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PATTERN OF DISTRIBUTION OF ANTHOCYANIN PIGMENTS IN THE DIFFERENT TAXA OF *LANTANA*SPECIES (VERBENACEAE)

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ABSTRACT

Lantana species are widespread around the world and they are plants ound as ornamental as well as wild varieties. The colors of the flowers vary largely and the variation in colour is due to the presence of Anthocyanins. Anthocyanins are pigments that are responsible for the coloring of the plant parts; they are localized in various parts of the plants and are known to impart brilliant colors.Anthocyanins are known to occur in different colors based on the pH and thereby anthocyanins are used as pH indicators. Study of anthocyanins in varying pH can be done using spectrophotometric methods. This study involves extraction of anthocyanin pigments of *Lantana* flowers at different pH ranges by pH differential method (pH1 and pH5) and estimation of the anthocyanin content at 520nm, the result is an estimate of the total monomeric content of anthocyanins. Anthocyanin pigments are also estimated at two different wavelengths at 530nm and 657nm and the relative anthocyanin content expressed. The study clearly indicated the varying anthocyanin content with the change in colour of the flowers. This change in the chemical nature of the anthocyanin pigments may be due to genetic variation shown by the plants and thereby, leading to differing anthocyanins in the plant.

KEY WORDS: **Anthocyanins, *Lantana*, pH, variation, pigments**

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INTRODUCTION

Lantana species are native to South America. They are widespread and distributed around the world[1]*Lantana* the name is derived from the genus Viburnum which resembles a little in foliage and inflorescence. *Lantana* is found in cultivated as well as wild varieties. The wild type of *Lantana* are woody shrubs which have flowers of different colors like red, pink, white, orange, violet, yellow. The stem and branches could be thorny[2]. The cultivated variety is generally a short shrub that is known to spread and it is non-thorny. Leaves are generally smaller than the wild type. The flowers in *Lantana* have shown to vary in the colors and this colour variation is directly related to the age of the flowers.The flower colors have shown to undergo colour change after anthesis. Pollination was a trigger for this change in colour[3]. This colour variation in flowers is due to the anthocyanin content in the flowers and pollination is known to be a trigger for the synthesis of anthocyanins[3]Anthocyanins are the most important pigments that are responsible for the coloring of plant parts. These are intensely colored pigments and are water soluble[4]. The colour of fruits, vegetables, flowers, grains etc,. is primarily due to anthocyanins. These pigments impart various colors like red, violet, blue, scarlet, purple[5]. Chemically, anthocyanins are all based on cyanidin which is an aromatic compound. Anthocyanins are basically derivatives of cyanidin by addition or subtraction of hydroxyl groups or by methylation or by glycosylation[4]Anthocyanidins which are of six different kinds vary in their structures based on the glycosidic substitution at the 3 and 5 positions. Anthocyanidins are anthocyanin aglycones formed when anthocyanins are hydrolyzed with acid.[4,5]The most common of the anthocyanidins are cyanidin which is magenta in colour.Pelargonidin are orange-red colored and are known to have one hydroxyl group less than cyaniding. Mauve, purple and blue colors are generally due to delphinidin that are known to have one more hydroxyl group than cyanidin. The other anthocyanins are anthocyanidin methylesters like Peonidin that is derived from Cyanidin, Petunidin and Malvidin that are based on delphinidin. These six anthocyanidins are known to occur with various sugars attached as a range of glycosides (as anthocyanins), the main variation is the nature of sugar and it may be glucose, galactose, rhamnose, arabinose or xylose[4]Studies have shown that anthocyanins can occur in different colours based on pH [6] Anthocyanins are the largest group of flavonoids distributed in plants. Anthocyanins are used as pH indicators due to their varying response to different pH ranges, from red in acids to blue in bases[6]. The pH differential method has been used extensively by food technologists and horticulturists to assess the quality of fresh and processed fruits and vegetables. The method can be used for the determination of total monomeric anthocyanin content[7]Sondheimer and Kertesz in 1948 introduced the concept of determining the amount of anthocyanin present in a material by measuring the absorbance at 2 different pH values (3.4 and2.0)[8]. This concept was then further modified by various researchers and analyzed that monomeric anthocyanins undergo a reversible structural transformation as a function of pH. They appear as a coloredoxonium form at pH 1.0 and colorlesshemiketal form at pH 4.5. The difference in the absorbance at 530nm of these two pH samples would be directly proportional to the concentration of the pigmentAnthocyanins belong to a large family of more than 4000 flavonoid compounds found in plants and are known to be a part of the plants secondary metabolism. Anthocyanins refer to a pigment molecule called Anthocyanidin with one or more sugar residues attached to it. The anthocyanins are known to absorb light in both the visible and UV parts of the electromagnetic spectrum that gives them the colour[9]. Theanthocyaninsthat are known to be synthesized in the cytoplasm of plant cells are transported to the vacuole through the tonoplast and they accumulate in the vacuole.The colors of the flowers, fruits, vegetables that are seen due to the pigments are due to a whole range of interactions which include[9]:

