Extraction and Stability of Natural dyes from Selected Ornamental Plant Species

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(2016-ES-26-M)

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Master of Science in Environmental Sciences

2019


Dedicated
To My
Dear Parents

Sher-e-Kashmir
University of Agricultural Sciences and Technology of Kashmir
Faculty of Horticulture, Division of Environmental Sciences

Certificate – I

This is to certify that the thesis entitled **"Extraction and Stability of Natural dyes from Selected Ornamental Plant Species"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Environmental Sciences** to the **Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** is a record of bonafide research work carried out by **Ms. Mubeena Manzoor (Regd. No. 2016-Es-26-M)** under my supervision and guidance. no part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or information received during the course of investigation have duly been acknowledged.

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We, the members of the Advisory committee of **Ms. Mubeena Manzoor (Regd. No. 2016-ES-26-M)**, a candidate for the degree of **Master of Science in Environmental Sciences**, have gone through the manuscript of the thesis entitled, **"Extraction and Stability of Natural dyes from Selected Ornamental Plant Species"** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of degree.

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Certificate – III

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ABSTRACT

The present investigation was carried out during 2017-18 to study the dye yielding potential of some selected ornamental plant species. The experimental material, comprising of 4 plant species, were collected from different locations of Srinagar. The stability and colour quality of pigments (anthocyanins and carotenoids) were assessed by quantifying the pigments and recording their colour quality after an interval of 30 days for 06 months under normal storage conditions. Antimicrobial activity of the flower extracts was determined against *Asperrigillus niger* and *Escherichia coli*. It was revealed that highest value of anthocyanin was recorded in Pansy (*Voila tricolor* var. *hortensis* DC.) and lowest was recorded in in Chrysanthemum (*Chrysanthemum morifolium* L.). Highest total carotenoid content was recorded in Chrysanthemum (*Chrysanthemum morifolium* L.) and lowest in Globe Amaranth (*Gomphrena globosa* L.). Highest pigment content was recorded during 0 days of storage in all selected plant species. The pigment content decreased with increase in the storage time. Colour quality of the plant species changed with increase in storage time. The hue angles of the plant species showed their respective colors on colour wheel. Highest antimicrobial activity was recorded in Chrysanthemum (*Chrysanthemum morifolium* L.)

Key words: Natural dyes, Anthocyanin, Carotenoids, Antimicrobial activity, Pigments.

Signature of Student Signature of Major Advisor

Dated : _____ Dated: _____

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Chapter–1

INTRODUCTION

A dye can be described as a coloured substance that has affinity to the substrate to which it is applied. The art of dyeing is as old as human civilization. Natural colourants or dyes were available to people during the Greco-Roman periods. The use of dyeing material is evident even in the wall paintings of Ajanta, Ellora and Sithannvasal. Textile dyeing is an age old process which has been followed right from the Neolithic period. People have used locally available materials to dye clothes. The natural invertebrate dyes such as tyrian purple and crimson kermes were highly prized items in the ancient and medieval world. Plant based dyes such as wood, indigo, saffron and madder were important trade goods in Asia and Europe. During the earlier times locally available materials like vegetables, flowers, fruits and also animals or minerals were used to prepare dyes with little to no processing. The first synthetic dye, mauveine, was discovered accidently by William Henry Perkin in 1856. It is also among the first dyes to have been mass produced (Hubner, 2006).

The use of synthetic dyes is increasing with every passing day. These dyes are derived from petroleum, sometimes in combination with mineral derived components. Synthetic dyes are used widely in textile manufacturing industries for dyeing cotton clothing, wool and nylon. These dyes became popular because of their long lasting colour and wide range of colour choices. There are more than ten thousand dyes available commercially and seven lakh tonnes of dyes are produced annually (Ratna and Padhi, 2012). A large percent of dyes are being used up by textile industries. However, these dyes have harmful effects on environment and human beings.

Synthetic dyes are made of chemical compounds which are harmful to humans, especially those who work in their production. Some chemicals in synthetic dyes contain mercury, lead, chromium, copper, sodium chloride,

toluene, benzene etc. Exposure to large doses of these dyes proves toxic and cause severe effects in the human body such as cancer and skin troubles. During the colouration process, a large percentage of the synthetic dyes does not bind and is lost to the waste stream. Approximately 10-15 percent dyes are released into the environment during dyeing process making the effluent highly coloured and aesthetically unpleasant (Ratna and Padhi, 2012) The effluent from the textile industries carries a large quantity of dyes and other additives which are added during the colouring process (Wang *et al.,* 2002). Moreover, untreated effluents from the industries where synthetic dyes are used, when dumped directly into the water bodies result in water pollution. These are difficult to remove in conventional water treatment procedures and can be transported easily through sewers and rivers because they are especially designed to have high water solubility. They undergo degradation to form highly toxic and carcinogenic products causing potential hazard to living organisms.

As an alternative to this problem, natural dyes can be used. These dyes are derived from plants, animals, fruits, insects and other natural resources and are usually harmless and safe for the environment. Natural dyeing is an ancient practice. Plant parts used in the preparation of these dyes include leaves, bark, fruits, roots, rhizomes, wood, seeds, and gum. Sometimes the plant is used as a whole for dye extraction. About fifteen hundred plant species are used for dyeing in the world and nearly one hundred fifty dye yielding plants have been found able to compete with synthetic dyes (Nidhi and Nitan, 2014). Natural dyeing is safer than synthetic dyes as they are less injurious to human health. These dyes are more skin friendly and safe for the environment. They are eco-friendly, biodegradable, non-carcinogenic and are soft, non-toxic and generally non allergic. These dyes are beneficial in preventing cancer which is one of the main causes of death in the world. The shades produced by natural dyes are usually soft, lustrous and soothing to the human eye. Turmeric, the naturally occurring

yellow dye is a powerful antiseptic which revitalizes the skin and gives a cooling sensation.

Natural dyes are used in the colouration of textiles, food, drug and cosmetic industries. They are also used for the colouration of paper, leather, shoe polish, candles, wood etc. There are many plants which are used for extracting natural dyes. Some of the commonly used dye yielding plants are Marigold (*Tagetes erecta*), Indigo plant (*Indigofera tinctoria*), Saffron (*Crocus sativus*), Indian Madder (*Rubia cordifolia*), Woad (*Isatis tinctoria*), Pomegranate (*Punica granatum*), Larkspur (*Delphinium* sp.), Turmeric (*Curcuma longa*), Henna plant (*Lawsonia inermis*), Sumac (*Rhus* sp.), Jamun (*Eugenia jambalana*), Tamarind (*Tamarindus indica*), Awla (*Emblica officinalis*), Walnut (*Juglans regia*), Kamala (*Mallotus philippensis*), Catechu (*Accacia catechu*), Butternut (*Juglans cinerea*), Black walnut (*Juglans nigra*), One seeded juniper (*Juniperus monosperma*) etc.

The majority of natural dyes are vegetable dyes from plant sources such as roots, berries, leaves etc. The art of making natural dyes is one of the oldest practices known to humans. In India, it was used for colouring fabrics and other materials. The earliest dyes were discovered accidently when berries and fruits were used during experiments and these experiments led to the development of highly refined art. In the recent days the inherent advantages of vegetable dyes and its awareness has resulted in the revival of demand of the dyes.

Ornamental plants generally grown for decorative purpose have less functional use and most of them do not have any value beyond the fact that they are attractive. Some ornamentals have brightly coloured foliage and flowers and are rich in anthocyanins and carotenoids and can prove source of dyes. In Kashmir valley annual flowers are usually cultivated during late spring and early winter and such species bloom in summer and spring respectively.

The garden Pansy (*Viola tricolour*) – a type of large flowered hybrid plant, with their endless variety of colours from white to blue, purple to orange, yellow

and red, as well as the long flowering period, garden pansies can be used as a good material for dyeing purposes. Phlox (*Phlox paniculata*), native to the Eastern and Central United States and Eastern Canada. Globe amaranth (*Gomphrena globosa)* and Chrysanthemums (*Chrysanthemum morifolium*) are cultivated throughout Kashmir valley and bear flowers of wide array of colours.

Keeping in view the availability and wide spectrum of colours of above mentioned ornamental species cultivated in Kashmir valley, the study was undertaken with the following objectives:

- Extraction and quantification of pigments from selected species.
- To work out the stability and colour quality of pigments.

Chapter–2

REVIEW OF LITERATURE

The use of natural dyes is increasing rapidly due to environmental problems and toxic and allergic reactions associated with synthetic dyes. Efforts are done worldwide to extract natural dyes from different plant sources. A brief review of work done in India and abroad is presented below:

Grover *et al*. (2005) evaluated the dye yielding potential of flowers of *Jatropha integrimme* Jacq. and used it to dye silk yarn. The mordants used were alum, copper sulphate, potassium dichromate and ferrous sulphate. The dyeing was carried out by using all three methods of mordanting i.e. pre mordanting, simultaneous mordanting and post mordanting. The colour fastness properties such as light, washing, crocking and perspiration were tested for each sample which were dyed. The results showed that post mordanting gave good results in washing fastness, light fastness, rubbing fastness and perspiration fastness. While comparing acidic and alkaline perspiration fastness *Jatropha integrimme* dye showed good perspiration fastness for acidic solution. A wide range of colours was obtained on silk using various mordants and mordanting methods. Since the flowers showed good shades with satisfactory fastness to light, washing, crocking and perspiration on silk it can be used for textile dyeing.

