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NATURAL PIGMENT: CHLOROPHYLL, CAROTENOID AND ANTHOCYANIN IN THE LEAVES OF FOUR ORNAMENTAL PLANTS.

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ABSTRACT
The quantities of some essential plant pigments: chlorophyll, anthocyanin and carotenoid in the leaves of four ornamental plants were assessed. These ornamental plants were: Acalypha wilkesiana, Acalypha hoffmannii, Ficus panda and Gardenia jasminoides-variegata. Standard procedures were followed in the collection, identification and assessment of these plants. Among the plants, A. wilkesiana had the highest content of anthocyanins (2783.10 mg/kg), which could be responsible for the red color of its leaves, while A. hoffmannii had the highest quantity of chlorophyll (932.15 mg/kg) and chlorophyll is responsible for the green colour of plants. Ficus panda which has yellow leaves had the highest carotenoid content (389.40 mg/kg) and the carotenoid pigment is responsible for the yellow colour of plants. Gardenia jasminoides-variegata which has cream to white leaves had the lowest contents of all three pigments when compared across the plants, but among the pigments, the quantity of carotenoid responsible for the yellow color of plants was the highest (116.70 mg/kg) compared to that of chlorophyll (55.16 mg/kg) and anthocyanin (18.50 mg/kg). The study showed variation in the concentration of pigments in the leaves across the plants and these concentrations are probably responsible for the different colours exhibited by these ornamental plants.
Keywords: anthocyanin, carotenoid, chlorophyll, natural, ornamental plants

INTRODUCTION

Gardens and houses are beautified with ornamental plants having different colours. These colours are as a result of or combination of pigments. Delgado-Vargas *et al*. (2000) described these pigments as chemical compounds that absorb light in the wavelength range of the visible region. Hence, Heldt (2005) reported that any chemical compound which absorbs visible radiation between about 380 nm (violet) and 760 nm (ruby-red) is considered a pigment. According to Hoffman and Puszynski (2009), a pigment is any material capable of absorbing light. Plant pigments give colour to leaves, flowers, and fruits and are also important in controlling photosynthesis, growth, and development. Natural occurring plant pigments possess different structures and biochemical features. Consequently, they are responsible for the capture of lights at different wavelengths.

There are several types of pigments in leaves, mostly chlorophyll, carotenoid, and anthocyanin (Davies, 2004; Sousa, 2022). Chlorophyll, as the major type of plant pigment, captures yellow and blue light required for photosynthesis. Davies (2004) reported that pigments responsible for the appearance of colours in higher plants are

classified in several groups: chlorophylls, carotenoids (carotenes, xanthophylls), flavonoids (chalcones, anthocyanins, flavones, flavonols) and betalains (betaxanthin, betacyanin). Also, Dzugan (2006) documented that there are two kinds of chlorophyll in higher plants: green-blue chlorophyll *a*, and green-yellow chlorophyll *b*. Their amounts depend on the species of plant (Shweta and Agrawal, 2006), light conditions, and the availability of minerals such as magnesium (Mg). Replacing Mg with Fe ions gives a grey-brown chlorophyll product, and the presence of Zn and Cu ions increases the stability of the natural green colour. Plants growing in shade contain less chlorophyll *a* and more chlorophyll *b*. However, Nishio (2000) reported that the success of green pigment results not only from chlorophyll's ability to absorb light under changing irradiance and maximal utilization of light energy in photosynthetic electron transport, but also from its ability to protect chloroplasts from light excess by dissipating it in the form of heat or fluorescence radiation. Thus, the greenness of plants is a result of complex reactions of chlorophyll biosynthesis.

On the other hand, carotenoids absorb light of 400–500 nm wavelength, which accounts for their orange colouring (yellow, orange or red). In addition to their role in energy transfer to chlorophylls, carotenoids also have a key function in chloroplast protection under excess light conditions. By dissipating the excess energy, they prevent reactive oxygen species production, photo-oxidation and damage to the photosynthetic system (Britton, 1995; Niyogi, 2000; Cazzonelli, 2011). Carotenoids are an important part of the diet, comprising a source of vitamin A and antioxidants. One of the earliest genetic modifications was the introduction of β-carotene, a vitamin A precursor, into rice, which does not contain vitamin A or any of its biochemical precursors. Rice lines genetically modified with genes encoding phytoene synthase and lycopene β-cyclase originating from *Narcissus pseudonarcissus* and *Erwinia uredovora* were produced. The transformed lines produced β-carotene manifested phenotypically as orange pigmentation of rice grains, giving rise to the name "Golden Rice" (Giuliano *et al*., 2000; Al-Babili and Beyer, 2005).