- The number of hydroxyl and methoxyl groups attached to ring B
- Attachment of different sugars and aromatic acids to ring B
- pH of the vacuole in which they are present
- Shape of the cell in which they are present
- The formation of stacked complexes with one another, and with other pigments and ions
- The presence of various carotenoid pigments and flavanols
- The names of various anthocyanin's would be from the flowers the source from where they have been collected
- Some flowers have only one anthocyanin plus its derivatives, whereas others may contain two or more anthocyanin's and their derivatives[10]

It is believed that the coevolution of the flowering plants and insects could have involved strong selection pressures, by different kinds of pollinators, on the production of particular colors in flowers and fruits. This may have contributed to the anthocyanins becoming most widespread group of colored flavonoid pigments. They are responsible for the red, pink, mauve, purple, and blue colors found in the flowers, fruits and leaves of angiosperms, as well as some gymnosperms and some bryophytes[4]. It is the colour provided by the anthocyanins that attract the pollinators. The anthocyanins are located in the vacuole of the plant cell and do not participate in photosynthesis. .Anthocyanins are abundantly present in fruits, especially the fruit wall. They are richly present in flower petals also[10].Pigment production is often timed to coincide with flower fertility. Thus, pigments may appear just prior to the flower becoming fertile or in some cases flower colour may fade or the flower may turn from white to colored (leucoplast to chromoplast) or even change in colour following fertilization. These transformations may be the result of changes in petal cell pH as the flower ages, degradation of the pigment or de novo pigment biosynthesis. An example of the latter can be found in *Lantana*. The flowers are yellow initially when fertile but change to purple over an ageing period of 3 days. The yellow flowers contain grains whereas the older flower do not, and are preferred by butterfly pollinators.

Retention of purple older flowers maintain a large inflorescence which is more attractive to pollinators at a distance while still directing pollinators to fertile flowers[7]

MATERIALS AND METHODS

The flower samples of *Lantana* were collected from different locations in Karnataka and Kerala. The samples included 5 ornamental varieties and 5 native varieties. The collected samples were stored in methanol acidified in 1% HCl over night and was extracted in the same solvent system.

Extraction of anthocyanins

Anthocyanins can be extracted from plant tissues by various methods. One of the best method is to use methanol that is acidified with concentrated HCl (99% acidified methanol). The flower samples are collected and preserved in acidified methanol. They are then crushed with acidified methanol to extract the pigments. Anthocyanins were extracted and were estimated for their total monomeric content as well to know the amount of relative anthocyanins in the sample.

Method I: Anthocyanins at different pH

The technique used to estimate the anthocyanin content in the different samples of *Lantana* is a pH differential method[9], The pH of the extracted samples were adjusted to pH1and pH5, the samples were centrifuged at 5000 rpm for 10 minutes to remove the particulate matter if any and the absorbance read at a wavelength of 520nm.Anthocyanin concentration was calculated in mg/100cm[3].

$$\text{Anthocyanin concentration} = \frac{(\text{Abs pH1} - \text{Abs pH5}) \times \text{DF}}{0.775}$$

Abs pH1 = Absorbance at 520nm for the sample at pH1
Abs pH5 = Absorbance at 520nm for the sample at pH5
DF = Dilution factor (final volume of the extract/initial volume of the extract)

This method works on the principle that there are other pigments also in the plant extracts apart from anthocyanins, the other pigments are chlorophyll and carotenoids. But these pigments absorb negligible amount of light at the wavelength of 520nm. Studies shown that when the pH of the plant extract is less than 3, Most of the anthocyanin pigments exist as colored positively charged oxonium ions which strongly absorb the 520nm light. Raising the pH to 5 will cause the conversion of most of the flavylium cations to a colorless pseudo base form. This is a result of the disruption of some of the conjugated double bonds and consequently, less 520nm light will be absorbed. The difference between the two 520nm readings provides a measure of the quantity of anthocyanins in an extract. This method is been extensively used to study the anthocyanin content of fruits and the same technique is been used for this study[10]. The difference in the absorbance between the two different pH buffer solutions is due to the monomeric anthocyanin pigments.

