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RESEARCH ARTICLE

Isolation and Analysis of various Anthocyanins from the Corolla of Different Varieties of *Lantana* by TLC Method

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ABSTRACT

Anthocyanins are the most spectacular plant pigments that are localized in various parts of the plants like fruits, vegetables and flowers that give these plants their brilliant colour. The separation of these pigments present in different petals of *Lantana* was carried out using thin layer chromatography (TLC). The pigments were extracted from different varieties of *Lantana* collected from various locations; they include wild variety as well as ornamental variety. The samples collected include flowers of different colours like white, pink, yellow, scarlet red, red, orange, white and purple. The pigments were extracted with Methanol:HCL and then spotted on TLC plate. Single dimensional TLC was done using Butanol: Acetic Acid: Water as the mobile phase. The characteristic colours in the petals of *Lantana* is due to a mixture of anthocyanin pigments. The results revealed that the pigment that predominates in yellow flowers are carotenoids along with anthocyanins. The anthocyanins are found in epidermis and carotenoids are found in sub-epidermis. It is observed that the colour of the inflorescence changes from yellow to orange, scarlet and pink.

Key words: *Anthocyanins, pigments, Lantana, TLC.*

INTRODUCTION

Anthocyanins are natural colorants which have raised a growing interest due to their extensive range of colours, innocuous and beneficial health effects including anti-cancer, anti-inflammatory and vasoprotective effects preventing coronary heart diseases and improving visual activity [1]. They are water soluble polysaccharides found as pigments that give colour. They are basically secondary metabolites and the parent class of molecules referred to as flavonoids and their base color changes as the cell environment changes [2]. Anthocyanins generate red, pink, purple and blue colors. The wide variety of colours seen in trees, flowers and fruits is due to the presence of Anthocyanins. The red colour of cherries, cranberries, apples and blue colour of grapes, blueberries and plums are contributed by Anthocyanin. The more common forms of Anthocyanin yield orange-red, purple-red, bluish-purple, rosy-red, and a host of purple colors. Each pigment ranges widely in appearance based upon the conditions of the cell and the capacity to dissolve.

The genus *Lantana* (Verbenaceae) is mostly native to subtropical and tropical America; few taxa are indigenous to tropical Asia and Africa and occur approximately in 50 countries. Studies have shown that the genus is a difficult one to classify as the species are generally not very stable and is very wide spread. *Lantana* is a plant that was introduced in India as an ornamental plant by the British in 1807 for the Calcutta botanical garden. It has spread across India and there are around 150 known species of *Lantana*[3]. These plants are grown as ornamental, wild varieties and morphological differences exists between them. The wild variety of *Lantana* is seen as a woody shrub with thorns, that can grow as tall shrubs up to 6ft or more, while the cultivated

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variety is generally a short shrub that spreads in the ground, it is non thorny, and the leaves are known to be smaller than the leaves of the wild variety .[4]

The plant has been used in many parts of the world to treat a wide variety of disorders, cancers and tumors. A tea prepared from the leaves and flowers was taken against fever, influenza and stomach-ache. In Central and South America, the leaves were made into a poultice to treat sores, chicken pox and measles. Fevers, cold, rheumatisms, asthma and high blood pressure were treated with preparations from the plant. In Ghana, infusion of the whole plant was used for bronchitis and the powdered root in milk was given to children for stomach-ache [7]. In Asian countries, leaves were used to treat cuts, rheumatisms, ulcers and as a vermifuge. Decoctions were applied externally for leprosy and scabies.The plants show inflorescence of different colours, varying from pink, lavender, scarlet red, orange, white, and there has been a significant observation of colour change in the inflorescence from yellow to various colours or from pale colour to the darker colour. The colour of the flowers is mainly due to varying amounts of Anthocyanins and carotenoids. These are mainly distributed among flowers, fruits, and vegetables and are responsible for most of the red, blue, and purple color [5]. *Lantana* has been attractive because the colour of the inflorescence, also interesting is the colour change in the flowers. [6]has reported the colour change in the flowers of *Lantana*, and found that old nonfunctional flowers would increase optical attractiveness of the inflorescence and become distinct from young functional flowers . The shape of the inflorescence is known to change with the age and flower colours are known to vary with age and maturity.