Shanker and Vankar (2005) reported about a natural colouring material betalain from *Celosia cristata* (murgkesh) for wool yarn dyeing. The dye was extracted by boiling the crushed flower in a beaker kept over a water bath for 3 hours. The wool yarn was treated with 2% (w/v) aqueous solution of sodium hydroxide, ethylene diamine and morpholine separately. The pretreatment was carried out for 1 hour at 27^{o}C, keeping the M: L ratio as 1:20. Then the samples were washed and dried. A known weight of wood sample was treated with different metal salts. After that the wool yarn was dyed with dye extract, keeping M: L ratio as 1:30 and the pH was maintained at 4, at a temperature of 60^{o}C for 30

minutes. The dyed wool yarn was then rinsed with water thoroughly, squeezed and dried. It was inferred that the wool which was pretreated with ethylene diamine showed darker shades as compared to morpholine and sodium hydroxide. The findings of colour fastness test of wool samples to rubbing under dry conditions showed that the samples had fair to excellent fastness as compared to dry rubbing. The samples when subjected to wet rubbing exhibited a decrease in colour fastness. So *Celosia* flower can be used as a good material for wool dyeing.

Vankar and Shanker (2006) worked to produce dye from *Cayratia carnosa* which is a natural resource growing wild and in abundance. The ripe fruits of the plant were used to dye cotton, silk and wool. Deep blue, purple coloured berries of *Cayratia carnosa* were used. The fruits were crushed and dissolved in distilled water and allowed to boil in a beaker kept over a water bath for 3 hours for quick extraction. The solution was filtered for further use. Cotton fabric was pretreated with tannic acid for better adherence of dye. The pretreatment was carried out for 1 hour at 27^{o}C keeping the M: L ratio as 1: 20. Then the samples were washed and dyed before mordanting. Silk and wool samples were mordanted directly. The mordants used were stannous chloride, stannic chloride, alum, ferrous sulphate, copper sulphate and potassium dichromate. The accurately weighed cloth sample was treated with 2 to 4% of the above mordants. Then the fabrics were dyed with a dye extract keeping M: L ratio as 1: 30 and pH 4 to 5 at a temperature of 60^{o}C for 1 hour. The shades obtained were bright and had very good fastness properties. These dyes can be used for dyeing in various shades of blue to dark blue and purple shades for cotton, silk and wool. The samples had fair to excellent colour fastness.

Jothi (2008) evaluated the dyeing potential of African marigold flower (*Tagetes erecta* L.) using the dye extracted from the flowers on 100 percent cotton, and silk fabrics under normal dyeing conditions using alum, copper sulphate, stannous chloride and ferrous sulphate as mordants. Studies on dyeing ability, wash fastness, light fastness and colour hue were done. Changes in some

of the colours were noticed after washing with soap. Most of the metal salts exhibited the highest K/S values due to their ability to form co-ordination complexes with dye molecules. The findings reveal that the extract of marigold flower can be used for colouration of 100 percent cotton and silk fabrics.

Vankar and Shanker (2009) used flowers of *Delonix regia* for natural dyeing of silk using bio mordants (pyrus) and enzymes (lipase, diasterase, protease and amylase). The aqueous extract obtained from the dried flowers was used for dyeing silk fabrics. Bright reddish brown hue colour was observed when 30% of *Delonix regia* extract was used on the pretreated silk material. The silk fabric was heated with an enzyme or a bio mordant and the resultant dyed fabric showed resistance to fading. All the dyed specimens were tested for wash and light fastness properties. It was found that Delonix is a viable alternative to synthetic red dyes. Desorption studies were conducted and the order of reactivity of enzymes towards dye uptake in the one step process was found to be lipase > diasterase > protease = amylase = pyrus. For two step process the order of reactivity was found to be protease = amylase > lipase > pyrus > diasterase. It was concluded that the two step process of treatment was better in terms of large colour yield values, fastness properties and dye adherence ability.

Ahmad (2010) measured the colour stability of red cabbage using different solvents. The solvents used for the extraction were methanol, ethanol (ethanol-water mixture 80:20 (v/v)), acidified water, alcohol in acidified water (the most used and efficient acids are acetic, citric, tartaric and hydrochloric acid. Colour was determined using CIE system L*, a*, b*. Based on the results, it was concluded that the colour of red cabbage is most stable when extracted with 40% methanol.

Gachovska *et al.* (2010) conducted a study to evaluate the effect of pulsed electric field (PEF) treatment on anthocyanin extraction from red cabbage using water as a solvent. A PEF of 2.5 kV/cm electric field strength; 15 μs pulse of 50 and a specific energy of 15.63 j/g were used to treat the mashed cabbage which

was placed in a batch treatment chamber. By using the HPLC method the anthocyanin concentration (16-889 μg/ml) was determined from the mashed cabbage. Same initial concentration of the material was used to study the heat and light stability of the control and PEF samples. It was found that the PEF treatments increased the total concentration using water by 2.15 times with nonacylated forms showing higher proportions than control ($P<0.05$) The heat and light stabilities of the PEF-treated samples and control samples were not significantly different ($P > 0.05$).

Wanyama *et al.* (2010) selected nine dye-yielding plants namely *Albizia coriaria* (bark), *Vitellaria paradoxa (*bark), *Curcuma longa* Linn (roots), *Indigofera arrecta* (leaves), *Syzygium cordatum* (bark), *Morinda lucida* (bark), *Morinda lucida* (roots), *Rubia cordifolia* (roots), *Mangifera indica* (bark) and *Justicia betonica* (leaves), collected from Jinja, Kampala, Luwero, Mbarara, Mbale, Mukono, Pallisa, Arua and Wakiso districts of Uganda. These plant parts were evaluated for colour absorption and fastness on cotton fabrics. Colours were evaluated based on the CIE Lab colour order system. The highest value of chroma was found in C. *longa* Linn. (68.34) and lowest for *J. betonica* (10.66). When the spectral reflectance curve was studied, it indicated towards a wavelength of 550 to 700nm I.e. an indicative of yellowish green to red colour in C. *longa* Linn. and *J.betonica* reflected violet blue light (65%) in between wavelength of 410-495nm. The colour coordinates in colour space diagram were found to lie in the yellow-red quadrant for *R. cordifolia, M. lucida, S. cordatum, C. longa, M. indica, V. paradoxa* and *A. coriaria*; in the yellow-green quadrant for *I. arrecta* and *M. lucida* (roots) and in the red-blue quadrant for *J. betonica, A. coriaria, V. paradoxa, M. lucida* and *S. cordatum* had fairly good fastness ratings of 3 to 4. Lightness values varied from 58.57 for *A. coriaria* to 77.63 for *I. arrecta*. Thus dyes obtained from selected dye-yielding plants in Uganda can be good sources of natural dyes for the textile industry.

Grover and Patni (2011) exploited medicinally much used dye yielding plant *Woodfordia fruticosa* (Linn.) Kurtz in perfume, leather and textile industry and three methods for the extraction of dye from the flowers were evaluated to determine the best extraction method. Three types of fabrics (cotton-synthetic mix, cotton-jute mix and pure cotton) and three different types of yarn (silk, cotton, wool) were used in the experiment. The first method used for extraction was the aqueous extraction from fresh flowers. In the second method of dye extraction, 10g of uncrushed flowers were treated with 100ml of distilled water to extract the dye. This pasty mass was kept for 10-15 days to get the dye. Then the extract was filtered and used for dyeing. In the third method of dye extraction the flowers (crushed, 10 g) were put in an earthen pot to which 100ml of distilled water was added and was kept undisturbed for 20-25 days and the extract was filtered through a piece of cloth to yield the dye. The second method of dye extraction gave the best colouration and was found to be the best extraction method. Cotton-jute mix and pure cotton showed the best colouration and the silk yarn developed a shiny golden-brown colour.

Mishra *et al.* (2011) conducted an experiment to find out the performance of ultrasound assisted extraction (UAE) technique for the extraction of colourant from *Dahlia variabilis* and compared it with conventional solvent extraction method. The study revealed that UAE is more efficient as compared to conventional solvent extraction. The most suitable conditions for UAE were found to be the pH of 2-3 and extraction time 25-30 minutes. The FTIR and UV-visible spectra indicate that the colourants are mainly flavonoids and anthocyanins. The extracted dye showed good fastness properties on wool yarn, generally used in carpet industries. So it was concluded that the colourant from Dahlia flower may be a promising ecofriendly indicator which can be used in the place of phenolphthalein.

Baishya *et al.* (2012) carried out an investigation to extract natural dye from flowers of Bottle brush (*Callistemon citrinus*). The dye was extracted by

boiling method. Then a part of the extract was autoclaved. The autoclaved and non-autoclaved flower dye was used to dye scoured cotton cloth using two mordants *viz*. copper sulphate and ferrous sulphate. The fastness test of dyed cloth was undertaken and relative colour strength of the dye was determined in terms of K/S value with respect to autoclaved and non-autoclaved extract. It was found that the dye content and colour strength of the autoclaved extract was higher than its non-autoclaved counterpart. Good light fastness, rub fastness and wash fastness were observed in fabrics mordanted with ferrous sulphate. The relative colour strength was also found to be more in case of cotton clothes mordanted with ferrous sulphate.

Das and Mondal (2012) studied about the uses of dye yielding plants with the help of local people in two famous handicrafts - 'Patchitra' in Pingla and 'Mat craft' in Sabang areas of Paschim Medinipur district, West Bengal. The indigenous knowledge of using the natural dye from plants was carried out. In this investigation fifteen dye yielding plants belonging to 11 families were recorded *viz*. *Acacia catechu* (L.f) Wild. *Aegle marmelos* (Linn.), *Correa ex* Roxb., *Basella alba* Linn., *Bixa orellana* Linn., *Butea mo*no*sperma* Taub., *Clitoria ternatea* Linn., *Curcuma longa* Linn., *Enhydra fluctuans* Lour., *Erythrina suberosa* Roxb., *Lawsonia inermis* Linn*., Nyctanthes arbortristis* Linn., *Peristrophe tinctoria* Nees., *Tagetes erecta* Linn., *Tectona grandis* Linn. f., *Wedelia chinensis* Merril,. The study was conducted to focus on the uses of natural dye in the traditional job in the district and to make people conscious of actual need of conservating the indigenous knowledge.