Method II: Estimation of anthocyanins at two different wavelengths

The process of extraction of anthocyanins is as follows[11]. Flower samples collected were crushed and ground using 300µl of Methanol 1% HCl, this allowed extraction of pigments to happen. To 300µl of the extract, added 200µl of Milli-Q-H_2O, followed by 500µl of Chloroform to each tube. The tubes were centrifuged at 10,000 rpm for 5 minutes. Transferred 400µl of the upper layer to new vials. The volume in each vial was made up to 800µl by adding 400µl of 60% methanol 1% HCl: 40%, Milli-Q- H_2O solution to each vial. The absorbance of the sample is read at 530nm and 657nm using a spectrophotometer. 480µl methanol 1% HCl and 320µl Milli-Q H_2O for a total volume of 800 µl was used as a blank.The difference between the two wavelengths gave an estimate of the relative anthocyanins in the sample.

RESULTS

Extraction of anthocyanins at two different pH and the total amount of monomeric anthocyanins (mg/ml) in each sample.

Table 1
List of Ornamental plants

Sample	Flower colour	Absorbance at 520nm, pH 1	Absorbance at 520 nm, pH5	Anthocyanin (mg/mL)
I	Yellow	1.9745	0.0598	44.470
II	White	0.8786	0.0449	21.514
VIII	Crimson red	1.5958	0.1046	25.013
IX	Purple	1.1002	0.5041	11.537
X	White	0.7104	0.1637	10.581

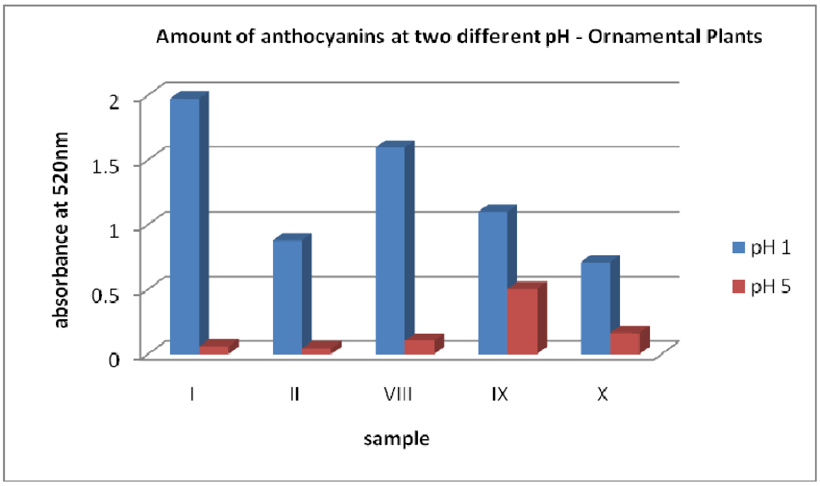

Figure 1
Extraction of anthocyanins at two different pH

Table 2
Wild type

Sample	Flower colour	Absorbance at pH1	Absorbance at pH5	Anthocyanin (mg/mL)
III	Red	0.9982	0.0205	27.754
IV	Pink	1.9120	1.6700	9.367
V	Pink	2.2801	0.3327	37.691
VI	Pink – Orange	2.6300	2.5024	2.469
VII	Orange	2.3952	0.3265	40.039

Result 2: Estimation of anthocyanins at two different wavelengths

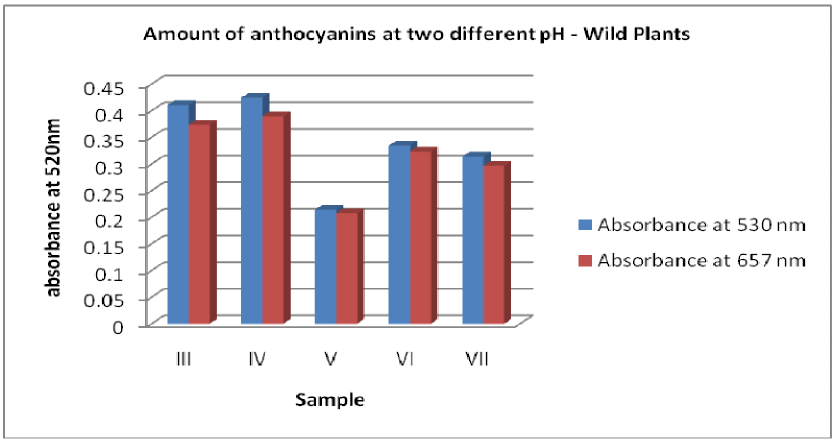

Figure2
Estimation of wild plant Anthocyanins at different wavelengths 530 nm and 657nm respectively.

