CAROTENOID SCREENING IN SELECTED FLOWERS

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Abstract

Flowering plants have interesting potential as sources of pigments for use in food products.This research focuses on the carotenoid composition of five commonly grown garden flowers that bloom year-round and that have adapted well to the environmental conditions in Tamil Nadu and may be of commercial value as sources of carotenoids. The five selected flowers are divided into five different families: Apocynaceae, Boraginaceae, Fabaceae, Cucurbitaceae and Malvaceae. The colors of the selected flowers are varying from yellow to red. In this work the carotenoid pigments were extracted, separated and identified and quantified for their potential use as low-cost and abundant sources in the food industries.The majority methods of extraction of carotenoids from plant sources make use of organic solvents such as hexane, ethanol, methanol, tetrahydrofuran, benzene, and petroleum ether. It has been observed that the stability of carotenoid extract obtained with hexane, acetone, ethanol was higher than that of extracts obtained with other organic solvents, such as chloroform, methanol or dichromate. The proposed work was done by Soxhlet method and all metrics were analyzed by high performance factor Concentration of β-carotene (μg/g).

Keywords:

Colored Flowers,Carotenoid, Organic Solvents, Screening, β-carotene

Introduction

A flower, sometimes known as a bloom or blossom, is the reproductive structure found in flowering plants. Flowers have long held an important role in human societies. They have been used for ornamental purposes as well as in diverse dishes, mainly due to their appealing and diverse colours. In addition, flowers have been used in traditional medicine. In recent years, there has been a growing interest in the study from different points of view of the health-promoting secondary metabolites present in flowers, including carotenoids and phenolics Flowers inherit their appearance from genes. Flower colour is one of the most important traits of ornamental plants and is attributed to various pigments. As a plant looks aesthetic, the colours of a flower must attract us first. Therefore the brighter the flower, the more likely it will be visited .Plants have a number of different means to attract pollinators, with bright showy colours being one of the most common ways to maximize their visual effect. While other flower features, such as texture and fragrances, are also used to attract pollinators, a plant's colour is vital to its survival from one generation to the next. The colour observed in flowers is actually the result of reflected light from various chemical compounds called "Plant Pigments".

Pigments are born into these plants, producing a range of colours across the spectrum. There are many different plant pigments, and they are found in different classes of organic compounds. The selective absorption of different wavelength determines the color of a pigment. The molecular

structure of a pigment determines its absorption spectrum. When a pigment absorbs radiation, it is excited to a higher energy state. A pigment molecule absorbs some wavelengths and not others simply because its molecular structure restricts the energy states which it can enter. Once a pigment has absorbed radiation and is excited to a higher energy state, the energy in the pigment

can be emitted as heat. Some like chlorophyll, phytochrome, rhodopsin, and phycobilin are plant pigments which use much of their absorbed light energy to produce chemical changes within the plant. On the other hand, some pigments which typically emit most of their absorbed light energy as heat.They can be divided into three major classes including flavonoids, carotenoids, and betalains.

Flavonoids and Betalains

Flavonoids are widely distributed plant pigments. They are soluble in water and commonly occur in vacuoles, membrane-enclosed structured within cells which also store water and nutrients. Anthocyanins are the most common class of flavonoids and they are commonly orange, red, or blue in colour. Betalains are a class of red and yellow tyrosine-derived pigments found in plants of the order Caryophyllales, where they replace anthocyanin pigments. Betalains also occur in some higher order fungi.

Carotenoids

Carotenoids, a group of hydrophobic pigments, are stored in differentiated plastids. In thechloroplasts of green tissues, carotenoids are essential in photosynthesis for functions such as photosystem assembly, light harvesting, and photoprotection, whilenon-greentissues, such as flowers, fruits and seeds, accumulate carotenoids in chromoplasts and display vivid colours. Carotenoids are precursors for vitamin A and play important roles not only in plants but also in human health (Asensi-Fabado and Munné-Bosch, 2010). More than 750 naturally occurring carotenoids which are mainly divided into carotenes and xanthophylls, have been identified from plants, animals, and microorganisms (Nisar *et al*., 2015). The carotenoid profiles in green tissues of most plant are similar, whereas those in non-green tissues are distinct and vary considerably depending on the plant species (Yuan*etal*., 2015). Therefore, different regular mechanisms forcarotenoid accumulation exist in various plant species or their tissues.

