World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

DETERMINATION OF RETENTION FACTOR IN PIGMENTS OF PETAL

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Article Received on 04/10/2020 **Article Revised on 25/10/2020** **Article Accepted on 15/11/2020**

ABSTRACT

The investigation was carried out on separation of pigments from petals of flower of different plants. The five flowering plants such as *Ixora, Portulaca*, *Hibiscus, Clitoria* and *Lilium* were selected for pigment separation.The paper chromatography technique was used for separation of pigments.The separation of coloured pigments was observed and its values were recorded on the basis of distance traveled by specific pigment along with solvent. The distance of pigments traveled was measured in centimeters .The variation in retention factor was recorded in all the five plants The distance traveled by stationery phase and mobile phase on chromatography paper was used to determine retention factor (Rf) of pigments, in all the flower of selected plants .The Rf of was noted in *Ixora* (0.58) , *Portulaca* (0.53) , *Hibiscus* (0.72),*Clitoria* (0.69) and *Lilium* (0.79) The minimum Rf (0.53) was noted in petals of *Portulaca* flower and maximum Rf (0.79) in petals of Lilium flower.

KEY WORDS: *Flowers, Anthocyanin, Carotenoid, Chromatography, Retention factor.*

INTRODUCTION

The pigments are the chemical compounds that reflect only at a certain wavelengths of visible light. This makes them appear colourful. The corolla is a whorl of flower made up of petals The petals constitute various colours in different plants. The pigments of petals interact with light to absorb certain wavelengths .The aerial parts of plant, except flowers are consisted of chlorophyll pigments which is green coloured. The chlorophyll pigment can be separated by various chromatographic techniques. The chemical compounds in petals gives rise to yellow, orange, red and purple colour in wide variety of plants, animals and microorganisms[1] Anthocynins is a naturally occurring chemical compound which imparts colour to the flower .The chemical compounds of pigments are the glycosides of polyhydroxy and polymethoxy derivatives of 2- phenylbenzopyrylium belong to the class flavonoids. The flavonoids is synthesized via the phenylpropanoid pathway. They occur in all tissues of higher plants, including leaves, stems, roots, flowers and fruits.[2]

Paper chromatography is an analytical method used to separate colored chemicals or substances.[3] This technique is used to separate coloured chemical components or pigments from petals of flower with the help of solvent. The analytical technique of separation of pigments has a wide range applications in different field. The separation of pigments can be made by other types of techniques also such as TLC Column chromatography, HPLC etc. The expression of retention factor (*Rf*) is generally used to depict the chromatographic performance of solute [4]. In the present investigation, natural pigments of flowers especially from petals were separated from different flowering plants of different families.

MATERIALS AND METHODS

Collection of Sample: The collection of samples of healthy and fresh flowers of five different plants were made from the campus of Govt.Vidarbha Institute of Science and Humanities ,which is located in Amravati city .This Institute is a premier institute in vidarbha region of Maharashtra state in India . It lies between north latitudes 20 ^{O} 55 ' 33" N and 77 ^{O} 45 ' 53 " E. The Institute constitutes a big campus with lush floral vegetation. The fresh flowers of the plants like *Ixora, Portulaca, Hibiscus, Clitoria and Lilium* were collected in the month of January and February 2020. The samples were brought to the laboratory. They were washed with running tap water to remove dust, other foreign particles and debris which is deposited on the petals .These petals were blotted to dry with the help of blotting paper and used freshly for pigment extraction.

Extraction of Pigment: The pigments present in the petals of flowers were extracted with the help of 80 % acetone as a solvent. 25 gm petals were weighed on sensitive balance and kept in mortar and pestle for grinding of tissues. 10 ml of acetone was poured in

mortar. The tissues were thoroughly grinded. The extract was filtered through Whatman no.1 filter paper .The filtrate was taken in a separating funnel and 20 ml of distilled water was added along with 10 ml of 10% KOH. The mixture was shaken vigorously and kept undisturbed .The extract was then kept in glass vial for loading purpose.

Preparation of Chromatograph paper: A white coloured Whatman no.1 paper was used for separation of pigments. A 15 cm long rectangular strip of chromatograph paper was prepared which served as stationery phase to separate the pigments of petals. Touching of fingers to chromatography paper was avoided strictly. From the bottom edege of paper, a faint line about 1 inch was marked with the help of lead pencil The apex of chromatograph paper was tagged with a thread to avoid touching by hands.

Loading of Pigment: A centre point to the bottom line of chromatograph paper was marked with a faint dot made by lead pencil. A drop of pigment extract was loaded with the help of capillary tube at the centre of bottom line. The drop was allowed to settle and let it air dry for 02 to 03 minutes. Sub sequentially about 10 such drops was loaded and allowed to dry. After drying of the loaded drops the chromatograph paper strip was labelled as per the sample plant.

Preparation of Solvent: The solvent system was freshly prepared and used for running of pigments on stationery phase. The solvent system was preapared with the help of n- butanol + acetic acid + distilled water in the ratio of 4:1:5. All these chemicals were mixed thoroughly and kept in sealed glass container meant for running of pigments or components.

Developement of Chromatogram: The loaded chromatograph paper was kept safely in clean glass rectangular container containing solvent system, the care was taken to immerse the bottom edege of paper in solvent system . Then the glass container was covered by a glass lid to avoid evaporation of solvent. The chromatograph paper was allowed to run in solvent system. The running was kept for 1 hour After sufficient separation of pigments, the paper was taken out and let it dried with the help of air drier .Then the separated pigment was marked with lead pencil and distance traveled by components or pigments was measured in centimetre with the help of scale. Like wise the distance traveled by solvent was also measured and recorded.