Raja *et al*. (2012) utilized the petals of Saffron flower to extract dye for application on Pashmina. The saffron flower waste was dried and grounded to powder form. Dye was then extracted by aqueous method at boiling conditions (85^{o}C). The extract was applied on pashmina wool at two different pH (4-5 and 7-8) with and without mordants. It was inferred that the dye extracted from the saffron flower waste were used to dye pashmina shawl and also shows washing

and light fastness properties satisfactorily. The dyed fabric at acidic pH without mordant showed zone of inhibition for the growth against *Staphylococcus aureus*.

Upadhayay and Choudhary (2012) studied about some common plants having dye yielding potential. As many as 100 species were screened, out of which 15 angiosperm plant species belonging to 12 genera and 12 families were studied in detail for dye yielding capacity and fixed after treating with mordents. It was observed that most of the dyes were mainly obtained from the bark of plants and were used for dyeing cotton, silk and fiber. The plants mainly used were *Abution indicum* (flower), *Acacia catechu* (bark), *Butea monosperma* (flower), *Butea superba* (flower), *Cordia dichotoma* (bark), *Chloroxylon swietenia* (bark), *Delonix regia* (bark), *Eucalyptus globules* (bark), *Lannea coromandelica* (bark), *Maninkara hexandra* (bark), *Morinda citrifolia* (bark), *Murraya koenigii* (bark), *Punica granatum* (rind of fruit).

Geetha and Sumathy (2013) extracted dyes from peacock flower, beetroot, onion skin, red cabbage, bougainvillea and papaya leaf. The dye was then used on the cotton fabric for colour fixation. The dye was fixed with the help of mordants and different mordants were used. The mordants used were vinegar for peacock flower, salt for beetroot, alum for onion skin, vinegar for red cabbage, alum and cream of tartar for bougainvillea and salt for papaya leaf. It was observed that when the cloth was pretreated with mordants and then immersed into the dye gave better results than directly adding the mordant into the dye. An analytical study such as IR spectroscopy was also performed on the extract. It was found that all the dyes had phenol in the range of 3350-3400 nm. The highest quantity of phenol was found in beetroot. Alkanes were found in the range of 2920-2930 nm and the highest was found in red cabbage. Alkenes were found in the range of 1070-1700 nm and the highest was found in beetroot. In the extract of peacock flower, red cabbage and papaya leaf alkenes were absent. In papaya leaf extract amines were found at 1121 nm. It was concluded that all the plant extract can be used for dyeing in the textile industry.

Li *et al.* (2013) studied the photo-electrochemical optimal conditions for red cabbage extract as natural dye to develop dye-sensitized solar cells (DSSC). Red cabbage extract was characterized by various methods, such as electrochemical impedance spectroscopy (EIS), UV–Visible spectroscopy and cyclic voltammetry (CVs). DSSC was fabricated from a combination of relatively popular materials containing TiO_2 photo-electrode, natural dye, electrolyte containing I^-/I_3^- redox mediator and counter electrode. The DSSC performance for red cabbage significantly improved when the pH was suitable. When the purification and immersion time of natural dye increased, it directly increased the specific activity and total load volume i.e. the efficiency of the dye to impart colour increased.

Qazi (2013) carried out an investigation to study the dye yielding potential of some wild and cultivated plant species of Kashmir valley. The study comprised of 10 plant species collected from different locations of Kashmir valley include Burr marigold (*Bidens tripartita* L.), Pot marigold (*Calendula officinalis*), Cockscomb (*Celosia argentia*), European blackberry (*Rubus fruticosus* L.), Himalayan indigo (*Indigofera hetrantha*), Indian madder (*Rubia cordifolia*), Mountain fleece flower (*Bistorta amplexicaulis*), Red amaranth (*Amaranthus hybridus*), Selfheal (*Prunella vulgaris*) and Wild strawberry *(Fragaria nulicola*) were studied. From the study it was concluded that the European blackberry fruits contained higher levels of anthocyanin content (521.04 to 1109.97 mg/100g) and are non-toxic and show antimicrobial activity. So it can be used as a raw material for the extraction of reddish to pink edible food colour. Highest value of total carotenoids was recorded in Pot marigold (*Calendula officinalis* L. var. Gitana orange) which are 170.00 to 288.33 mg/100g are non-toxic and have good anti-microbial activity. Thus the petals of these varieties can be used heal as a raw material for the extraction of edible food colours. It was also found that self (purple) and red amaranth (magenta) can also be used as potent dye yielding plant.

Sati and Chandra (2013) applied the natural mordants obtained from *Myrica esculenta, Symplocos* recemosa*, Juglans regia* and *Hippophae rhamnoids* and natural dyes obtained from *Myrica esculenta, Quercus floribunda, Rhus parviflora* and *Berberis kumaonensis* on wool yarns. The plant parts were air dried and crushed and were extracted in water at 100^{o}C for 30, 60 and 90 minutes separately. It was cooled to room temperature and optical density was checked separately. Now 10 g of wool sample were dipped in six different beakers containing dye solution. The dye bath was heated for 1 hour at 100^{o}C and then cooled to room temperature. Then the wool sample was removed and the optical density of the left over solution was checked. The extract of the mordant plants (10 ml) were obtained by crushing the air dried plants and boiling it in water at 100^{o}C for 30, 60, 90 minutes separately. 10 ml of the mordant was used for dyeing. The mordanting was done by three methods i.e. premordanting, simultaneous mordanting and post mordanting. It was optimized separately with each dye. The wool sample was then dried and colour fastness to light and washing was tested. The experimental finding shows that mordant extracted from plants impart different colour shades with different plant dyes and it was stable to light and washing.

Deshpande and Chaturvedi (2014) found out that the flowers of *Plumeria rubra* to be a good source of natural dye for producing various green and ivory shades for silk cloth. An aqueous medium was suitable for extraction of dye from the flowers. The fresh flowers collected were dried and grinded. Then the dye was extracted in pure water (500ml) by boiling 3 g of material for 1 hour. Various combinations of mordants like alum, acid, sodium hydroxide, sodium chloride, copper sulphate, ferrous sulphate and potassium dichromate was used. Now the cloth was dipped in 1% mordant solution for 1 hour (pre-mordanting). Then the pre mordanted cloth was dried by soaking the cloth in extract for 2 hours. Excellent fastness to sunlight was found in all mordant combinations. Pre-treatment of *Terminalia chebula* to silk cloth enhanced the shade and improved

the colour fastness property of dye. Colour change was found in all samples subjected to dry and wet crocking. It was found that only 30 g of material is required for dyeing 5 m of silk cloth. This dye is biodegradable and the technology was found to be economically viable.

Geelani (2014) studied the fabric dye yielding potential of some herbaceous and arboreal plant species of Kashmir valley. Six plant materials of 5 species (*Quercus robura* L. (fruit cups), *Juglans regia* L. (leaves), *Juglans regia* L. (fruit hull), *Prunus cerasifera* Ehrh. var. *atropurpurea* (leaves), *Nasturtium officinale* R.Br. (leaves) and *Tagetes patula* L. (petals) were selected for dye extraction and 4 plant material of 3 species *Salix alba* L. (wood extract and wood ash), *Populus deltoides* Bartram ex-Marsh. (wood ash) and *Punica granatum* L. (peel) for mordant extraction. Natural dyes were extracted by Soxhlet method and mordants by soaking method using distilled water as solvent. The dyes extracted were applied on pashmina, wool, silk and cotton fabrics adopting pre, simultaneous and post mordanting method with and without the mordants. Highest value of anthocyanins were found in *Prunus cerasifera* Ehrh. var. *atropurpurea* (leaves) i.e. 219.80 mg/100 gm and lowest in *Nasturtium officinale* R.Br. (leaves) (69.29 mg/100 gm). Highest carotenoid (0.16 mg/g) and chlorophyll (0.51 mg/g) were recorded in *Juglans regia* L. (leaves), and lowest in *Qurecus robura* L. (fruit cups) i.e. 0.50 mg/g carotenoids and 0.10 mg/g chlorophyll. Leaves of *Prunus cerasifera* Ehrh. var. *atropurpurea* recorded highest percent yield and lowest was recorded in petals of *Tagetes patula* L. The highest average adsorption in pashmina and wool was due to pre-mordanting and that of cotton and silk were due to simultaneous mordanting. The highest average colour strength (K/S) of mordants was recorded due to *Punica granatum* L. (peel) in pashmina, wool, silk and cotton fabric. The lowest value of colour strength in pashmina and wool fabric were recorded due to *Salix alba* L. (wood extract). *Salix alba* L. (wood ash) recorded lowest value in silk and cotton fabric. It was found that all the extracted dyes and mordants recorded acceptable fastness grades and

all the extracted natural dyes and mordants recorded excellent results on selected fabrics except cotton.