Carotenoids also called tetrapenoids, are yellow, orange and red organic pigments that areproduced by plants and algae, as well as several bacteria, and fungi. Carotenoids serve two key roles in plants and algae: they absorb light energy for use in photosynthesis and they provide photoprotection via non-photochemical quenching (Armstrong and Hearst, 1996). Carotenoids are ubiquitous organic compounds, mainly of yellow, orange and red colour, soluble in fats and organic solvents (Watkins and Pogson, 2020). Currently more than 1000 carotenoids are known (Yabuzaki, 2017). Several of these pigments are retinoids because of their vitamin A activity (Becerra *et al*., 2020). From the chemical point of view, these compounds are preconjugated double bond system representing a light-absorbing chromophere that gives carotenoid dominantly tetraterpenes composed of eight isoprene units that gives carotenoids their characteristic colour (Rodriguez and Amaya, 2019). Carotenoids are identified from their visible absorption spectrum(Saini *et al*.2015).

Carotenoids have been widely accepted as safe chemicals for food supplementation and neutral chemical purposes due to their intense coloring abilities, their role as precursors of Vitamin A (Romer *et al*., 2000), and their antioxidant activity in animals (Stahl and Sies, 2003). They are linked to photosynthesis, photoprotection and plastid structure (Bartleyand Scolnik, 1995). Carotenoids consist of long chains of alternating double and single carbon-carbon bonds, with cyclic end groups and various keto-hydroxyl-, and acid-functional groups

in different positions (Goodwin, 1980). They have been shown to be beneficial in preventing major health problems indeveloping and developed countries, including cancer, cardiovascular and coronary heart disease(Kritchevsky, 1999), and ophthalmological disease (Mayne, 1996) including age-related macular degeneration (Landrum*etal*., 1997). About 60 different carotenoids occur in plant tissue, including
β-carotene, α-carotene, and lycopene. (Palace *et al*. 1999).

Carotenoids are a class of more than 750 naturally occurring pigments synthesized by plants, algae, and photosynthetic bacteria. These richly colored molecules are the sources of the yellow, orange, and red colors of many plants. Fruits and vegetables provide most of the 40 to 50carotenoids found in the human diet. α-Carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene are the most common dietary carotenoids.Some are provitamin A carotenoids can beconverted by the body to retinol.

Carotenoids are divided into two basic groups – Carotenes and Xanthophylls. Carotenes are formed by a hydrocarbon chain (lycopene, α carotene, β carotene) while xanthophylls are oxidized derivatives of carotenes (β cryptoxanthin, lutein, zeaxanthin) (Becerra *et al*., 2020). Lutein and its isomer zeraxanthin are natural pigments of yellow colour. These substances are labile and they easily isomerize and degrade in the presence of light, heat and oxygen; thus, preventive procedures have to be taken to adapt both storage and processing conditions (Saini *etal*.,2015). Human bodies are not able to synthesize carotenoids themselves, so they have to take themin food or food supplements.

Data from several laboratory studies and scientific reports illustrate that over six hundred different carotenoid compounds have been characterized from plants, algae, bacteria and animals(Olson and Krinsky, 1995). Carotenoids are found in all parts (root, leaf, flower, fruit and seed) but they are usually most noticeable in the flowers. By attracting insects and birds, plant pigmentsserve an important ecological function by mediating pollination and seed dispersal (Camara*et al.,* 1995). Several different classes of pigments are responsible for coloration but in many yellow,red, and orange flowers, the pigments synthesized by the plant are carotenoids (Schoefs, 2002). The predominant carotenoids vary widely among different species.

Statement of the Problem

Carotenoids are a class of more than 750 naturally occurring pigments synthesized by plants , algae, and photosynthetic bacteria These richly colored molecules are the sources of the yellow ,orange and red colors of many plants. Carotenoids are the potential sources of pigments. It prevents health issues like visions and skin diseases. Carotenoids, which are present in various foods, are capable of capturing free radicals that are in imbalance in our bodies and which cause negative effects to human skin such as muscular degeneration, cancer, mutations, premature aging, as well as cataracts and arteriosclerosis.