Anthocyanins play an important role in facilitating plant reproduction as they attract pollinators and seed dispersers by imparting bright colours (Harborne and Williams, 2000; Hoballah *et al*., 2007). In addition to their colourful characteristics, anthocyanins protect plants from several biotic and abiotic stresses (Chalker-Scott, 1999; Ahmed *et al*., 2014), which may provide them a better adaptation to climate change. Anthocyanins are photoprotective agents which shade and protect the photosynthetic apparatus by absorbing excess visible and UV light and scavenging free radicals (Guo *et al*., 2008). Joseph *et al.* (1999) suggested that anthocyanins may be physiologically relevant in aging leaves by being very strong anti-oxidants and acts as a sunscreen, protecting chloroplasts that are vulnerable as they take the chlorophyll molecules apart under autumn conditions of cold temperatures and bright sunlight. Anthocyanin, a water-soluble pigment whose colour varies depending on pH, from red (when acidic) to blue (when neutral) absorb visible light in the blue-green wavelengths. They are stored in the vacuoles and are responsible for the pinkish-red colour of most flower petals, fruits and almost all red leaves in the fall. Other essential plant pigments include flavonoids, xanthophyll, betalain, porphyrins and much more. All these pigments stimulate the process of chemical reactions by reflecting the different wavelengths.

This study is aimed at quantifying some essential pigments which include chlorophyll, carotenoid and anthocyanin in the leaves of four ornamental plants; *Acalypha wilkesiena* Mull Arg., *Acalypha hoffmannii* Mull Arg., *Ficus panda* L. and *Gardenia jasminoides-variegata* J. Ellis.

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MATERIALS AND METHODS

The leaves of the plants (*A. wilkesiena* Mull Arg., *A. hoffmannii* Mull Arg., *F. panda* L. and *G. jasminoides-variegata* J. Ellis) were collected within the University of Port Harcourt and were properly identified (Plates I – IV). The plant samples were separated for chlorophyll, carotenoid and anthocyanin determination.

Plate I: *Acalypha wilkesiena*

Plate II: *Acalypha hoffmannii*

Plate III: *Ficus panda*

Plate IV: *Gardenia jasminoides variegata*

Chlorophyll Determination

The chlorophyll was determined using the Comar and Zachelle (1942) method. Thesample (0.1 g) was homogenized or macerated by adding 10 ml 85% acetone. The sample was filtered on Buchner funnel and washed with 85% acetone. Homogenization and filtration were repeated until filtrate and washings were colourless. Homogenization was done once again with 10 ml 85% acetone and after filtration, water was added to adjust acetone filtrate concentration. The combined filtrates and washings were then transferred to

a suitable volumetric flask and diluted to volume with 85% acetone.

Twenty-five (25) ml or suitable aliquot of extract was added to 50 ml diethyl ether in separating funnel. It was well mixed and water was added until the chlorophyll passed into the ether layer. The water layer was discarded and the ether layer was washed 5 times with water. The ether phase was then transferred to a volumetric flask, diluted to volume with ether and mixed well. 2 g of Na_2SO_4 was added and allowed to stand with occasional shaking until a clear solution was obtained. The optical density was then measured at 660 nm and 643 nm. The total chlorophyll content was calculated using the following formula:

$$If\ C = total\ chlorophyll\ in\ ether\ solution\ (mg\ l^{-1})$$

$$= 7.12 \times optical\ density\ at\ 660\ nm + 16.8 \times optical\ density\ at\ 643\ nm\ then:$$

$$Total\ chlorophyll\ (\%) = \frac{C(mg\ l^{-1}) \times ether\ solution\ (ml) \times acetone\ extract\ (ml)}{10^4 \times acetone\ aliquot\ (ml) \times sample\ weight\ (g)}$$

Carotenoid Determination

Fifteen grams (15 g) of the sample, plus 3 g of celite 454 (Tedia, Ohio, USA) were weighed in a mortar on a digital balance (Bel Engineering, model MA0434/05). For the carotenoid extraction, successive additions of 25 mL of acetone were made to obtain a paste, which was transferred into a sintered funnel (5 µm) coupled to a 250 mL Buchner flask and filtered under vacuum. This procedure was repeated 3 times until the sample became colourless. The extract obtained was transferred to a 500 mL separatory funnel containing 40 mL of petroleum ether. The acetone was removed through the slow addition of ultrapure water (Milli-Q - Millipore) to prevent emulsion formation. The aqueous phase was discarded. This procedure was repeated four times until no residual solvent remained, then the extract was transferred through a funnel to a 50 mL volumetric flask containing 15 g of anhydrous sodium sulfate. The volume was made up by petroleum ether, and the samples were read at 450 nm. The total carotenoid content was calculated using the following formula:

$$\boldsymbol{Carotenoids\ Content}\ (\mu \boldsymbol{g/g}) = \frac{\boldsymbol{AxVx10000}}{\boldsymbol{A1\%\ xP(g)}}$$

where A = Absorbance; V = Total extract volume; P = sample weight; $A_{1cm}^{1\%}$ = 2592 (β-carotene Extinction Coefficient in petroleum ether