Nidhi and Nitan (2014) documented the dye yielding and associated knowledge from the district Kathua of Jammu and Kashmir state in India. Sixty four dye yielding plant species belonging to 43 families were reported from the study area. It was found that wealth of plant resources for natural dyes exist in the study area but most of it is underutilized. So the work was aimed to open new ways for future studies on various aspects of dye yielding and extensive exploration in other districts of the state. Cesalpiniaceae was the most dominant with 4 dye yielding plant species followed by Euphorbiaceae, Fabaceae, Malvaceae, Rosaceae and Solanaceae with 3 dye yielding species each and Anacardiaceae, Asteraceae, Combretaceae, Lythraceae, Mimosaceae, Moraceae, Myrtaceae and Rutaceae with 2 species each. All the remaining families were represented by a single species. Flowers of *Woodfordia fruticosa*, leaves of *Lawsonia inermis*, rhizome of *Curcuma longa*, flowers of *Butea monosperma*, leaves of *Adathoda vasica*, *Morus alba* and dried fruits of *Emblica officinalis* and *Sapindus trifoliatus*, fruits of *Terminalia arjuna* were used as herbal dyes.

Jha *et al.* (2015) extracted natural colourants mainly flavanoids and carotenoids from marigold flowers (*Tagetes erecta* L.) using Soxhlet extaction method and other conventional techniques under different operating conditions. Spectrophotometric method based on the Aluminium complex formation was used for the determination of total flavanoid concentration in the extracts of different solvents used. They also evaluated the dye potential of colourants obtained from the marigold by colouring the cotton fibers and the yarns of pure cotton and wool. The maximum strength of dye was found in the ethanol water mixture (70:30 v/v) as a solvent. The maximum dye extraction was recorded at 95˚C using aqueous extraction method.

Utchanah and Joyram (2015) conducted a comparative study of extracts of beetroot and turmeric. The betalains extracted from beetroot and curcuminoids

extracted turmeric were systematically evaluated using conventional magnetic stirring and microwave assisted extraction techniques. When 50% aqueous ethanol was used along with an agitation speed of 160 rpm, microwave power of 320 W with 0.44 mm particle size and a solvent to solid ratio of 35:1 was used, it was found that the plant species showed maximum yield. Under the optimized conditions, the result showed that the extraction yield for microwave assisted extraction techniques were higher and more efficient than the conventional one as it reduces the extraction time drastically compared to the classical system. Consequently for magnetic stirring extraction of beetroot and turmeric, the maximum yields recovered were 69.55 percent and 28.00 percent while the yield for microwave assisted extraction rose to 89.64 percent and 79.73 percent respectively.

Yeniocak *et al.* (2015) developed an ecofriendly wood stained extract from beetroot and determined the colour stability in UV radiation. The dye was extracted from beetroot (*Beta vulgaris*) by ultrasonic assisted method. It was prepared from aqueous solution with ferrous sulphate, aluminum suulphate, copper sulphate and vinegar mordant mixes. The wood specimens which were used to study was Scots pine (*Pinus sylvestris*), Oriental beech (*Fagus orientalis*), Oak (*Quercus petraea*) and Walnut (*Juglans regia*). After treating with the stain, these wood specimens were then exposed to UV radiation for 50, 100 and 150 hours. All wood specimens showed darker colour with beetroot and ferrous mixed i.e. ferrous sulphate was observed to be the mordant type with the highest colour change among all the wood types. It was found that compared to synthetic dyes, the beetroot stained wood specimens showed better performance. So it was concluded that beetroot dyes can be used as a more economical and ecofriendly wood paint as compared to synthetic dyes.

Das *et al.* (2016) attempted to utilize the petal parts of chrysanthemum (*Dendranthema grandiflora*) flower and peel of badam fruit (*Prunus dulcis*) to extract dye and its application on fabrics. The dyes were extracted by aqueous

extraction method i.e. 20 g of dried flowers were added to 100 ml distilled water and was boiled at a temperature range of 70 to 80^{o}C for 2 hours. After the extraction procedure, the flowers and peels were discarded. The extracts were then applied on cotton and silk. Both the fabrics used for dyeing were boiled with 10% sodium hydroxide solution for 15 minutes to remove the starch and then washed with cold distilled water. For mordanting, copper sulphate (20-30 g/l) was used. The fabrics were then transferred in mordant for 30 minutes followed by treatment in dye bath for 1 hour. Studies were also conducted on the effect of dye without mordanting the fabrics. All the treated fabrics were washed with water, detergent and dried in sunlight. The natural dye extracted from flowers of chrysanthemum showed two different colours; dark purple and red and badam peel showed a pink colour. Cotton and silk showed significant result in dyeing with or without mordants. The dyes showed strong colour properties even after washing with detergent. According to experimental results, the performance was better with all the natural dyes and they can be used as an alternative to synthetic dyes.

Ali and Nishkam (2016) extracted a new natural dye from walnut shell and then applied it to cotton and wool fabrics. They investigated about developing a process for the extraction of natural dyes from inedible walnut shells. The study shows that the source can produce different shades of colour with and without mordents. Extraction of dye from the walnut shell can be done through both acidic and alkaline medium. The result indicates that the dye extracted through acidic medium shows darker shades on wool fabric as compared to cotton fabric.

Ghurde *et al.* (2016) isolated a dye from floral petals of *Ixora coccinea* Linn. with aqueous and methanol. The dyeing potential of the extract was evaluated by dyeing on cotton fabric using alum, copper sulphate, ferrous sulphate and stannous chloride as mordant under normal conditions. An aqueous extract without mordant was used as control. Colour fastness and washing properties were also tested. Shades generated in mordents were all unique and different than control. Results revealed that methanolic extract of the pigments exhibit dark

shades on cotton fabric as compared to aqueous. The spectroscopic analysis of the dye was made for identification of certain functional groups which helped in identifying the interaction of dye with mordants. Mordanting with different metal salts exhibited variation in colour hue because of the formation of coordination complexes with dye molecule. So it was inferred that extract of floral petal of *Ixora coccinea* Linn. can be used for dyeing of cotton fabrics.

Patil *et al.* (2016) investigated the dyeing pigments present in flowers of red rose. The pigments were extracted using four different solvent extraction methods i.e. aqueous extraction method, alkaline extraction method, acidic extraction method and alcoholic extraction method. Three different mordants ferrous sulphate, stannous chloride and copper sulphate were used to dye the cotton fabric. The results revealed that different shades of pink and yellow colours were obtained from the dye when subjected to mordant. The investigation showed that red rose can be used as a source for cotton dyeing.

Pervaiz *et al.* (2016) devised a study to utilize floral waste as an inexpensive source of natural dyes. They used the waste petals of *Rosa damascena* as natural dye and its potency on chrome tanned goat crust leather. Ecofriendly, aqueous method was adopted for the dye extraction. Various shades were obtained with pre-mordanting and post-mordanting methods using ten different mordents. Spectrophotometer (Spectraflash SF-650X) was used for evaluation of colour coordinates of dyed substrates. Very good colour fastness properties with respect to rubbing and light were found with copper sulphate, ferrous sulphate, ferric chloride and acetic acid mordents. Good colour fastness properties were also obtained without mordant. The findings of the study reveal that *R. damascena* petals are a good source of natural dye for leather dyeing, which will help local tanning industry to minimize environmental problems, lessen dermal issues by providing eco-friendly, non-carcinogenic and non-allergic dyes at low cost.

Sabarudin *et al.* (2016) extracted the red dye betacyanin from bougainvillea flower bract using solvent extraction method. It was conducted to investigate the factors contributing for pigment extraction from the flower bracts. The solid liquid ratios used were 0.05, 0.07, 0.09, 0.11, 0.13 and 0.15. The absorbance readings were recorded for every time interval of 15 minutes until 3 hours of the experimental process. The colour intensity of the dye was evaluated using UV-Vis spectrophotometer. This study revealed that the best solid liquid ratio for the extraction process was at 0.11. While the equilibrium time reached for the extraction process was at 60 minutes. The highest absorbance reading of a betacyanin pigment dye extract was 2.3. It was concluded that this pigment can be used as a potential dye.

Tasneem and Maria (2016) obtained natural dyes from beetroot and used it to dye woolen yarn and thread. 50 g of beetroot were cut into small pieces and 450 mL of water was added into it. It was then boiled in a water bath for 2 hours. After that the dye bath was cooled and then 0.2 g of each thread and woolen yarn was soaked in mordant dye mixture and were kept on the boiling water bath for about half an hour with constant stirring. The mordants used were alum, potassium dichromate, vinegar, ammonia and copper sulphate. The dyes wool and thread samples were then rinsed and dried in shade. It was evaluated that betacyanin in beetroot can be used as a potential material for dye. Different shade were observed with different mordants at different temperature and time. It also depends on the amount of mordant and dye.

Arora *et al.* (2017) made an attempt to extract natural dyes from a variety of plant parts such as bark of sandalwood (*Pterocarpus santalinus*), root of madder (*Rubia tinctorum*), rhizome of ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*), dried petals of safflower (*Carthamus tinctorius*), leaves of mulberry (*Morus nigra*), eucalyptus (*Eucalyptus globulus*), basil (*Ocimum basilicum*), henna (*Lawsonia inermis*), hibiscus (*Hibiscus rosa-sinensis*), spinach (*Spinacia oleracea*), dried peels of onion (*Allium cepa*), fruits of harda

(*Terminalia chebula*), fruit pulp of jamun (*Syzygium cumini*) etc. using specific techniques such as pre-mordanting, simultaneous mordanting and post-mordanting wherein different mordents such as alum, copper sulphate and ferrous sulphate etc. were used to fix the dye on to the textile material. A variety of natural dyes were obtained from the above plant parts and it was found that they produce a rainbow of natural dyes from varied shades of each colour. These dyes were tested for their dyeing potential on different textile materials (cotton, silk and wool). The intensity of colour of dye extracted from the same plant material changes with the pH of the medium. They prepared shade cards for each dye and found that the colour obtained varied depending on the type of mordant applied and the mordanting technique used. Best colours were obtained on the silk fabric followed by wool and lastly cotton. Therefore, it was concluded that modern techniques of extraction are a better approach which is cost and time efficient and a good yield of dye can be obtained.