Objectives of the study

Carotenoid extraction and quantification was carried out from flowers of *Allamanda blanchetii* L., *Clitoriaternatea* L., *Cordia sebestena* L., *Cucurbita pepo* Duch.and *Gossypium arboretum*L. The main aim of the study was to find the amount of β-carotenes present in the flowers of the above plants. Flower color was strongly correlated with carotenoids content in flowers and weakly correlated with carotenoids content in fruits, which suggest that regulation ofcarotenoids biosynthesis in flowers and fruit is independent.

Review of Literature

Carotenoids are a class of hydrocarbons consisting of eight isoprenoid units in reverse manner at the center of the molecule. The major carotenoids are derived from 40-carbon polyene chain, which would be considered as backbone of the molecule. This chain may be terminated bycyclic end-groups and may be complimented with oxygen containing functional groups. **Rodriguez and Amaya (2019)** reported that Carotenoids are natural pigments, constituents of foods, some of which are precursors of vitamin A. Investigation of the factors that influence carotenoid composition in foods are: the existence of a very large number of carotenoids, the qualitative and quantitative variation in the composition of food, the very wide range of concentrations in which the carotenoids are found in food, the susceptibility of carotenoids to the isomerization and oxidation during storage and before sample analysis. Besides, these factors canbe considered: the variety or cultivar, stage of maturation; climate or geographic location; plant part used and production technique.

Fahy reported that Carotenoids are categorized as prenol lipids, forming the group of C40 isoprenoids (tetraterpenes), which contains more than 330 biologically relevant members. Carotenoids are typically associated with brightly colored fruits and vegetables, as well as pigmentation in numerous animals such as birds and amphibians. Rodriguez stated that Carotenoids are singlet excellent oxygen scavenger and are used as food colorant, food additive, cosmetics, neutraceuticals etc. Animals are incapable of carotenoid biosynthesis, thus their carotenoids are diet derived, selectively or unselectively absorbed, and accumulated unchanged or modified slightly into typical animal carotenoids. Fruit and vegetables provide most of the carotenoids in the human diet.(Nisar et al., 2015) elucidated the Carotenoid biosynthesis, as well as the related genes and enzymes, in many plants. Generally,as a part of the terpenoid biosynthesis pathway, carotenoid biosynthesis originates from the condensation ofisopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which are generated in the methylerythritol phosphate (MEP) pathway.

Cazzonelli and Pogson (2010) reported that Carotenoids can be classified as 'chloroplast-type carotenoids' and 'chromoplast-type carotenoids,' based on their locations in diff erent types of plastids (Yamamizo et al., 2010). These carotenoids have distinct functions, Chloroplast-type carotenoids are mainly produced in photosynthetic tissues such as leaves and are essential pigments involved in photosynthesis, while chromoplast-type carotenoids which mainly accumulate in flowers and fruits, conferring yellow, orange and red colors to attract insects and animals for pollination or seed dispersal.

Cardoso (1997) reported that Carotenoids known as β-carotene is the yellow pigment found in fruits and vegetables and their antioxidant role of interact with radical ions and reactive oxygen species.**Bogacz-Radomska and Harasym (2018)** studied that β-carotene is used as an orange-reddye in various products of the food industry. β-carotene is used as a coloring agent for tablets in the pharmaceutical industry, while in the cosmetic industry it is used as a bioactive cream ingredient that protects skin lesions from oxidation and UV exposure.

Shirakami *et al* (2012) reported that β-carotene is found extensively in the human diet, it is readily detectable in human tissues, and has well-recognized pro-vitamin A and antioxidant properties. It is important to emphasize that β-carotene serves as a precursor molecule for vitamin A. This is particularly important in the context of liver disease as there is a growing and significant literature that links altered hepatic retinoid signaling with diseases of the liver, including NAFLD, and ALD .

Krinsky (2010) reported that Carotenoids are a class of more than 600 naturally occurring pigments synthesized by plants, algae, and photosynthetic bacteria. They comprise a class of natural lipid-soluble compounds which are found in numerous vegetables and fruits.