Studies also show that *Lantana* flowers undergo changes subsequent to anthesis. Flower pigments were chemically identified as delphinidinmonoglucoside and beta carotene. According to post pollination, shift in petal coloration is caused by masking of carotenoids by differential amounts of anthocyanins [7].

MATERIALS AND METHODS

Collection of samples:

The flower samples were collected from different locations in Karnataka and Kerala. The collected samples were stored in methanol acidified in 1% HCl overnight and was extracted in the same solvent system.

Extraction of Anthocyanin:

The given samples were macerated thoroughly extracted with acidified methanol (Methanol:HCl, 99:1,v/v) overnight at low temperature (4ºC) to avoid the hydrolysis and degradation of potential acyl groups in anthocyanin structure [8]. After extraction, the obtained extract was centrifuged and the clear supernatant was collected. The supernatant was concentrated by evaporating the samples at a relatively low temperature (<30 ºC).

TLC Technique:

For TLC silica gel plates (20 cm x 13 cm, Merck) were used. The solvent mixture used were n-Butanol: Acetic Acid: Water (4:1:5, v/v/v, Upper phase). The samples were spotted on the plate and maintained a distance of 1 cm between each spotted sample. The samples were allowed to run and at the completion of the run, the plate was viewed under UV and the florescent spots were marked in order to determine the Rf value of each pigment.

$$\text{Rf value} = \frac{\text{Distance travelled by sample}}{\text{Distance travelled by solvent}}$$

RESULTS AND DISCUSSION

The TLC was done on a set of ten different samples. The samples I, II, VIII, IX and X are ornamental varieties and samples III, IV, V, VI and VII are wild varieties. The TLC result of sample I shows five different spots and the probable pigments present are petunidin , malvidin and pelargonidin. Sample II shows three different spots and the probable pigments present are petunidin, peonidin and pelargonidin. Sample VIII shows eight different spots and the probable pigments present are delphinidin, petunidin, malvidin, pelargonidin.Sample IX shows six different spots and the probable pigments are delphinidin, cyaniding and pelargonidin.Sample X shows two different spots and the probable pigments are peonidin and pelargonidin

Sample III shows six different spots and the probable pigments present are petunidin, peonidin and pelargonidin. Sample IV shows five different spots and the probable pigments present are petunidin, peonidin and pelargonidin.Sample V shows seven different spots and the probable pigments preset are petunidin, malvidin, cyandin,

peonidin, pelargonidin.Sample VI shows five different spots and the probable pigments present are petunidin, peonidin and pelargonidin Sample VII shows four different spots and the probable pigments present are petunidin, peonidin and pelargonidin.

From the data obtained, we can understand that difference in colour of *Lantana* is due to difference in the anthocyanin pigments. The TLC analysis of the flowers from 10 different plants collected from ten different locations show a varying number of spot patterns that indicates varying separation and reflect genetic differences in different varieties. Some of the pigments in these flowers are similar while a variation shows that there could be different pigments and pigment concentration in different plants.

The analysis shows that the main pigments present could be Cyanidin, Delphinidin, Petunidin, Malvidin, Peonidin, Pelargonidin. The TLC data shows maximum occurrence of Petunidin, Pelargonidin, Peonidin in most of the samples. The pattern of change in colour of the flowers after pollination to different colours as scarlet, pink, violet etc may have a genetic link.

Studies have shown that the colour of the flowers in *Lantana* is known to change after the process of pollination. The fertile flowers are yellow which later turn to pink or purple. This observation is seen mostly in the varieties that are wild weed variety. This pattern is not so observed in the ornamental variety. In most of the ornamental variety studied here the colour of the older flowers is more intense compared to the younger ones. It is also observed that the pigment separation and the spot pattern shows maximum separation and occurrence of spots in darker flowers as Crimson red (seven spots) and the least number of spots in lighter colours like white. (Two spots). The present study shows that the number of spots and separation is much more in flowers that are darker and least in flowers that are lighter.