Determination of Retention factor: The retention factor (R_f) is the ratio of the distance traveled by the solute (pigments) to the distance traveled by the solvent. The Rf is used in chromatography to quantify the amount of retardation of a sample in a stationary phase relative to a mobile phase. The retention factor of pigment was determined by using the formula, in which the values of distance traveled by pigments and solvent were put.

$$Rf = \frac{\text{Distance traveled by component (Pigment)}}{\text{Distance traveled by solvent}}$$

RESULTS AND DISCUSSION

The five flowering plants possessing healthy and fresh flowers were selected for pigment separation (Table 1). The bright colour of flowers i.e. petals are a signal to attract insects for pollination.The floral parts stand out against a background of vegetation. The paper chromatography technique was selected due to its simple technique of pigment separation and due to less-expensive .The plant *Ixora coccinea* is a low growing tropical shrub notable for its bright coloured flowers forming a dense head of cluster. The colour of flowers especially of petals ranges from red, yellow, pink. The plant flowers throught the year, so used as ornamental plant The orange coloured petals were selected for pigment separation. The chromatogram has shown that the dark orange colour traveled to certain distance and separated to yellow pigment. The retention factor value after calculation was noted (0.58) (Table 2).The brown colour may be due to presence of carotenoid .The similar type of results were obtained in Petals of Chrysanthemum.[5]

The plant *Potulaca grandiflora* is an annual herb ,it grows up to 8 inches in height .The branches grows upto 1 feet wide The flowers of this plant are pink , red , orange, yellow, white. The petal colour selected for pigment separation was pink .On the chromatograph paper it has shown that the pink colour traveled along with solvent and separated into dark yellow pigmentation The retention factor calculated was noted (0.53)(Table 2)

Table 1: List of plants used for separation of pigments from petals of flower.

S. N.	Name of the plant	Family	Vernacular Name
1	*Ixora coccinea* L.	Rubiaceae	Bandhuka
2	*Portulaca grandiflora* H.	Portulacaceae	Rose moss
3	*Hibiscus rosa-sinensis* L.	Malvaceae	Jasvand
4	*Clitoria ternatea* L.	Fabaceae	Butterfly pea or blue pea
5	*Lilium philadelphicum* L.	Liliaceae	Wood lily

Hibiscus rosa-sinensis is an evergreen bush or shrub or may be small tree. It grows upto 8 feet tall and 6 feet wide. There are many specieds of *Hibiscus* that produces various coloured flowers .The flowers may be red ,

white, yellow, pink This plant bloom throught the year.The flowers are bell shaped and flared showing long central tube of stamens and pistils at the tip. Red coloured petals were used for separation of pigment .The chromatograph paper has shown that the red colour was traveled and separated along with solvent and shown brown yellow colour .The retention factor was noted (0.72) (Table 2) The yellowish colour indicates the presence of carotenoid The caretonoid plays an important role as colorant and vitamin A precursor.[6]

The *Clitoria ternatea* is a perennial herbaceous plant with elliptic and obtuse leaves. It grows as a vine or creeper in support of other plants .This plant species produces flowers of deep blue or white coloured.The flowers are about 4 cm long and 3 cm broad .The pigment separation of blue flowers were carrid out .The blue colour traveled along with solvent system and became brownish yellow colour .This colour shows maximum content of xanthophylls . The retention factor was noted (0.69) (Table 2).

Table 2: Determination of Retention factor (Rf) of pigments in petals of flower.

S. N.	Name of the plant	Colour of Flower	Plant part	Retention Factor
1	*Ixora coccinea* L.	Orange	Petals of flower	0.58
2	*Portulaca grandiflora* H.	Pink	Petals of flower	0.53
3	*Hibiscus rosa-sinensis* L.	Red	Petals of flower	0.72
4	*Clitoria ternatea* L.	Blue	Petals of flower	0.69
5	*Lilium philadelphicum* L.	Deep orange	Petals of flower	0.79

Lilium philadelphicum is a herbaceous flowering plants .The leaves of this perennial plant are long and narrow, they are arranged in whorls. Generally it produces 1 to 3 flowers at the tip of the stem, occasionally up to 5 flowers .The flowers are 2 ½ inches across producing 6 petal like tepals, The tepals are spatula shaped and are deep orange to red coloured or may be yellow as per the species character . The flowers are yellow at the base and marked by dark maroon brown spots over petals. The prominent orange coloured flowers were selected for pigment separation. The chromatograph paper has shown that orange colour pigment was traveled and separated along the solvent and it was found to become brown to yellow coloured. The retention factor of pigments separated was noted (0.79) (Table 2) As the solvent rises up by capillary action through the paper chromatography then the components of the pigment mixture are partitioned between the stationery phase and mobile phase By compairing the retention factors of pigments of five selected plants the minimum Rf was noted in *Portulaca* (0.53) The minimum Rf may be due to heavy or large sized molecules of pigment compound .The maximun Rf was shown in *Lilium* (0.79). The maximum value of retention factor may be due to light or small sized molecules of pigments.

CONCLUSION

The current investigation has shown that, pigment of petals can be effectively separated with the help of paper chromatography technique by using solvent. The pigment separation of petals can be based upon the size of molecules of chemical compounds .The component pigments was separated and changed its originality while travelling with solvent .It was found that seperation of each pigment or component was gradually rised up The greater retention factor indicates better resolution of pigments with respect to solvent and size of chemical compounds or molecules The smaller retention factor indicates poor resolution of pigments with respect to solvent and size of molecules The distance travelled by the pigment component or solute may depends upon adsorption and solubility of chemical compounds. The micro components of pigment able to spread along solvent very efficiently .The solvent system is proved to be satisfactory for pigment separation of petals of flowers.

ACKNOWLEDGEMENT

The author is greatful to the Director of parent Institute for their kind help regarding providing library and laboratory facilities throught the research work

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