Harrison (2012) Vitamin A deficiency is the most common vitamin deficiency in the world

and affects an estimated 190 million preschool-age children and 19.1 million pregnant women worldwide. In areas of endemic vitamin A deficiency, people obtain vitamin A almost exclusively as provitamin A carotenoids found in foods of plant origin . Provitamin A carotenoids are enzymatically converted to retinal (vitamin A aldehyde) by the enzyme β-carotene 15–15′-oxygenase (BCO1). Hence, understanding the mechanism and regulation of this enzyme is important.

Christensen investigated the strongest data regarding dietary carotenoid intake and Non-Alcoholic Fatty Liver Disease (NAFLD) comes from analysis of the National Health and Nutrition Examination Survey (NHANES, 2003–2014) dataset. This analysis shows that individuals who consume the highest amounts of carotenoids have the lowest risk of having NAFLD, this effect was true for the intake of α-carotene, β-carotene, β-cryptoxanthin, andlutein or zeaxanthin, but not lycopene. This study also showed a lower risk of having NAFLD in those individuals with the highest circulating levels of all carotenoids measured (i.e., α-carotene, β-carotene, β-cryptoxanthin, lycopene, and luteinn or zeaxanthin).

Research Methodology

The flowers used for the study were *Allamanda blanchetii* L., *Clitoria ternatea* L., *Cordia sebestena* L, *Cucurbita pepo* Duch. and *Gossypium arboreum* L. The flowers used were collected from the Rani Anna Government College for women campus and washed under tap water, cleaned with sterile water and air dried at room temperature. The raw materials were chosen based on their availability and also on the basis of information gathered during literature studies. The scientific names and families of the selected flowers that were used in this study are mentioned in the below table.

Table 1. Selected Flowers in different families with different colours

Scientific Name	Family Name	Petal colour
Allamanda blanchetii L.	Apocynaceae	Violet
*Clitoriaternatea*L.	Fabaceae	Blue
*Cordia sebestena*L.	Boraginaceae	Orange
*Cucurbita pepo*Duch.	Cucurbitaceae	Yellow
Gossypium arboretum L.	Malvaceae	Red

FLOWER DESCRIPTION

Allamanda blanchetii L .(APOCYNACEAE)

Eng: Purple allamanda, violet Allamanda

*Allamanda blanchetii*L. (Violet allamanda) is a species of perennial flowering plant in the family Apocynaceae native to Brazil. It can be cultivated as an ornamental plant. This plant's violet, bell shaped blooms are about 3" across. Fibre is extracted from stalk with high strength and silky white after chemical treatment. The plant is also known to deal with heat and different toxic products; it activates blood circulation and diuresis. The plant possesses various secondary metabolite substances like carotenoids, flavonoids, polyphenols, iridiods, tannins and alkaloids.
Distribution: Cultivated as ornamental species in India.

Flowers and Fruits: June-September.

Uses: Treating malaria, Jaundice, Cough, Wounds and Constipation.

Clitoria ternatea L. (FABACEAE)

Eng: Butter fly pea, Winged leaved *Clitoria*.

Twining herbs; branchlets tomentose, Leaves odd-pinnate, leaflet; 3-7, ovate, entire, obtuse. Flowers white or deep blue, in axillary racemes. Sepals tubular, membranous. Petals exserted; standard erect, wings falcate-oblong, connate at the middle of the keel. Stamens 9+1. Pods linear, flattened, oblong, compressed, apically beaked; seeds 10-15, reniform.
Distribution: Cultivated in gardens everywhere, but also common self-sown in hedges and thickets.
Flowers and Fruits: March-August

Uses: Memory enhancer, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing and sedative agent.

*Cordia sebestena*L.(BORAGINACEAE)

Eng: Geiger Tree, Sebesteans, Scarlet Cordia
Evergreen, large shrubs or small trees, Leaves thick-coriaceous, large, ovate, or elliptic-lanceolate, entire or undulate, acute, base rounded or obtuse, rough hairy above, smooth and puberulous below, lateral nerves 5-7 pairs. Flowers scarlet-orange, pedicelled, in large, open, terminal clusters. Sepals 5, tubular, green. Petals 6-7, tubular, as long as sepals. Stamens 6-7, styles 2, each style is branched; stigmas 4, recurved. Ovary bicarpellary, tetralocular; ovule 1 per locule, axile. Fruits lanceolate.
Distribution: Cultivated as an ornamental in gardens and in habitations. Tirumala, Tirupati.