The pigment that predominates in yellow flowers is carotenoids along with anthocyanins. The Anthocyanins are found in epidermis and carotenoids are found in sub-epidermis. It is observed that the colour of the inflorescence changes from yellow to orange, scarlet, pink. Similar results were reported in the TLC chromatogram of Purple Petunia , white and violet petunia showed the presence of 4 spots and 2 spots respectively [9]. Separation and Identification of Anthocyanins extracted from the Mulberry fruit by TLC method was also reported [10].

The TLC is very rapid, convenient and economical and it can be used for the separation of different pigments from the plants. It is proved to be a good tool for the screening of pigments from flowers, and utilized for the identification of different species.

The TLC chromatogram obtained is shown in (**Figure 1 & 2**) and the results are tabulated in (**Table 1& 2**).

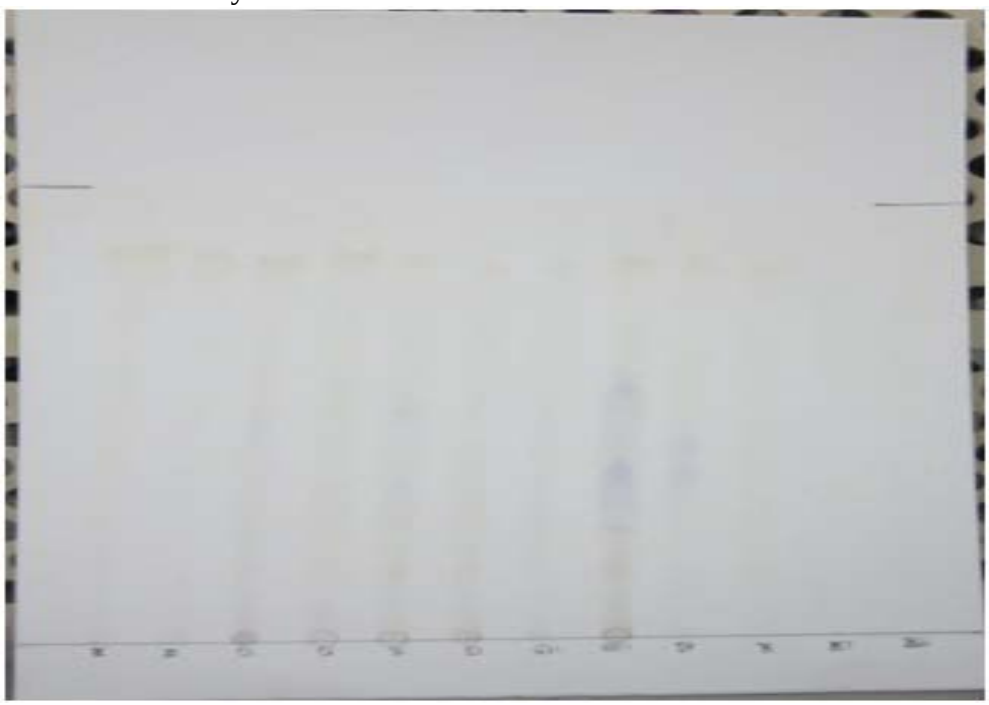

Figure 1: TLC chromatogram before UV exposure

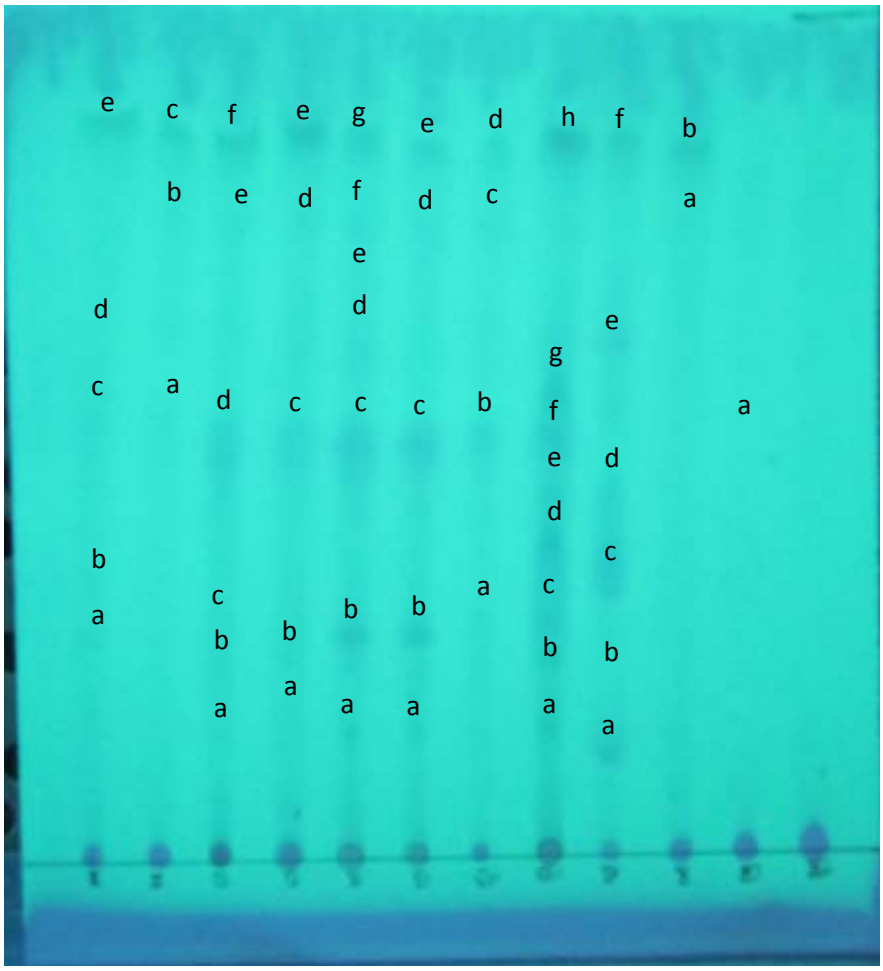

Figure 2: TLC chromatogram after UV exposure

