

## Spectroscopic Characterization of *Phyllanthus muellerianus* Plant Leaves Dye

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**Abstract:** The aim of this investigation is to characterize the *Phyllanthus muellerianus* plant dye used as a colour source in painting walls and craft works for ages in Oghé, Ezeagu Local Government Area of Enugu State. Spectroscopic (UV-Vis, FT-IR and <sup>1</sup>H NMR) techniques were employed to characterize P. muellerianus dye. UV-Vis absorption spectrum shows peaks at 313 nm and 482 nm being attributes of anthocyanine. According to FT-IR and <sup>1</sup>H NMR investigations the tested sample have cyaniding glycoside and peonidin glycoside.

**Keywords:** *Phyllanthus muellerianus*; Spectroscopic; dye; absorption; functional groups; cyaniding glycoside.

### INTRODUCTION

Most natural dyes are based on vegetable origins which are agro-renewable, bio- and environmentally friendly. Natural dyes produce compatible vibrant colours creating a palette that blends with each other to give various shades to colour natural fibers and art works due to the nature, and structure of the dye, the compatibility of mordants and the conditions of dyeing methods [1].

Natural degradable dyes find application chiefly for colouration of textiles, food, drugs, and cosmetics and for colouration of wood carvings, candle, leather, paper etc [2].

The production of synthetic dyes and dyeing of textile are considered a risk and harmful to the environment, and to the people working in dyes production industries and textile processing and potential consumers due to the hazardous chemicals and finishers [3]. In spite of the brilliant colours and high quality colour fastness properties of synthetic dyes there is a huge concern due to the petrochemicals that are problematic and contain toxic or carcinogenic amines which are not environmental friendly. Now that it has been scientifically proven that synthetic dyes does more harm than good the environmentalist and World Health Organization have called for the replacement of synthetic dyes with natural dyes, which are less hazardous to health and environmental friendly [4-8].

Various quality attributes of fruits and vegetables such as colour and taste are due to the presence of phenolic compounds especially anthocyanins that absorb in the visible region of the electromagnetic spectrum [9]. The anthocyanin ring system which carries eight conjugated double bonds and a positive charge is responsible for the intense colour under acidic conditions [10]. Also the presence of at least one of the following radicals called

chromophores (which provide colour) and auxochromes (which intensify and deepened the colour) in a structure of an organic molecules makes it coloured, which can selectively absorb and reflect incident light (tables 1) [11].

The specific states of unsaturation of chromophores enable them to absorb and reflect incident electromagnetic radiation within a very narrow band of visible light. Loosely held electrons in the conjugated system of the organic radicals called chromophores and the influence by auxochromes on the orbitals causes these electrons to absorb and reflect intensified incident light energy only of specific wave lengths [11]. The colour fastness properties of the fiber polymer improves with auxochromes that increase the overall polarity of the dye molecule and make it more readily soluble in water and more readily attracted to the fiber polymer (Figure-2.ππ1) [11].

The majority of dyes can be regarded as resonance hybrids with the colours obtained depending on the energy state of the orbitals, lengthening the conjugated chains increases the number of double bonds and decreases the energy gap between the π-

orbitals that is required to excite the electrons and greater will be the wavelength of the absorbed light. As the number (n) of double bonds increase, there is a clearly defined shift of the light absorbed towards red, with a corresponding relative increase in the proportion of blue reflected [3].

Many of the natural dyes absorb in UV region and therefore fabrics dyed with such dyes should offer good protection from UV light [12-14].

*Phyllanthus muellerianus* is one of the plants of phyllanthaceae and is a small often stunted tropical plant that is found all over West Africa. The plant, *Phyllanthus muellerianus* is a glabrous shrub or woody climber found in savannah and drier secondary forest common in coastal thickets and scrub and widespread in parts of tropical African [15].

Among other uses of the plant, is in East Africa where the brown dye extract from the bark is used to dye mats and fishing lines. From the whole plant a black dye is obtained used to colour fibers [16].

## MATERIALS AND METHODS

### Plant sample collection and processing

*Phyllanthus muellerianus* leaves were collected from Agbani near Faculty of Applied Natural Sciences of Enugu State University of Science and Technology. They were dried in a subdued sunlight and ground into powder form with the aid of Kika Grinding Mill (Model MP300-20 A11 basic). The powder form was used to extract the corresponding dyes.