Flower and Fruit: March-June.

Uses: Bark-Dyspepsia, acne, skin disorders. Fruit-worm infestation, boils, diuretic, purgative, expectorant, maturant.

Cucurbita pepo Duch. (CUCURBITACEAE)

Eng: Pumpkin, Summer Squash.

Climbers with sharp stiff translucent hairs; stems long running. Leaves stiff and somewhat rigid, erect, triangular or ovate-triangular, irregularly sharp-serrate. Calyx lobes short and narrow. Corolla with erect or spreading pointed lobes. Fruits large, orange, furrowed, perishable; seeds white, elliptic with raised obtuse margin.

Distribution: Cultivated for its fruits.

Flower and Fruit: Throughout the year.

Uses: Fruit, seed and seed oil-Debility, tuberculosis, febrifuge, burning sensation, psychosis, cough, abdominal disorders, dysuria, mental disorders, ulcers, leucorrhoea, wasting diseases, haemoptysis, drug reactions.

Gossypium arboretum L.(MALVACEAE)

Eng: Tree cotton., Asian cotton.

Subshrubs, Leaves 5-7 lobed, stipules caducous; involucral bracts triangular. Flowers solitary, axillary, pale yellow, Corolla 2 or 3 times as long as involucre. Staminal column long, antheriferous throughout. Capsules tapering, pitted, mostly 3-celled, seeds fuzz, linted.
Distribution: Cultivated.

Flower and Fruit: Throughout the year.

Uses: Whole-plant-Rheumatism, gonorrhea, gleet, chronic cystitis. Seed-demulcent, laxative, galactogogue, expectorant, dysentery, intermittent fevers.

Chemicals and glasswares

Standard Petroleum ether and ethanol was taken from the lab. The other apparatus like beaker, measuring cylinder, separating funnel, pipettes, test tubes were taken and handled with high care. Distilled water was purified using a water purification system and used. The Spectrophotometer was used to read the optical density of the solution.

Extraction of carotenoids

The petals of the selected flower were taken in 2 grams and macerated with 95% of ethanol. Centrifugation was done at 3000 rpm for 15 minutes. The supernatant was collected. The total volume of supernatant was measured and made up to known volume with distilled water. The set up was kept in ice box for 5 minutes. The mixture was then transferred into a separating funnel and 10 ml of petroleum ether was added and ethanol was poured over it. The funnel was swirled gently to obtain a homogenous mixture and it was later allowed to stand until two separate layers were obtained. The bottom layer was run off into a beaker while the top layer was collected.

Measurement of Absorbance

The final extract was measured and the absorbance of the extracts was measured using a spectrophotometer at a wavelength of 436 nm. A cuvette containing petroleum ether was used to calibrate the spectrophotometer to zero point.

Concentration of carotenoids was calculated using the following formula:

$$\text{Concentration of } \beta\text{-carotene (C)} = A/EL$$

Where A=Absorbance, E=Extraction coefficient, L=Thickness of cuvette (path length). The extraction coefficient of carotene (absorbance at a given wavelength of a 1% solution in spectrophotometer cuvette with a 1-cm light path) used in the calculation of the concentration also varies in different solvents.

Cucurbita pepo **Duch.**

Gossypium arboreum **L.**

Cordia sebestena **L.**

Clitoria ternatea **L.**

Allamanda blanchetii **L.**

Fig.1 Selected Colored Flowers

Results and Discussion

The concentration of carotenoids (β-carotene) was estimated. The color of the flower petals used in the study varies from red to yellow. Extraction duration, solvent-solid ratio and extraction temperature were assumed to be the most important factors affecting solvent extractionfor the determination of carotenoids. The determinations of β-carotene content in the selected flowers are listed in the table below.