The distance travelled by the solvent was 12.8 cm as measured from the origin. The Rf values obtained are tabulated.

Table 1: Ornamental plants

Sample	Flower colour	Spot	Rf value x 10	Probable pigments
I	Yellow	A	26	
		B	32	
		C	51	Petunidin
		D	57	Malvidin
		E	87	Pelargonidin
II	White	A	52	Petunidin
		B	75	Peonidin
		C	86	Pelargonidin
VIII	Crimson red	A	16	
		B	24	
		C	28	
		D	37	
		E	42	Delphinidin
		F	50	Petunidin
		G	55	Malvidin
		H	85	Pelargonidin
IX	Purple	A	13	
		B	25	
		C	32	
		D	42	Delphinidin
		E	62	Cyanidin
		F	85	Pelargonidin
X	White	A	75	Peonidin
		B	85	Pelargonidin

Table 2: Wild type

Sample	Flower colour	Spot	Rf value x 10	Probable pigments
III	Red	A	14	
		B	23	
		C	28	
		D	49	Petunidin
		E	75	Peonidin
		F	85	Pelargonidin
IV	Pink	A	17	
		B	25	
		C	49	Petunidin
		D	75	Peonidin
		E	86	Pelargonidin
V	Pink	A	15	
		B	26	
		C	49	Petunidin
		D	60	Malvidin
		E	69	Cyanidin
		F	71	Peonidin
		G	86	Pelargonidin
VI	Pink – Orange	A	15	
		B	26	
		C	49	Petunidin
		D	71	Peonidin
		E	85	Pelargonidin
VII	Orange	A	28	
		B	50	Petunidin
		C	75	Peonidin
		D	89	Pelargonidin

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