### Extraction of Plant dyes

The powdered leaves material (1.5 kg) was percolated in acetone and water (50/50 v/v) at the ratio of 1:3 for 48 hours and filtered. The filtrate was made free of acetone by distillation and the aqueous filtrate was partitioned successively with n-hexane, chloroform, ethyl acetate and n-butanol. The fractions were concentrated using rotary evaporator. The concentrate was dried at 65°C in a Pickstone Thermostatic Oven series 30/300 (Model BD/AL) to a constant weight [17].

### Column chromatographic separation of dyes

A column prepared with chloroform and silica gel was impregnated with a solution of ethyl acetate fraction in (1g in 7.5 mL) methanol. This was eluted with gradient of n-hexane increasing the polarity with ethyl acetate. Eight fraction were collected and TLC analysis conducted [18].

### Determination of UV-Vis. absorption spectra

Peak pick method was employed using Thermo Electron UV spectrometer equipped with vision pro-software's v4.10. Spectroscopic grade chloroform

was used for the dissolution of the samples while chloroform without added reagent was used as reference solutions. The fractions of the dyes in chloroform (mg/ml) were scanned through UV-visible spectrophotometer [19, 20].

### FT-IR spectroscopy analysis

FT-IR – 8400S Fourier transform infrared spectrophotometer was employed in functional group determination since different functional groups in molecule vibrate at distinctly different frequencies. IR spectra are useful for qualitative identification of molecular compound [21, 22].

### NMR studies

In this study NMR (Model Agilent-NMR-vnmrs400) was used to determine the peak and chemical shift of the dye.

Nuclear magnetic resonance (NMR) is an analytical technique was used to probe the nature and chemical structure of the dyes employing proton (<sup>1</sup>HNMR) [23].

## RESULTS AND DISCUSSIONS

The UV/visible spectra of the F1 sample revealed that it absorbs at (313 and 482 nm) (Fig-2). The wavelength of maximum absorption of the F1 sample at 313 nm is within the range 315-325 and is attributed to the absorption from the B-ring cinnanoyl system [24]. Most natural dyes that absorb between 280nm and 320nm can be expected to offer good to excellent protection from the harmful ultraviolet radiation [25]. The F1 dye of *P. muellerianus* plant leaves going by the UV/ visible absorption at the ultraviolet region (313 nm) will probably confer excellent protection against UV-A to B radiation thereby preventing penetration UV-A radiation to the skin and development of cancer.

The F1 dye also shows absorption in the visible region at 482 nm that agrees the fact that most plant natural dye shows absorbance in the visible region that usually occurs in between 465 and 550 nm [26, 27].

### The result of FTIR analysis of dye F1

The area between the bands at 1300 – 1000 cm<sup>-1</sup> represents presence of stretching vibration of alcoholic and phenolic group's containing C-H, and C-O vibration of aromatic compounds [28]. Ring stretching vibrations occur in the region between 1600-1300 cm<sup>-1</sup> that represents C-O and C-C stretching vibrations of glucoside bonds. The absorption involves stretching and contraction of all of the bonds in the ring and interaction between these stretching modes. The absorption at 1512 cm<sup>-1</sup> of F1 dyes therefore are due to stretching and contraction of all of the bonds in the ring of a hetero aromatic compound. This is region (1500-

