are present in the thylakoid membranes of the leaf absorb the energy from the sunlight and act as a photocatalyst in the process of photosynthesis [2]. Leaves also contain other pigments such as anthocyanin, betalain, and various classes of carotenoids. The existence of these pigments in the leaf depends on the age of the leaf, biotic and abiotic stress factors. Plants have their own mechanism to protect themselves against various stress factors. Certain pigments protect the young leaves from excess damage caused due to photoinhibition [3]. The leaves of *Mangifera indica* are one of the most commonly used herbs in Ayurvedic medicine. Mangiferin, a polyphenolic antioxidant extracted from the leaves of *Mangifera indica* has immense antiviral, antibacterial, antifungal, anti-inflammatory, anti-microbial, anthelmintic and anti-allergenic, antiparasitic, anti-tumor, anti-HIV, antispasmodic and antipyretic activity. It is an important chemical in pharmacology and medicine [4]. The Mangiferin content in the leaves of *Mangifera indica* changes depending on the age of the leaf [5]. It is necessary to pick the leaves at the right stage to extract a considerable amount of Mangiferin for medical applications. The photosynthetic pigment chlorophyll is a good indicator of leaf age. This pigment in the chloroplast of the leaves absorbs maximum light in the blue and red wavelength range and emits fluorescence at the wavelength of 684 nm and secondary fluorescence at 720 nm respectively. In this paper, both the absorbance and fluorescence spectroscopy studies have been conducted in the fresh and healthy leaves of *Mangifera indica* and identified the changes in the pigment concentration at different developmental stages.

MATERIALS AND METHODS

Fresh and healthy leaves of *Mangifera indica* collected at different developmental stages during summer were used in this experiment. The color of these collected leaves varies based on the developmental stage as shown in Figure 1. The leaf extract has been prepared for optical absorbance studies as per the workflow indicated in Figure 2.

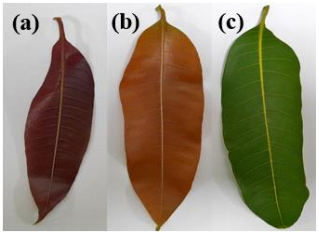

Figure 1: Leaves of *Mangifera indica* at different developmental stages (a) young, (b) intermediate, and (c) mature.

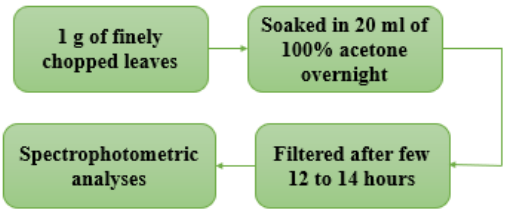

Figure 2: Workflow of pigment extraction from leaves of *Mangifera indica*.

The absorption spectrum of each extract was obtained using a spectrophotometer (Holmarc HO-SP-3480, India). The spectrophotometer arrangement for absorbance studies of the sample after extraction is shown in figure 3.

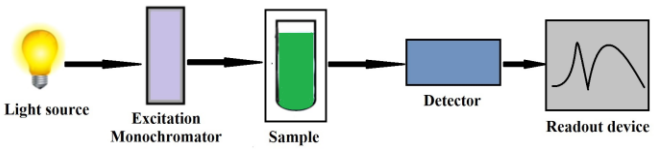

Figure 3: Experimental setup for absorbance studies.

The fluorescence spectrum of all these leaves has been obtained by illuminating the leaves at a particular point using a 360 nm LED source which is connected to one end of the Y optical fiber probe with the probe tip angled at 45^0 placed normal to the sample. The other end of the probe is connected to a spectrometer (Maya 2000, Ocean optics – USA), connected to a PC and the spectrum is displayed on the monitor as shown in Figure 4.

The collected spectra were then analyzed using MATLAB (9.12.0.2009381 (R2022a), Mathworks, USA).