Ganie (2017) carried out an investigation on the paper making and dye yielding potential of some herbaceous and arboreal taxa of Kashmir valley. The selected species were *Amaranthus hybridus* L. and *Datura stramonium* L. stem (stalks) for paper making. *Calendula* offcinalis L. petals, *Prunus cerasifera* L. Ehrh. var. *atropurpurea* leaves and *Nasturtium officinale* leaves for dye extraction and *Salix alba*. L. wood stalks for mordant extraction. Highest value of anthocyanins was obtained in *Prunus cerasifera* Ehrh. var. *atropurpurea* (215.88 mg/100 g) and lowest in *Calendula officinalis* (10.29 mg/ 100 g). The carotenoid content were recorded higher in *Calendula officinalis* L. (1.40 mg/g fw) and lowest in *Nastartium officinale* (0.07 mg/g fw). The paper obtained from *Amaranthus hybridus* L. recorded highest percent dye absorption with *Prunus cerasifera* Ehrh. var. *atropurpurea* and lowest with *Nastartium officinale*. Similarly the paper obtained from *Datura stromonium* L. showed highest percent dye absorption value with *Prunus cerasifera* Ehrh. var. *atropurpurea* and lowest with *Nastartium officinale*. The result revealed that *Amaranthus hybridus* L. and

Datura stromonium L. stalks have a promising potential for pulp and paper production. *Prunus cerasifera* Ehrh. var. *atropurpurea, Nasturtium officinale* and *Calendula* offcinalis L. can be used as potent dye yielding plants for pulp and paper industry.

Roriz *et al.* (2017) aimed to optimize the condition that helps to maximize betacyanin extraction from *Gomphrena globosa* as an alternative source for food colourants. An experimental design was developed for testing the extraction variable i.e. time, temperature, water ethanol proportion and solid liquid ratio using response surface methodology. The powdered samples of the pigmented parts were extracted at different time, temperature, water ethanol proportion and solid liquid ratio. The solvent volume was fixed at 20 ml and the samples were stirred using a CIMAREC magnetic stirrer at an agitation speed of 500 rpm. The mixture was then filtered and centrifuged at 14000 rpm for 10 minutes. The supernatant was then collected and divided into two parts. One part was used to quantify yield and its light intensity. The second part was employed to quantify the total betacyanin. The betacyanin identified were gomphrenin and isogomphrenin II and II. The highest betacyanin content (45mg/g) was obtained by 165 minute at 25^{o}C, 0% ethanol and 5 g/l of solid liquid ratio. It was observed that betacyanin content from the floral parts of *Gomphrena globosa* is higher than those normaly found in other sources.

Chapter 3

MATERIALS AND METHODS

3.1 Locations

The investigation entitled "Extraction and Stability of Natural dyes from Selected Ornamental Plant Species" was conducted during 2017-2018 at Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Shalimar, Srinagar (J&K).

3.2 Experimental material

The experimental material consists of four plant species collected from different areas of Kashmir valley are as under:

S. No.	Plant species	Common name	Parts used
1.	*Viola tricolour* var. *hortensis* DC	Pansy	Flowers
2.	*Phlox paniculata* L.	Phlox	Flowers
3.	*Gomphrena globosa* L.	Globe Amaranth	Inflorescence
4.	*Chrysanthemum morifolium* L.	Chrysanthemum	Flowers

3.3 Quantification, stability and colour quality of pigments

3.3.1 Quantification of pigments (anthocyanins and carotenoids)

3.3.1.1 Total anthocyanin content (mg/100g)

The total anthocyanin content present in the flower sample was extracted using the method given by Rangana (1986) spectrophotometric method. The procedure involved extraction of anthocyanin by using ethanolic HCL. One gram of sample was placed in a beaker and 100ml of ethanolic HCL (95% Ethanol + 1.5 N HCL) was added to it. Then the sample was kept in refrigerator overnight at 4 ^{o}C. The pigment was then filtered using Whatman Filter Paper No.1. Then the filtrate was taken and the colour was measured at a wave length of 535nm against a blank of ethanolic HCL using UV spectrophotometer.

Pansy

Phlox

Globe Amaranth

Chrysanthemum

Plate 1: Selected species

Plate 2: Collection of samples

Calculations

The total anthocyanin content was calculated using the formula given below:

$$\text{Total optical density} = \frac{\text{Optical density} \times \text{volume made}}{\text{Weight of sample}} \times 100$$

$$\frac{\text{Optical density} \times \text{volume made}}{\text{Weight of sample}} \times 100$$

$$\text{Total anthocyanin content (mg/100g)} = \frac{\text{Total optical density}}{98.20} \times 100$$

3.3.1.2 Total carotenoid content (mg/100g)

Total carotenoid content was calculated by the method given by Arya (1981). In this method, known weight of sample was taken and the pigment was extracted with 10 ml methanol, 10 ml acetone and 20 ml hexane by using pestle and mortar. Then the extract was washed in a separating funnel continuously with 5% NaCl solution. The content was then allowed to stand for some time, as a result two layers were separated. The lower layer was discarded and the upper layer I.e. supernatent was collected in a 25 ml volumetric flask. The volume was made with hexane upto 25 ml and then a pinch of anhydrous sodium sulphate was added to it. Then the extract was filtered using Whatman filter paper No.1. Finally the absorbance was noted at 449nm using spectrophotometer against hexane as blank.

Calculations

The total carotenoid content was calculated using the following formula:

$$\text{Total carotenoid content (mg/100g)} = \frac{\text{Optical density} \times \text{volume made}}{250 \times \text{weight of sample}} \times 100$$

3.3.2 Stability of pigments (anthocyanins and carotenoids)

The stability of pigments was assessed by quantifying the pigments after an interval of 30 days for six months under normal storage conditions.

Plate 3: Measuring colour attributes

3.3.3 Colour quality of pigments (anthocyanins and carotenoids)

The colour quality of pigments was assessed by recording their colour quality after an interval of 30 days for six months under normal storage conditions.

3.3.3.1 L*, a*b* values (By colour analyser)

A chromometer (Model CR-2000, Minolta, Osaka, Japan) was used to measure the colour attributes of the sample. This meter was calibrated using the manufacturer's standard plate. Three measurements were taken and results averaged.

L* is the lightness or darkness ranging from 0-100 i.e., black to white. Positive values of a* are in the direction of redness and negative in the direction of green. Positive values of b* are in the direction of yellowness and negative in the direction of blueness.

3.3.3.2 L*, C*, h* values

a) L* = lightness with 100 = absolute white; 0 = absolute black.

b) C* stands for Chroma which is a measure of intensity and represents colour saturation from dull (low value) to vivid colour (high value) and was calculated as:

$$\text{Chroma } (C^*) = [(a^*)^2 + (b^*)^2]^{1/2}$$

c) H^o stands for Hue angle which is defined as a colour wheel, with red-purple at an angle of 0°, yellow at 90°, bluish-green at 180° and blue at 270° and was derived from two coordinates a* and b* and calculated as:

$$\text{Hue angle } (H^\circ) = \tan^{-1} b^*/a^*$$

3.3.3.2.1 Total colour change (ΔE)

Total colour change was calculated as below:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

3.4 Antimicrobial activity determination

3.4.1 Collection of plant material

Freshly collected plant samples were air dried. Then it was grinded to a fine powder using a mixer grinder. This powdered material was then used for extraction of dyes.

3.4.2 Preparation of plant extracts

Acetone was used as an extractant in the ratio 1:10 of flower material to extractant. Then this material was filtered using Whatman filter paper No. 1 and the supernatant was collected. The filterate was evaporated till it gets completely dried. DMSO was used to dissolve the extract and it was stored at 4^{o}C in a sterile air tight eppendorf tubes.

3.4.3 Bacterial and Fungal strains

Pure culture of bacterial strain (*Escherichia coli*) was obtained from Division of Microbiology, Faculty of Veterinary Sciences & Animal Husbandry, SKUAST, Shuhama, Srinagar and pure culture of fungal strain (*Asperrigillus* spp.) were collected from Division of Plant Pathology, Faculty of Horticulture, SKUAST, Shalimar, Srinagar.

3.4.4 Preparation of bacterial inoculums

The stock culture was maintained on plates of Mueller Hinton Agar at 4^{o}C. The bacterial strain was sub cultured by transferring cells using inoculation loop from the stock culture to the test tubes containing Nutrient Broth Medium. It was then incubated for a period of 24 hours at 37^{o}C.

3.4.5 Preparation of fungal inoculums

The isolates were maintained on plates of Sabourand Dextrose Agar at 4^{o}C until the experiment was performed. Active cultures were prepared by mixing carefully small amount of test organisms in a sterilized petri plate with autoclaved water under UV laminar air flow and then spreaded.