Table 3
Ornamental plants

Sample	Flower colour	Absorbance at 530 nm	Absorbance at 657 nm	Relative anthocyanin content
I	Yellow	0.5176	0.4787	0.0389
II	White	0.3875	0.3733	0.0142
VIII	Crimson red	0.5040	0.4103	0.0937
IX	Purple	0.5475	0.4253	0.1222
X	White	0.3434	0.3336	0.0098

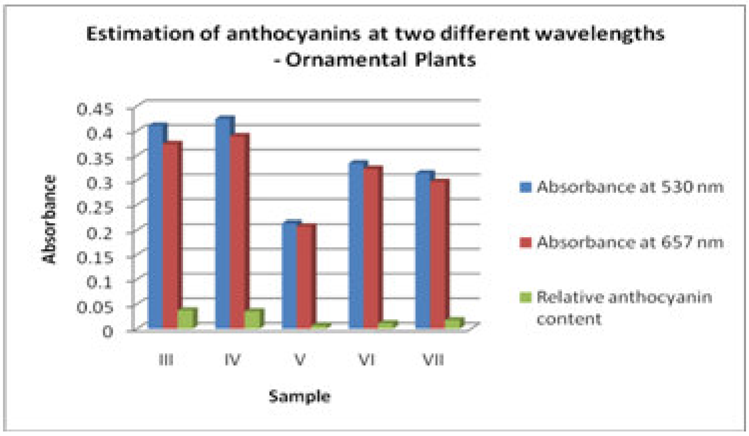

Figure 3
Estimation of ornamental plant Anthocyanins at different wavelengths 530 nm and 657nm respectively.

Table 4
Wild type:(With reference to figure.4)

Sample	Flower colour	Absorbance at 530 nm	Absorbance at 657 nm	Relative anthocyanin content
III	Red	0.4103	0.3732	0.0371
IV	Pink	0.4242	0.3891	0.0351
V	Pink	0.2136	0.2073	0.0063
VI	Pink – Orange	0.3341	0.3230	0.0111
VII	Orange	0.3139	0.2965	0.0174

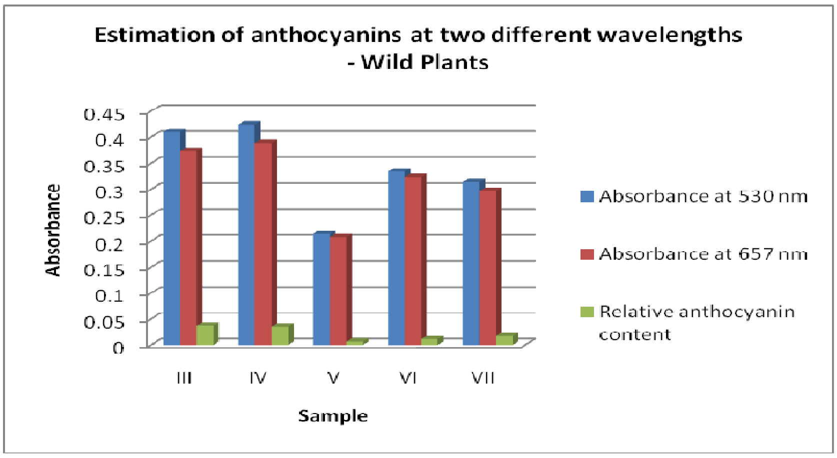

Figure 4
Estimation of wild plant Anthocyanins at different wavelengths 530 nm and 657nm respectively

Lantana species were introduced to India as a garden plant for their beautiful flowers. When they were introduced they had basic colour pattern like yellow, white, orange etc. As the years passed, many varieties of *Lantana*'s came into existence and they started spreading in every open space available as weed. The colour pattern in the flowers also has undergone marked changes. In many varieties, the basic colour pattern has changed to variegation of different shades. This may be due to the genetic variation of the plants that have undergone by cross pollination and change of habitats.

SUMMARY AND DISCUSSION

The present investigation which included the analysis of anthocyanin pigments and their distribution pattern among the various taxa using spectroscopy also revealed the change of chemical nature of anthocyanins (Table 1-4; Figure 1-4) The investigation was made by keeping a constant value of absorbance spectrum (520 nm) with various pH ranges 1-5. The experiment was repeated 2-3 times to ascertain the exact values. In all the cases, same values were obtained. This clearly indicated that the colour pattern of flowers which are morphologically visible correspond to the type of anthocyanins analyzed. The change of chemical nature of anthocyanins may be due to genetic variationof the plant that has undergone changes leading to change in the type of biosynthesis of anthocyanins in the plant body[12]. Further investigation on the distribution of genes may throw some light on the changed pattern of anthocyanins distribution in both the cultivated as well as wild varieties of *Lantana* species.

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