Table 2.Concentration of β-carotene content in the selected flowers

Flowers	Colour	Absorbance	Concentration of β-carotene (μg/g)
Allamanda blanchetii L.	Violet	0.127	0.0009 (low)
Cordia sebestena L.	Orange	0.183	0.0014
Cucurbita pepo Duch.	Yellow	0.238	**0.0018 (high)**
Gossypium arboretum L.	Red	0.217	0.0016
Clitoria ternatea L.	Blue	0.169	0.0013

In the present study five different coloured flowers were selected for the carotenoid estimation. The results stated that the β-carotene content is comparatively high in *Cucurbita pepo*Duch.0.0018 μg/β-carotene belong to the family Cucurbitaceae. It implies that the β-carotene content is large in yellow-coloured flowers. The yellow coloured flower showed maximum carotenoid content and this may be due the high contents of β-carotene, lutein and α-carotene. Similar results were also recorded in carnation cultivars. The pale yellow colour of carnation petals was due to accumulation esterified carotenoid (Ohmiya, 2019). In *Zantedeschia* hybrid yellow, the petal colour was due to the presence of the beta carotene (Lewis, 2003). Similarly in *Lantana camera* also, the yellow colour petals was due to the accumulation of beta carotene (Ram and Mathur, 1984). In *Canna indica* and *Narcissus perudomarcissus* the yellow colour was due to the presence off lutein (Tinoi *et al*., 2006; Valadon and Mummery, 1968). Similar to the present study, the carotenoid pigment was extracted from the petals of *Cucurbita moschata* as food colourant with antioxidant property using acetone and hexane. The pure carotenoid was separatedby giving water, brine and solvent wash. These carotenoid extracts were further used for the TLC analysis. The carotenoid extract was compared with the β- carotene standard and resulted with thetwo spots. The Rf value was 0.786 (dark orange) and 0.12 (light yellow). The absorbance was scanned for the carotenoid extract using UV/VIS spectrophotometer and was found to have four compounds at 662.5, 613.0, 497.0, and 436.5 nm. Carotenoid test was carried out for the respectivesample. Acetone disappearance confirm that the sample contain carotenoids. (Yan*et al*. 2017).

Next to yellow colour flower the red colour flower *Gossypium arboreum* L. of family Malvaceae showed maximum carotenoid content with the concentration of 0.0016 μg/β-carotene. This may be due the mainly contained β-carotene and α-carotene. Similarly in petals of the orange-red cultivars of *Osmanthus fragrans* is mainly presence of β-carotene and α-carotene (Wang *et al*., 2018). Similar results were recorded in the red colour of *Hibiscus* due to the presence of β-carotene, auroxnthin and chrysanthemaxanthin. (Hanny *et al*. 1972).

The next level of carotenoid content was found in *Cordia sebestena* L. with the concentration of 0.0014 µg/β-carotene. This may be due to the high content of anthocyanins. Alike present study, Orange coloration is generated by a combination of yellow and red pigments in many cases. In *Alstroemeria* (Tatsuzawa *et al*., 2004), *Rosa hybrida* (Yokoi and Saito, 1973), and *Zinnia elegans* (Boyle and Stimart, 1989).Orange petals contain both carotenoids and anthocyanins, as yellow and red pigments, respectively. On the other hand, orange is produced only with carotenoids in some species, such as *Calendula officinalis* (Kishimoto *et al*., 2005), *Eschscholzia californica* (Strain, 1938), *Lilium lancifolium* (Deli *et al*., 1999), and *Tagetes erecta* (Moehs *et al*., 2001). Many plants belonging to the Compositae (Asteraceae) family, including *chrysanthemum (Chrysanthemum morifolium Ramat*.), which is one of the most important ornamental plants in the world, have both yellow- and orange-coloured petals. Carotenoids are responsible for the deep yellow coloration in these plants, so the same carotenoids are probably produced in both orange and yellow petals along with anthocyanins.(Hayashi, 1988).

The present study showed least amount of carotenoid contents in blue and violet flowers in the families of Fabaceae and Apocynaceae. This may be because blue coloured flowers are highly associated with anthocyanins which are members of flavonoid metabolites. Especially delphinidin type anthocyanin is responsible for the blue colour of petals. Similarly a study investigated the relative concentration of anthocyanins based on molecular polarity for different seasons of harvesting of blue and red flowers namely- *Canna indica, Clitoria ternatea, Delonix regia, Hibiscus mutabilis, Impatiens balsamina, Ixora chinensis, Jatropha integerrima, Lagerstroemia indica, Mirabilis jalapa, Nerium oleander, Portulaca graniflora, Quisqualis indica, Rosa indica, Ruellia tuberosa and Thunbergia erecta.* The stability of anthocyanins extracted from these flowers at their different developmental stages, was evaluated mainly for theeffects of pH, storage period, temperature, storage period as well as light and dark conditions were evaluated. It concluded the presence of anthocyanin in blue, Orange and red coloured flowers. (Wang & Lin, 2000).