Plate 4: Anti-microbial susceptibility test of selected species

3.4.6 Antimicrobial susceptibility test

Agar disc diffusion was used to evaluate antimicrobial activity of the plant extract. The antimicrobial susceptibility of the plant extracts were tested against the bacterial (*E. coli*) and fungal (*Asperrigillus* spp.) pathogenic strains. 20 ml of sterilized Mueller Hinton Agar for bacteria and 20 ml of sterilized Sabourand Dextrose Agar for fungi were poured into sterilized petri plates and the plates were allowed to solidify for 5 minutes. Then the previously inoculated bacterial and fungal suspensions were swabbed on the agar surfaced petri plates by streaking it in horizontal and vertical directions to ensure the uniform distribution of the organisms. The paper discs of 6 mm diameter was prepared from Whatman filter paper No.1, placed in a petri dish and sterilized. The sterile paper discs were soaked in plant extract and it was placed in bacterial and fungal inoculated agar plates by using sterile forceps. Then the paper discs were adhered to the agar by gently tapping. Before inoculating, the agar plates were allowed to stand for 5 minutes to ensure the diffusion of plant extract. The plates were then incubated at 37^{o}C for 24 hours for bacteria and 25^{o}C for 72 hours for fungi in an inverted position. The zone of inhibition which was formed around the discs was measured including diameter of discs with transparent ruler from back of the plates. The experiment was replicated three times.

The experiment was performed under aseptic conditions. The work surface area of laminar air flow was cleaned with alcohol and UV light was left on 15 minutes before starting the working. The experiments were performed wearing gloves and masks to avoid contamination.

Chapter 4

EXPERIMENTAL FINDINGS

The investigation titled 'Extraction and Stability of Natural dyes from Selected Ornamental plant species' was carried out during 2017-18 at Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar (J&K). The results of the experiment are as under:

4.1 Quantification of pigments (anthocyanins and carotenoids) of selected plant species

4.1.1 Total anthocyanin content

4.1.1.1 Pansy (*Viola tricolour* var. *hortensis* DC.)

In Pansy (*Viola tricolour* var. *hortensis* DC.), total anthocyanin content ranged between 150.38 to 471.08 mg/ 100g under normal storage condition. Highest anthocyanin content (471.08 mg/100g) was recorded in the flower extract during T_0 i.e. on zero day of storage and lowest anthocyanin content (150.38 mg/100g) was recorded after 5^{th} month of storage interval. Decreasing trend of anthocyanin was recorded in table 1 and every storage interval was significantly different from each other.

4.1.1.2 Phlox (*Phlox paniculata* L.)

According to the data presented in table 1, it was revealed that the total anthocyanin content in Phlox (*Phlox paniculata* L.) was found in a range between 10.28 to 398.45 mg/100g. It can be inferred from table 1 that the highest value of anthocyanin content (398.45 mg/100g) was recorded during zero day of storage i.e. on T_0. The lowest value of anthocyanin content (10.28 mg/100g) was recorded after 5^{th} month of storage interval. The total anthocyanin content also showed a decreasing trend and every storage interval was statistically significant from each other.

4.1.1.3 Globe Amaranth (*Gomphrena globosa* L.)

In case of Globe Amaranth (*Gomphrena globosa* L.), total anthocyanin content ranged from 9.71 to 110.74 mg/100g (Table 1). The pigment extracted from the flowers of Globe Amaranth showed the maximum anthocyanin content of 110.74 mg/100g during T_0 i.e. on zero day of storage interval and it was significantly higher than all other storage intervals. The minimum value of anthocyanin content (9.71 mg/100g) was recorded after 5th month of the storage interval. Decreasing trend of anthocyanin was recorded after each storage interval.

4.1.1.4 Chrysanthemum (*Chrysanthemum morifolium* L.)

Anthocyanin extracted from Chrysanthemum (*Chrysanthemum morifolium* L.) ranged between 0.19 to 5.74 mg/100g (Table 1). Highest pigment content (5.74 mg/100g) was recorded during zero day of storage and lowest pigment content (0.19 mg/100g) was recorded in the flower extract after 3th month of storage. No anthocyanin content was recorded after the 4th month of storage in flower material. Here also a decreasing trend of anthocyanin content was recorded in each month of storage and every storage interval was statistically superior from each other except the value of total anthocyanin recorded from the flower extract after 4th and 5th month of storage.

Out of all selected test species, the highest anthocyanin content (471.08 mg/100g) was recorded in Pansy (*Viola tricolour* var. *hortensis* DC.) followed by Phlox (*Phlox paniculata* L.) (398.45 mg/100g), Globe Amaranth (*Gomphrena globosa* L.) (110.74 mg/100g) and Chrysanthemum (*Chrysanthemum morifolium* L.) (5.74 mg/100g). The lowest content (5.74 mg/100g) was recorded in Chrysanthemum followed by Globe Amaranth (*Gomphrena globosa* L.) (110.74 mg/100g), Phlox (*Phlox paniculata* L.) (398.45 mg/100g) and Pansy (*Viola tricolour* var. *hortensis* DC.) (471.08 mg/100g).

Table 1: Changes in total anthocyanin content (mg/100g) at different storage intervals of test species under ambient conditions

Species / Timing	Pansy	Phlox	Globe Amaranth	Chrysanthemum
T_0	471.08 (21.73)	398.45 (19.98)	110.74 (10.57)	5.74 (2.59)
T_1	430.75 (20.77)	301.76 (17.40)	93.94 (9.74)	2.65 (1.91)
T_2	342.32 (18.52)	220.64 (14.88)	85.66 (9.31)	1.42 (1.55)
T_3	290.37 (17.07)	124.62 (11.21)	58.50 (7.71)	0.19 (1.09)
T_4	221.01 (14.90)	70.87 (8.47)	21.69 (4.76)	0.00 (1.00)
T_5	150.38 (12.30)	10.28 (3.36)	9.71 (3.27)	0.00 (1.00)
CD ($p \leq 0.05$)	**0.598**	**0.637**	**0.537**	**0.043**

Figures in parentheses are square root of transformed means.

4.1.2 Total carotenoid content

4.1.2.1 Pansy (*Viola tricolour* var. *hortensis* DC.)

In Pansy (*Viola tricolour* var. *hortensis* DC.) the total carotenoid content ranged between 0.0003 to 0.042 mg/100g. The data presented in the Table 2 revealed that the highest carotenoid content of 0.042 mg/100g was recorded during zero day of storage interval and the lowest carotenoid content (0.0003 mg/100g) was recorded after 4^{th} month of storage. On the 5^{th} month of storage interval, no carotenoid content was detected in the flower extract. Here a decreasing trend in total carotenoid content was recorded and each storage interval was statistically significant except the value recorded after 4^{th} and 5^{th} month of storage.

4.1.2.2 Phlox (*Phlox paniculata* L.)

In case of Phlox (*Phlox paniculata* L.) the total carotenoid content ranged from 0.084 to 0.56 mg/100g. The maximum value (0.56 mg/100g) of carotenoid content was found during zero day of storage and the minimum value (0.084 mg/100g) was recorded after 5^{th} month of storage interval. Decreasing trend in carotenoid content was recorded when the storage interval was increased. Here every storage interval was statististically significant from each other.

4.1.2.3 Globe Amaranth (*Gomphrena globosa* L.)

Data displayed in the Table 2 reveals that the total carotenoid content of Globe Amaranth (*Gomphrena globosa* L.) was found in between 0.0003 to 0.019 mg/100g. The highest value of carotenoid content (0.019 mg/100g) was recorded on T_0 i.e. zero day of storage and the lowest value of carotenoid content (0.0003 mg/100g) was recorded after 3^{rd} month of storage interval. With an increase in the storage intervals, there was a decrease in the carotenoid content. No carotenoid content was found after 4^{th} and 5^{th} storage intervals. Each value of carotenoid content was found to be statistically significant except the value after 4^{th} and 5^{th} month of storage interval.

4.1.2.4 Chrysanthemum (*Chrysanthemum morifolium* L.)

According to the data presented in Table 2, the total carotenoid content in Chrysanthemum (*Chrysanthemum morifolium* L.) ranged between 49.11 to 149.20 mg/100g. The maximum value of carotenoid content (149.20 mg/100g) was recorded during zero day of storage interval and the minimum value (49.11 mg/100g) was recorded after 5^{th} month of storage interval. It was revealed that with an increase in the storage intervals, there was a decrease in the carotenoid content. Every storage interval was found to be statistically different from each other.

The highest carotenoid content (149.20 mg/100g) was recorded in Chrysanthemum (*Chrysanthemum morifolium* L.) followed by Phlox (*Phlox paniculata* L.) (0.56 mg/100g), Pansy (*Viola tricolour* var. *hortensis* DC.) (0.042 mg/100g) and Globe Amaranth (*Gomphrena globosa* L.) (0.019 mg/100g) and the lowest content was recorded in Globe Amaranth (*Gomphrena globosa* L.) (0.019 mg/100g), followed by Pansy (*Viola tricolour* var. *hortensis* DC.) (0.042 mg/100g), Phlox (*Phlox paniculata* L.) (0.56 mg/100g) and Chrysanthemum (*Chrysanthemum morifolium* L.) (149.20 mg/100g) in all selected plant species.

Table 2: Changes in total carotenoid content (mg/100g) at different storage intervals of test species under ambient storage conditions

Species / Timing	Pansy	Phlox	Globe Amaranth	Chrysanthemum
T_0	0.042 (1.02)	0.56 (1.25)	0.019 (1.01)	149.20 (12.25)
T_1	0.023 (1.01)	0.42 (1.19)	0.008 (1.00)	130.98 (11.48)
T_2	0.018 (1.01)	0.27 (1.12)	0.003 (1.00)	125.57 (11.24)
T_3	0.007 (1.00)	0.20 (1.09)	0.0003 (1.00)	84.91 (9.26)
T_4	0.0003 (1.00)	0.13 (1.06)	0.00 (1.00)	66.20 (8.19)
T_5	0.00 (1.00)	0.084 (1.04)	0.00 (1.00)	49.11 (7.07)
CD ($p \leq 0.05$)	**0.001**	**0.012**	**0.001**	**0.168**

Figures in parentheses are square root of transformed means

4.2 Chromaticity of selected species

4.2.1 L* value of test species

4.2.1.1 Pansy (*Viola tricolour* var. *hortensis* DC.)

The L* value of Pansy (*Viola tricolour* var. *hortensis* DC.) ranged from 16.92 to 45.25 (Table 3) under normal storage conditions. Highest value of L*(45.25) was recorded after 5th month of storage and it was statistically superior to all other storage intervals. Lowest value of L* (16.92) was recorded during zero day (T_0) of storage interval. It was found that with increasing days of storage interval, L* value increased.