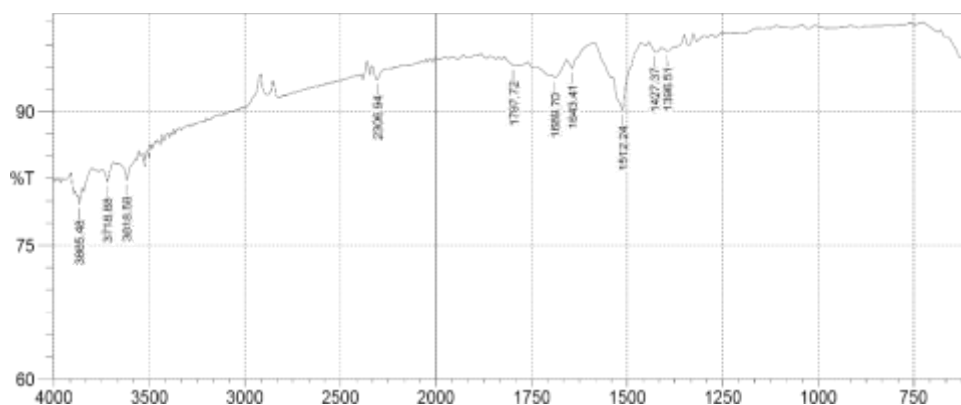
1520  $\text{cm}^{-1}$ ) of double bonds of which carbonyl group is the most important that give the very strong intensity in their IR spectra. Weak IR absorption bands in this region reveal the existence of carbonyl group as impurities. The band in F1 dye sample, within this region reveal strong absorption bands and represents

presence of carbonyl group ( $\text{C}=\text{O}$ ) from aldehydes and ketones [28].

The absorption at  $1797\text{ cm}^{-1}$  of the F1 sample is within the range of  $2000\text{-}1700\text{ cm}^{-1}$  that is an attribute of overtone and combination of aromatic compounds with a high intensity [29, 28].

**Table 1: IR spectra data result of purified (F1) dye**

Peak	Intensity	Assignment
1396.51	96.774	C-O and C-C stretching
1427.37	96.647	C—O Str.
1512.24	90.104	C=C Bending (Aromatic)
1211.34	94.791	C—O Str
1689.7	93.777	C=O Str.
1797.72	95.089	C=O (overtone and combination bands of aromatic compounds)
2306.94	93.551	C=C Accumulated double bonds
3618.58	82.275	—OCH <sub>3</sub> (para position) methoxyl group (-OCH <sub>3</sub> )
3718.88	82.065	X—H (X = H, O, S etc.) stretching vibrations
3865.48	79.587	X—H (X = H, O, S etc.) stretching vibrations



**Fig-2: FTIR analysis result of dye sample F1**

The absorption bands of hydroxyl groups are situated in the  $3650\text{-}3200\text{ cm}^{-1}$  region corresponds to stretching vibrations of OH groups. For the fact there is an absorption at  $1300\text{-}1000\text{ cm}^{-1}$  that represents the presence of stretching vibration of alcoholic and phenolic group's containing C-O vibration of aromatic compounds [30, 28] confirming that phenols is in the aromatic ring of the F1 dye.

The absorption at  $3618\text{ cm}^{-1}$  is an attribute methoxyl group ( $-\text{OCH}_3$ ) and its presence in F1 dye sample suggests a peonidin anthocyanin [20].

This is the region ( $4000\text{-}3600\text{ cm}^{-1}$ ) of stretching vibration frequencies of the functional groups containing hydrogen atoms, that is, the group of X—H (X: C, H, O, S, etc.) [30]. The absorption at 3718 and 3865 confirms the stretching vibrations of X—H in F1 dye sample.