Anthocyanin determination

Using an analytical balance, 150 g of the sample was blended using a normal kitchen blender to get a soft puree. A 1000 mL solution of 0.1 M HCl (hydrochloric acid) was prepared. Each puree was extracted with ethanol solvent: 0.1 M HCl (85:15%, v:v). The ratio used between the sample puree and the solvent for extraction was 1:2. The 225 mL of ethanol and 45 mL HCl were mixed together and then added to the sample puree. After that, a magnetic stirrer was used to mix the mixture for 1 hour, then the mixture was filtered using vacuum filtration by a Buchner apparatus to collect supernatant solutions. The extraction procedure was done in triplicate.

Preparation of buffers: The pH 1.0 buffer (0.025 M potassium chloride) and the pH 4.5 buffer (0.4 M sodium acetate) were prepared by using an analytical balance to weigh 1.86 g of KCl and 54.43 g of $CH_3CO_2Na{\cdot}3H_2O$ in a 1000 mL beaker respectively. After that, 980 mL of distilled water was added and mixed. The pH was measured using the pH meter and adjusted to pH 1.0 and pH 4.5 using HCl. The solution was transferred to a 1000 mL volumetric flask and the volume was made up to 1000 mL using distilled water.

Determination: The absorbance was measured within 20-50 minutes of preparation for the 2test solutions pH 1.0 and 4.5 using a spectrophotometer at 520 nm and 700 nm for the correction of haze. The diluted

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test solution that was found to be excessively turbid was filtered before measuring the absorbance using filter papers with ≤1.2 mm pore size to ensure that anthocyanins were not be absorbed. The blank used was distilled water and the test solutions were read. The anthocyanin pigment concentration was calculated using the following equation:

$$\text{Anthocyanin pigment} = \frac{A.MW.DF.10^3}{\varepsilon.l}$$

Where: A = (A520 nm - A700 nm) pH1.0-(A520 nm-A700 nm) pH4.5, MW = 449.2 g moL^{-1} for cyanidin-3-glucoside, DF= Dilution factor, l =Path-length (cm). ε = 26,900 molar extinction coefficient, in L/mol cm for cyanidin-3-glucoside, and 10^3 = Factor for conversion from g to mg.

RESULTS

The pigment concentrations differ within and amongst plants (Figure 1). The concentration of anthocyanin in *A. wilkesiana* was the highest (2783.1 mg/kg) compared to other plants, followed by *A. hoffmannii* (642.9 mg/kg), *F. panda* (45.2 mg/kg) and *G. jasminoides-variegata* (18.5 mg/kg) in that order. The chlorophyll content of *A. hoffmannii* (932.15 mg/kg) was higher than *A. wilkesiana* (216.39 mg/kg), *F. panda* (93.37 mg/kg) and *G. jasminoides-variegata* (55.16 mg/kg). Among the plants, *F. panda* had the highest carotenoid content (389.4 mg/kg) while *G. jasminoides-variegata* had the least (116.7 mg/kg).