4.2.1.2 Phlox (*Phlox paniculata* L.)

In case of Phlox (*Phlox paniculata* L.), the value of L* ranged between 31.92 to 51.55 (Table 3). The maximum value of L* (51.55) was recorded after 5th month of the storage interval and minimum value of L* (35.25) was recorded during zero day of storage interval. Here also with an increase in the storage interval, there was an increase in the L* value and each storage interval were statistically different from each other.

4.2.1.3 Globe Amaranth (*Gomphrena globosa* L.)

The L* value of Globe Amaranth (*Gomphrena globosa* L.) flowers ranged from 42.34 to 50.61 (Table 3). The highest value of L*(50.61) was recorded after 5th month of storage interval while the lowest value (42.34) was recorded during zero month of storage. Here also an increasing trend of L* value was recorded and every storage interval was statistically significant from each other.

4.2.1.4 Chrysanthemum (*Chrysanthemum morifolium* L.)

According to the data presented in the Table 3, it can be concluded that the L* value of Chrysanthemum (*Chrysanthemum morifolium* L.) ranged from 41.58 to 52.75. It was seen that with increasing days of storage, there was a decreasing trend of L* value. The maximum value of L*(52.75) was recorded during zero day of storage (T_0) and the minimum value of L* (41.58) was recorded after 5th month of storage interval. Each value of L* except T_0 i.e. zero day for different storage intervals was statistically different from each other.

Table 3: Chromaticity (L* value) of test species under ambient conditions

Species / Timing	Pansy	Phlox	Globe Amaranth	Chrysanthemum
T_0	16.92	31.92	42.34	52.75
T_1	22.21	38.85	44.69	52.69
T_2	26.04	41.62	46.55	48.50
T_3	30.56	46.60	47.45	47.78
T_4	35.28	49.00	48.18	43.57
T_5	45.25	51.55	50.61	41.58
CD ($p \leq 0.05$)	**0.57**	**0.53**	**0.58**	**0.60**

4.2.2 a* value of test species

4.2.2.1 Pansy (*Viola tricolour* var. *hortensis* DC.)

The a* value of Pansy (*Viola tricolour* var. *hortensis* DC.) flowers ranged from 32.25 to 47.04 (Table 4). Here it was observed that with increasing days of storage, there was a decrease in the a* value. The highest value of a*(47.04) was recorded during zero day of the storage interval and the lowest value (32.25) was recorded after 5th month of storage interval. Here all values of a* in each storage interval was statistically significant from each other.

4.2.2.2 Phlox (*Phlox paniculata* L.)

Data displayed in table 4 reveals that the a* value of Phlox (*Phlox paniculata* L.) flowers was found in between 48.27 to 59.71. The highest value of a* (59.71) was recorded during zero of storage interval and the lowest value of a* (48.27) was recorded after 5th month of storage. With the increasing days of storage intervals, the a* value decreased. Here all the a* values of storage interval was statistically different from each other.

4.2.2.3 Globe Amaranth (*Gomphrena globosa* L.)

The data given in the table 4 shows that the a* value of the flowers of Globe Amaranth (*Gomphrena globosa* L.) ranged between 22.58 to 28.63. The highest value of a* (28.63) was recorded during zero day of the storage and the lowest value (22.58) was recorded after 5th month of storage interval. The data revealed that the a* value gets decreased with increasing days of storage intervals. The value of a* for each storage intervals was statistically significant from each other.

4.2.2.4 Chrysanthemum (*Chrysanthemum morifolium* L.)

The a* value of Chrysanthemum (*Chrysanthemum morifolium* L.) ranged between 22.12 to 31.89 (Table 4). Highest value of a* (31.89) was recorded after 5th month of storage and the lowest value of a* (22.12) was recorded during zero day of storage interval. It was observed that with an increase in the storage interval the a* value also increased. Every storage interval was statistically different from each other.

Table 4: Chromaticity (a* value) of test species under ambient storage conditions

Species / Timing	Pansy	Phlox	Globe Amaranth	Chrysanthemum
T_0	47.04	59.71	28.63	22.12
T_1	42.40	58.23	27.23	25.75
T_2	38.22	55.37	26.24	26.24
T_3	35.29	53.19	25.37	27.35
T_4	33.73	50.70	23.40	30.19
T_5	32.25	48.27	22.58	31.89
CD ($p \leq 0.05$)	**0.40**	**0.43**	**0.52**	**0.47**

4.2.3 b* value test species

4.2.3.1 Pansy (*Viola tricolour* var. *hortensis* DC.)

The b* value of Pansy (*Viola tricolour* var. *hortensis* DC.) flower ranged between 42.35 to 54.16 (Table 5). The highest value of b* (54.16) was recorded during zero day of storage and the lowest value of b*(42.35) was recorded after 5^{th} month of the storage interval. Here a decreasing trend of the b* value was recorded as the storage interval increased. Every storage interval was statistically different from each other except the 2^{nd} month.

4.2.3.2 Phlox (*Phlox paniculata* L.)

The data displayed in the table 5 indicates that the b* value of Phlox (*Phlox paniculata* L.) ranged between 13.12 to 20.86. Here it was recorded that with increasing days of storage, there was a decrease in the b* value. The maximum value of b* (20.86) was recorded during the zero day of storage interval and the minimum (13.12) was recorded after 5^{th} month of storage. Every storage interval was significantly different from each other except 1^{st} and 3^{rd} month.

4.2.3.3 Globe Amaranth (*Gomphrena globosa* L.)

Data presented in table 5 shows that Globe Amaranth (*Gomphrena globosa* L.) has a b* value ranged between 6.42 to 12.62. Here the highest value of b* (12.62) was recorded during zero day of storage interval (T_0) and the lowest value of b* (6.42) was recorded after 5^{th} storage interval (T_5). Here all storage intervals were statistically significant from each other except 2^{nd}, 3^{rd} and 4^{th} month and a decreasing trend of b* value was observed with an increasing storage interval.

4.2.3.4 Chrysanthemum (*Chrysanthemum morifolium* L.)

From the data given in table 5, it was observed that the b* value of Chrysanthemum (*Chrysanthemum morifolium* L.) ranged between 25.94 to 53.98. The highest value of b* (53.98) was observed during zero day of storage interval (T0) and the lowest value of b* (25.94) was recorded after 5^{th} month of storage interval. The b* value in Chrysanthemum decreased with increase of the storage interval. It was also found that b* value of each storage interval was statistically different from each other.

Table 5: Chromaticity (b* value) of test species under ambient storage conditions

Species / Timing	Pansy	Phlox	Globe Amaranth	Chrysanthemum
T_0	54.16	20.86	12.62	53.98
T_1	51.76	18.34	11.10	48.07
T_2	49.33	17.28	9.63	42.77
T_3	49.29	5.52	8.56	38.36
T_4	44.37	14.35	7.38	32.29
T_5	42.35	13.12	6.42	25.94
CD ($p \leq 0.05$)	**0.98**	**1.20**	**1.31**	**1.52**

4.2.4 Chroma (C*) value of test species

4.2.4.1 Pansy (*Viola tricolour* var. *hortensis* DC.)

The C* value of Pansy (*Viola tricolour* Var. *hortensis* DC.) ranged between 53.24 to 71.52 (Table 6). The highest value of C* (71.52) was observed during the zero day of storage interval and the lowest value of C*(53.24) was recorded after 5th month. With increasing days of storage, C* value gets decreased and every storage interval was statistically different from each other.

4.2.4.2 Phlox (*Phlox paniculata* L.)

According to the data shown in table 6, the C* value of Phlox (*Phlox paniculata* L.) ranged from 50.27 to 63.24. It was also observed that with increasing days of storage interval there was a decrease in the C* value. The highest value of C* (63.23) was recorded during zero day of storage interval and lowest value of C* (50.27) was recorded after 5th month of storage interval. It was also observed that value of C* for each storage interval was significantly different from each other.

4.2.4.3 Globe Amaranth (*Gomphrena globosa* L.)

As shown in the table 6, the C* value of Globe Amaranth (*Gomphrena globosa* L.) ranged between 23.44 to 31.29. The highest value of C* (31.29) was recorded during zero day of storage interval and the lowest value of C* (23.44) was recorded during zero day of storage interval. It was observed that with increase in the storage intervals, there was a decrease in the C* value. The C* value of each storage interval was statistically significant from each other except 2nd and 4th month.

4.2.4.4 Chrysanthemum (*Chrysanthemum morifolium* L.)

In case of Chrysanthemum (*Chrysanthemum morifolium* L.), the value of C* ranged between 41.11 to 58.34 (Table 6). Here it was observed that with an increase in the storage intervals, there was a decrease in the value of C*. The maximum value of C* (58.34) was observed during zero day of storage and the minimum value of C* (41.11) was recorded after 5th month of storage. Each value of C* was statistically different from each other.