DISCUSSION

Flower color is one of the most important traits of ornamental plants and is attributed to various pigments that can be divided into three major classes including flavonoids, carotenoids, and betalains (Grotewold, 2006). Among these pigments, carotenoids are responsible for the colors ranging from yellow to red (Tanaka *et al*., 2008). Red flower can be produced by high concentrations of carotenoids, anthocyanins, or a combination of both pigments (Tanaka *et al*.,2008; Yuan *et al*., 2013; Ng and Smith, 2016a,b). Many plant species can accumulate special carotenoid constituents in their flowers, such as astaxanthin in the blood-red flowers of *Adonis annua* and *Adonis aestivalis* (Seybold and Goodwin, 1959; Cunningham and Gantt, 2011), capsanthin in orange and red tepals of lily (Jeknić *et al*., 2012), and lutein epoxides in and yellow petals of chrysanthemum (Kishimoto *et al*., 2004). In tomato and *Ipomoea* plants, CHYB is a key enzyme responsible for carotenoid concentrations in white petals and yellow petals (Galpaz *et al*., 2006; Yamamizo *et al*., 2010).

Carotenoids can be classified as 'chloroplast-type carotenoids' and 'chromoplast-type carotenoids,' based on their locations in different types of plastids (Yamamizo *et al.*, 2010). These carotenoids have distinct functions; chloroplast-type carotenoids are mainly produced in photosynthetic tissues such as leaves and are essential pigments involved in photosynthesis, while chromoplast-type carotenoids which mainly accumulate in flowers and fruits, conferring yellow, orange and red colors to attract insects and animals for pollination or seed dispersal (Cazzonelli and Pogson, 2010; Yuan et al., 2015). In present study, the yellow colors may be contributed by carotenoids and orange and other colors may be produced by the mixture of carotenoids and anthocyanins.

Conclusion

Flower color is mainly due to the presence and type of pigments. Pollinator preferences impose selection on flower colour that ultimately acts on flower pigments. The vast range of flower colors relies on four major pigment classes: chlorophylls, carotenoids, flavonoids, and betalains. Each pigment class has a distinctive chemical structure, which ultimately affects the specific wavelengths it absorbs and thereby the colour it generates. carotenoids mainly absorb in the blue region, giving rise to yellow-orange colourations.

The present study was made to estimate the amount of carotenoid content in five flowers from 5 different families. The chosen flowers were *Allamanda blanchetii* L., *Clitoria ternatea* L., *Cordia sebestena* L., *Cucurbita pepo* Duch. and *Gossypium arboretum* L. belong to the families of Apocynaceae, Fabaceae, Boraginaceae, Cucurbitaceae and Malvaceae respectively. The results The present study was made to estimate the amount of carotenoid content in five flowers from 5 different families. The chosen flowers were *Allamanda blanchetii* L., *Clitoria ternatea* L.,*Cordia sebestena* L., *Cucurbita pepo* Duch. and *Gossypium arboretum* L. belong to the families of Apocynaceae, Fabaceae, Boraginaceae, Cucurbitaceae and Malvaceae respectively. The results stated that the β-carotene content is comparatively high in *Cucurbita pepo*Duch.(0.0018 μg/g) of the family Cucurbitaceae. The less carotenoid content was reported from violet coloured flower of *Allamanda blanchetii*L. (0.0009 μg/g).From the present study, it can be concluded that the flowers investigated can be exploited for carotenoid extraction and the yield depends on the chemical nature of solvents. However, purification, optimization and scale up studies are needed for commercialization. For carotenoid extraction much work has been focussed on flowers, which was found to be the major source. Flower colour is strongly correlated with carotenoids content in flowers and weakly correlated with carotenoids content in fruits.

Carotenoids have wide range of applications in cosmetics, and one of them is protection against photo-oxidative processes. They can act as effective antioxidants through the capture of singlet oxygen and peroxyl radicals.

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