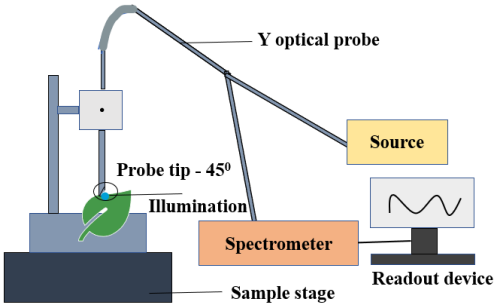

Figure 4: Experimental setup for fluorescence studies.

RESULTS AND DISCUSSIONS

Figure 5 represents the optical absorbance results of the leaf extracts. The peak at 666 nm in the absorption spectrum is due to the presence of chlorophyll molecules. The intensity of the optical absorbance increases as the leaf matures. The mature leaf has the highest optical absorbance intensity compared to the young and intermediate leaves of *Mangifera indica*.

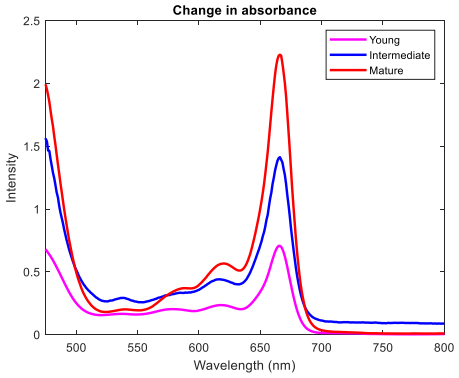

Figure 5: Optical absorbance results of the leaf extracts.

Figure 6 shows the fluorescence spectra of the leaf sample with fluorescence emission at 684 nm and re-emission at 740 nm upon excitation at 360 nm. It found that the fluorescence intensity of the young leaf is lesser than that of intermediate and mature leaves. The absorbance and fluorescence spectrum of the extracts and the whole leaf sample follows a similar trend which confirms that chlorophyll content in the leaves increases as they age. A peak at 418 nm is visible only in the young leaf which could be due to the presence of pigment anthocyanin that protects the leaves from photoinhibition due to intense light.

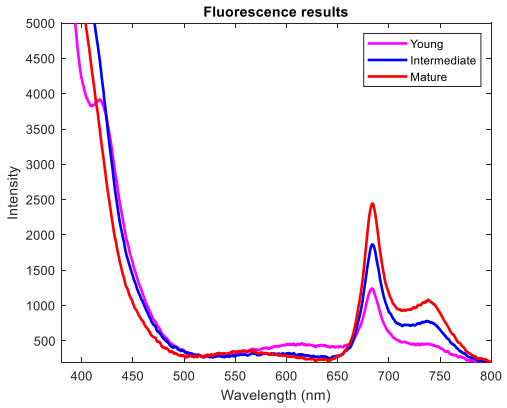

Figure 6: Fluorescence results of the leaf sample.

As the leaf matures, these protective pigments slowly degrade. The broad peak at 500 to 650 nm is mostly found in all the 3 developmental stages of mango leaves which could be due to the presence of carotenoids pigments. This follows a similar trend showing very less difference in all three developmental stages and may not be used to clearly distinguish between the leaf age in this case. But a clear difference in the intensity of this particular pigment can be seen in the ripening process of mango fruit [6]. Therefore, chlorophyll fluorescence at 684 nm in leaves can be used as a potential marker to identify the age of the leaf in *Mangifera indica.*

CONCLUSION

Fluorescence spectroscopy can be used as a potential tool in correctly identifying the leaf age in *Mangifera indica*. From the results obtained, it is understood that the chlorophyll fluorescence shows a clear difference in the intensities for all the 3 developmental stages. From this study, it can be concluded that the photosynthetic capacity depends on the age of the leaves and can be quantified based on chlorophyll fluorescence. By knowing the chlorophyll content along with the presence other pigments in leaves, it is possible to identify the leaf age and the right time to extract the phytochemicals with high medicinal value which can be used for various medical applications based on human needs. Further studies should be conducted to confirm the presence of other pigments in order to classify the developmental stages of leaves accurately.

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