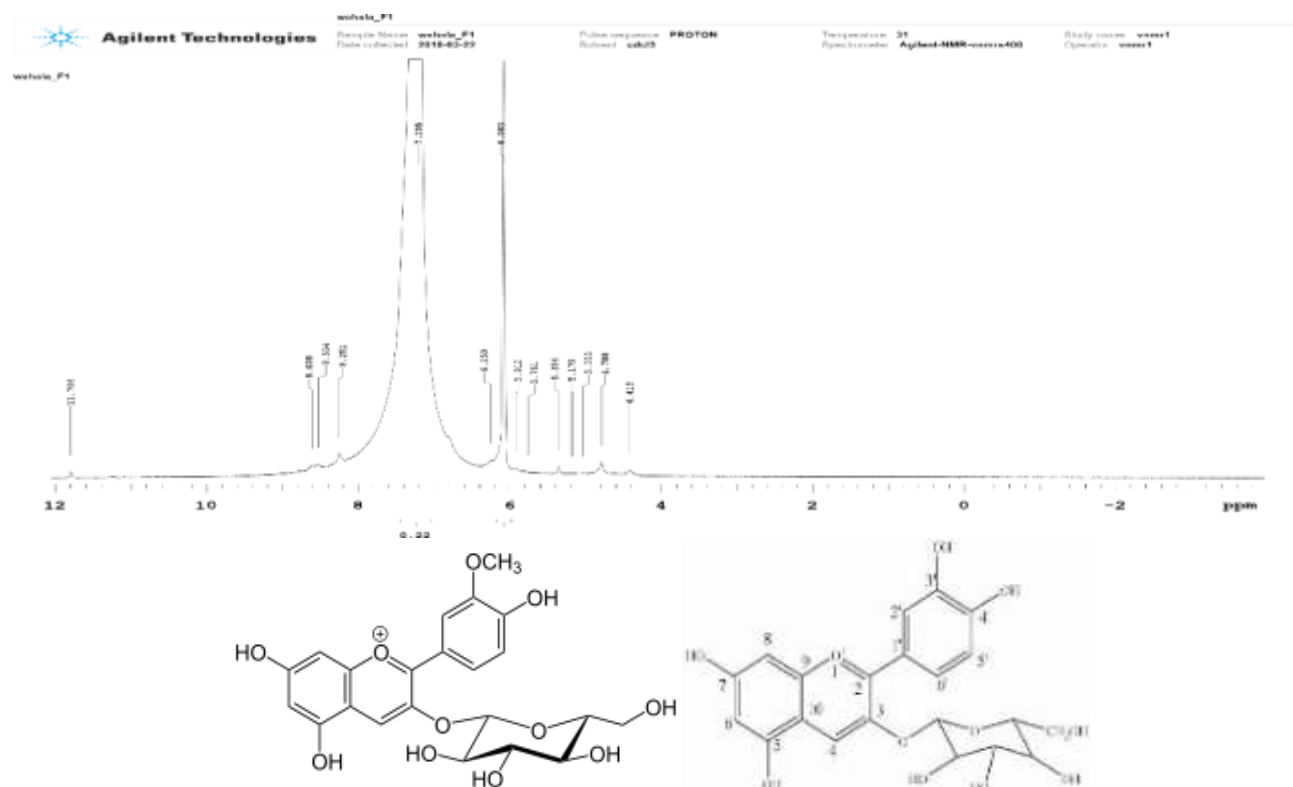
**The result of <sup>1</sup>H NMR analysis of dye F1**

The spectra of the F1 dye are shown in Fig-3. The spectra of the samples are suggestive of the

presence of an anthocyanin, with aromatic signal in the 6-9 ppm region [31, 32].

The signal in sample F1 (8.6, 8.5 and 8.3 ppm) could be due to the presence of aromatic protons of an anthocyanidin. The <sup>1</sup>H NMR spectra of F1 is dominated by the resonances from cyanidin 3- glycosides (cy 3 – gly). The signal at 8.3 ppm represent proton at carbon atom number 6 of cyanidin aglycone while 8.6 ppm are assigned to carbon atom number 4 of cyanidin aglycone. It can then be concluded that sample F1 contain cyanidin 3 – glycoside. The methoxyl group ( $-\text{OCH}_3$ ) in the sample at  $3618\text{ cm}^{-1}$  (FTIR peak) is suggestive of the presence of peonidin anthocyanin as another compound in dye sample F1 [20].

One of the major important achievements of this research is confirmation of the presence of cyanidin glycoside in *Phyllanthus muellerianus* leaves extract by the doublet in 8.3 ppm and 8.6 ppm being attributes of anthocyanin glycoside.



**Fig-3: Result of <sup>1</sup>H NMR of dye sample F1**

## CONCLUSIONS

A characteristic <sup>1</sup>H NMR spectra was obtained from the F1 dye samples of *Phyllanthus muellerianus* plant leaves. The analysis of F1 dye sample revealed the presence of signal at 8.6, 8.5 and 8.3 ppm. They are probably due to the presence of aromatic protons of cyanidin glycoside and the methoxyl group (-OCH<sub>3</sub>) in the sample at 3618 cm<sup>-1</sup> (FTIR peak) is suggestive of the presence of peonidin glycoside as another compound and are all anthocyanin respectively.

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