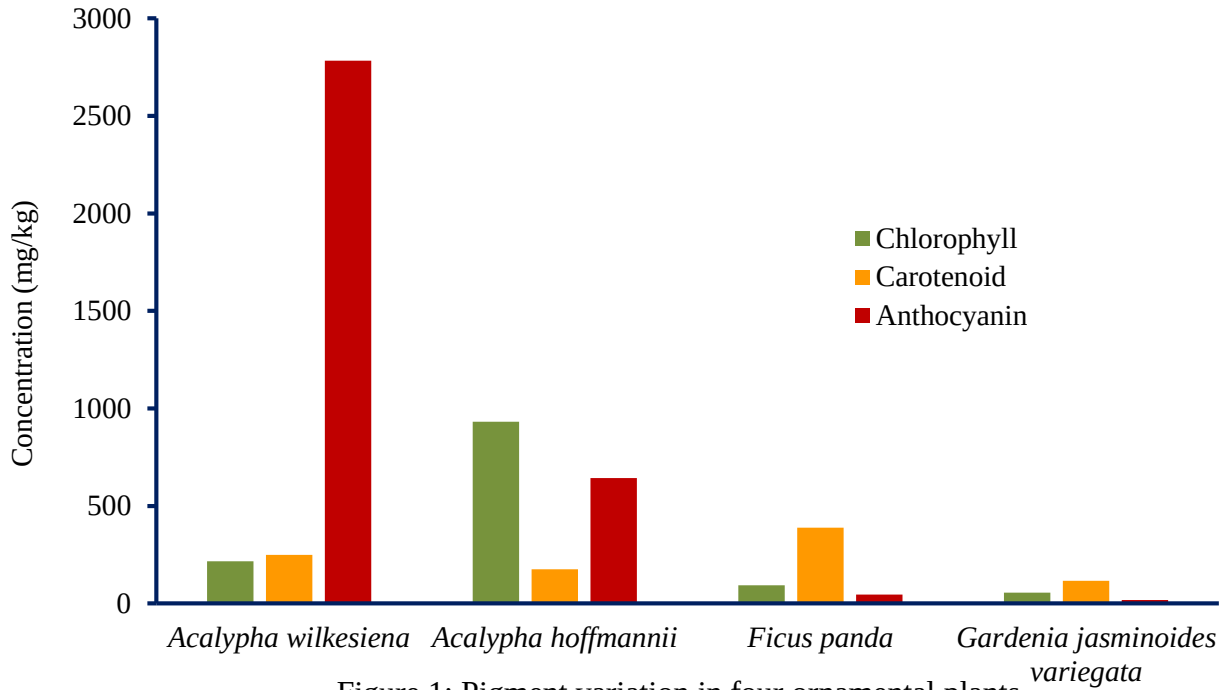

Figure 1: Pigment variation in four ornamental plants

DISCUSSION

The study showed that the quantity of pigments in the four ornamental plants differed, and this difference in colouration as seen among plants could be as a result of the varying concentration of the pigments (carotenoids, chlorophyll and anthocyanin) which is in alignment with Mlodzinska (2009), who stated that the intensive colours of some flowers, fruits and leaves are due to a combination of various pigments, often in complex structures called co-pigments. Earlier, Hatier and Gould (2007) reported that *Ophiopogon planiscapus* with very dark leaves contains an unusually high amount of chlorophyll *a* and *b*, and anthocyanins in surface layers of mesophyll. The quantity of

anthocyanin in *A. wilkesiana* was the highest among the plants, which could be responsible for its red colour and this agrees with the assertion of Nitarska *et al.* (2018), who documented that the red hues of *Poinsetta* are primarily influenced by its anthocyanin composition in his study of *Poinsetta* species (Euphorbiaceae). However, the concentration of chlorophyll in *A. hoffmannii* (932.15 mg/kg) was high compared to other plants. This support the work of Ghosh *et al.* (2018), who reported that the chlorophyll content of some green leafy plants like *Acalypha indica, Moringa oleifera, Ficus religiosa, Polyalthia longifolia* amongst others, were higher compared to than carotenoid content. This could suggest the green colour due to its high chlorophyll content. The total chlorophyll for *A. hoffmanni* (932.15 mg/kg) is higher than that of some green plants like *Moringa oleifera* (889.3 mg/kg), *Trigonella foenum-graecum* (437.40 mg/kg), *Alternanthera sessilis* (541.40 mg/kg), similar to *Spinacia oleracea* (0.9148 mg/g), and lower than that of *Sesbania grandiflora* (1.2731 mg/g) and *Piper betle*(1079.60 mg/kg) (Priyadharshana *et al.*, 2022). The concentration of carotenoid *F. panda* is high compared to other three ornamental plants assessed. Also, the quantity of carotenoid for *A. hoffmanni* (175.80 mg/kg) is higher than that reported by Priyadharshana *et al.* (2022): *Piper betle* (137.35 mg/kg), *Trigonella foenum-graecum* (127.55 mg/kg), *Spinacia oleracea* (95.53 mg/kg) and lower than *Moringa oleifera* (387.65 mg/kg), *Sesbania grandiflora* (360.87 mg/kg), *Alternanthera sessilis* (387.65 mg/kg). Again, the carotenoid contents of the tested plants were lower compared with the works of Pritwani and Manthur (2017) and Okonwu *et al.* (2018). Pritwani and Manthur (2017) reported the carotenoids value of 407.00 mg/kg while Okonwu *et al.* (2018) reported carotenoid value of 402.00 mg/kg for *Cucurbita moschata.*

CONCLUSION

From the study, it could be suggested that the difference in the colour of the ornamental leaves were as a result of variation in the concentration of the individual pigments present. The beauty of a garden with plants of different colours, is an indication of varying proportion of the pigment in the specific plants that make up the garden.

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