Table 6: Chroma (C*) of test species under ambient storage conditions

Species / Timing	Pansy	Phlox	Globe Amaranth	Chrysanthemum
T_0	71.52	63.24	31.29	58.34
T_1	66.90	61.04	29.40	54.55
T_2	62.40	58.03	27.94	50.51
T_3	58.82	55.05	26.77	47.11
T_4	55.07	52.69	24.54	44.22
T_5	53.23	50.27	23.44	41.11
CD ($p \leq 0.05$)	**1.04**	**1.23**	**1.36**	**1.59**

4.2.5 Hue angle, H° value of test species

4.2.5.1 Pansy (*Viola tricolour* var. *hortensis* DC.)

The H° value of pansy (*Viola tricolour* var. *hortensis* DC.) was observed to be in the range of 48.22˚ to 53.54˚ (Table 7). With an increase in the storage interval, there was a decrease in the H° value. The maximum value of H° (53.54˚) was observed during zero month of storage interval and the minimum value of H° (48.22˚) was observed after 5th storage interval. Each value of H° was statistically non-significant from each other.

4.2.5.2 Phlox (*Phlox paniculata* L.)

The data displayed in the Table 7 indicates that the H° value of Phlox (*Phlox paniculata* L.) ranged between 15.11˚ to 19.25˚. With an increase in the storage interval there was a decrease in the H° value. The maximum value of H° (19.25˚) was observed during zero day of storage interval and the minimum value of H° (15.11˚) was observed after 5th month of storage interval. Here all the values of H° during different storage intervals were found to be statistically significant except 1st, 2nd and 3rd month of storage interval.

4.2.5.3 Globe Amaranth (*Gomphrena globosa* L.)

The H° value of Globe Amaranth (*Gomphrena globosa* L.) was found to be in the range between 15.93˚ to 23.78˚. The highest value of H° (23.78˚) was recorded during zero day of storage interval and the lowest value of H° (15.93˚) was recorded after 5th month of storage. Here with an increase in the storage intervals the value of H° decreased. All the H° values for different storage intervals were found to be statistically significant except 2nd month.

4.2.5.4 Chrysanthemum (*Chrysanthemum morifolium* L.)

The H° value of Chrysanthemum (*Chrysanthemum morifolium* L.) ranged between 39.37˚ to 67.71˚. The highest value of H° (67.71˚) was found during zero day of storage interval and the lowest value of H° (39.12˚) was found after 5th storage interval. With an increase in the storage intervals, there was a decrease in the H° value for Chrysanthemum and every storage interval was found to be statistically different from each other except 1st month.

Table 7: Hue angle (H°) of test species under ambient storage conditions

Species / Timing	Pansy	Phlox	Globe Amaranth	Chrysanthemum
T_0	53.54	19.25	23.78	67.71
T_1	52.24	17.49	22.18	61.80
T_2	51.40	7.33	20.16	58.51
T_3	50.36	16.26	19.22	54.51
T_4	49.34	15.81	17.75	46.89
T_5	48.22	15.11	15.93	39.37
CD ($p \leq 0.05$)	**1.37**	**1.57**	**1.23**	**3.47**

4.2.6 Total Colour Change (ΔE) value of test species

4.2.6.1 Pansy (*Viola tricolour* var. *hortensis* DC.)

The ΔE value of Pansy (*Viola tricolour* var. *hortensis* DC.) ranged between 82.52 to 87.57. The maximum value of ΔE (87.57) was found during zero day of storage interval and the minimum value of ΔE (82.52) was found after 5^{th} month of storage interval. There was a decrease in the ΔE value with an increase in the storage intervals. All the values of ΔE except zero day and 3^{rd} month of storage were found to be statistically significant.

4.2.6.2 Phlox (*Phlox paniculata* L.)

In case of Phlox (*Phlox paniculata* L.), the value of ΔE was found in the range of 72.33 to 90.28. The highest value of ΔE (90.28) was found during zero day of storage interval and the lowest value of ΔE (72.33) was found after 5^{th} month of storage interval. With an increase in the storage intervals, there was a decrease in the ΔE value. Every storage interval was statistically significant except the 1^{st} month of storage.

4.2.6.3 Globe Amaranth (*Gomphrena globosa* L.)

In case of Globe Amaranth (*Gomphrena globosa* L.) the value for ΔE ranged between 104.52 to 109.79. With an increase in the storage intervals, there was a decrease in the ΔE value. The highest value of ΔE (109.79) was found during zero day of storage interval and the lowest value of ΔE (104.52) was found after 5^{th} month of storage interval. The ΔE value for each storage interval was found to be statistically different except the zero day and 1^{st} month.

4.2.6.4 Chrysanthemum (*Chrysanthemum morifolium* L.)

According to the data displayed in the table 8, the ΔE value for Chrysanthemum (*Chrysanthemum morifolium* L.) ranged between 80.10 to 94.30. It was observed that with an increase in the storage intervals, there was a decrease in the ΔE value. The highest and lowest value of ΔE was found to be (94.30) and (80.10) respectively. Here all the values of ΔE during different storage intervals was found to be statistically significant except 2^{nd}, 3^{rd} and 4^{th} month.

Table 8: Total colour change (ΔE) of test species under ambient storage conditions

Species / Timing	Pansy	Phlox	Globe Amaranth	Chrysanthemum
T_0	87.57	90.28	109.79	94.30
T_1	86.91	88.72	108.69	90.09
T_2	85.51	87.95	107.29	86.02
T_3	84.28	85.25	106.12	83.70
T_4	83.76	81.63	105.45	81.42
T_5	82.52	72.33	104.52	80.10
CD ($p \leq 0.05$)	**0.81**	**1.01**	**1.02**	**3.21**

4.3 Antimicrobial activity

All the extracts of the test plant species showed antibacterial activity against *Escherichia coli* and antifungal activity against *Aspergillus niger*. According to the data presented in the table 10, the flower extract of Chrysanthemum (*Chrysanthemum morifolium* L.) recorded the highest zone of inhibition (9.6 mm) against *E. coli* and the lowest zone of inhibition (7.9 mm) was recorded in the flower extract of Pansy flower.

In case of *Asperigillus niger* the highest zone of inhibition (10.8 mm) was recorded due to Chrysanthemum flower and the lowest zone of inhibition (7.4 mm) was recorded due to Pansy flower.

Table 9: Antimicrobial activity of natural dyes against bacteria and fungi

Parameters / Treatments	Zone of inhibition (mm)	
	Bacteria (*Escherichia coli*)	**Fungi (*Aspergillus niger*)**
Pansy	7.90	7.40
Phlox	8.40	10.00
Globe Amaranth	9.00	7.80
Chrysanthemum	9.60	10.80
CD ($p<0.05$)	**0.48**	**0.50**

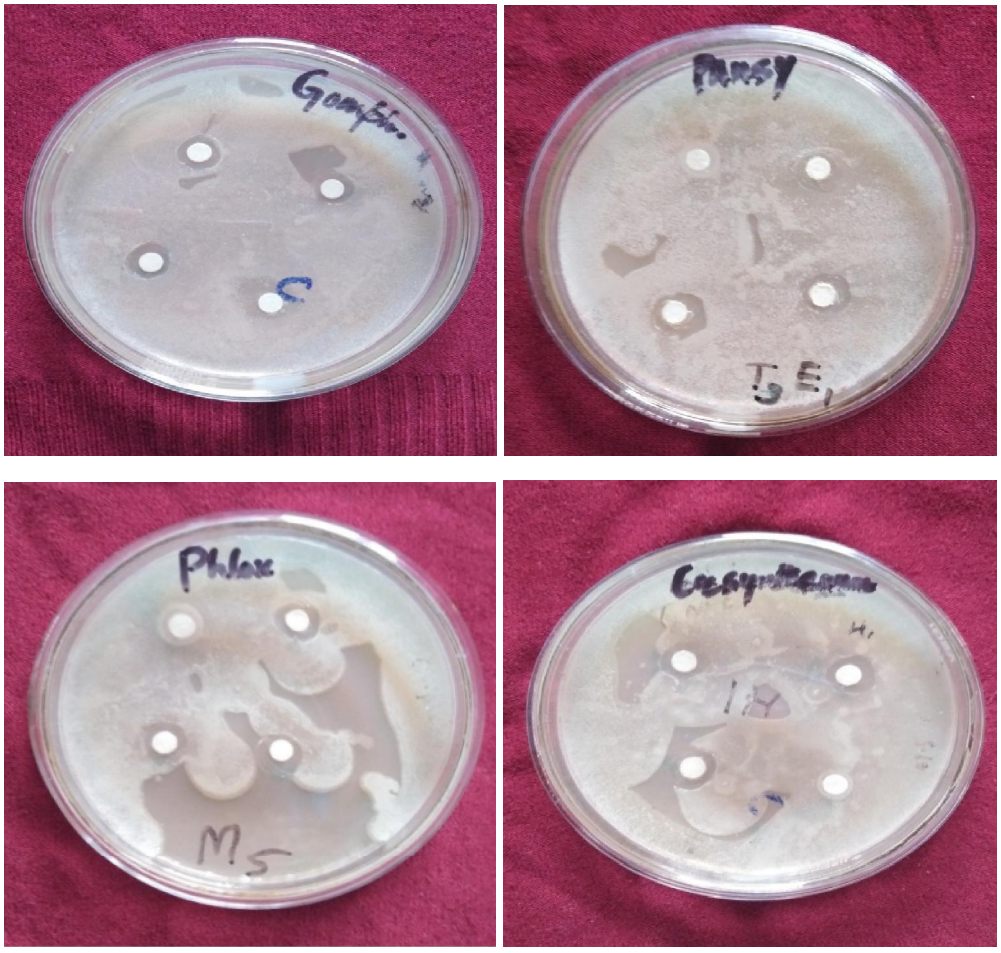

Plate 5: Zone of inhibition in *Escherichia coli*

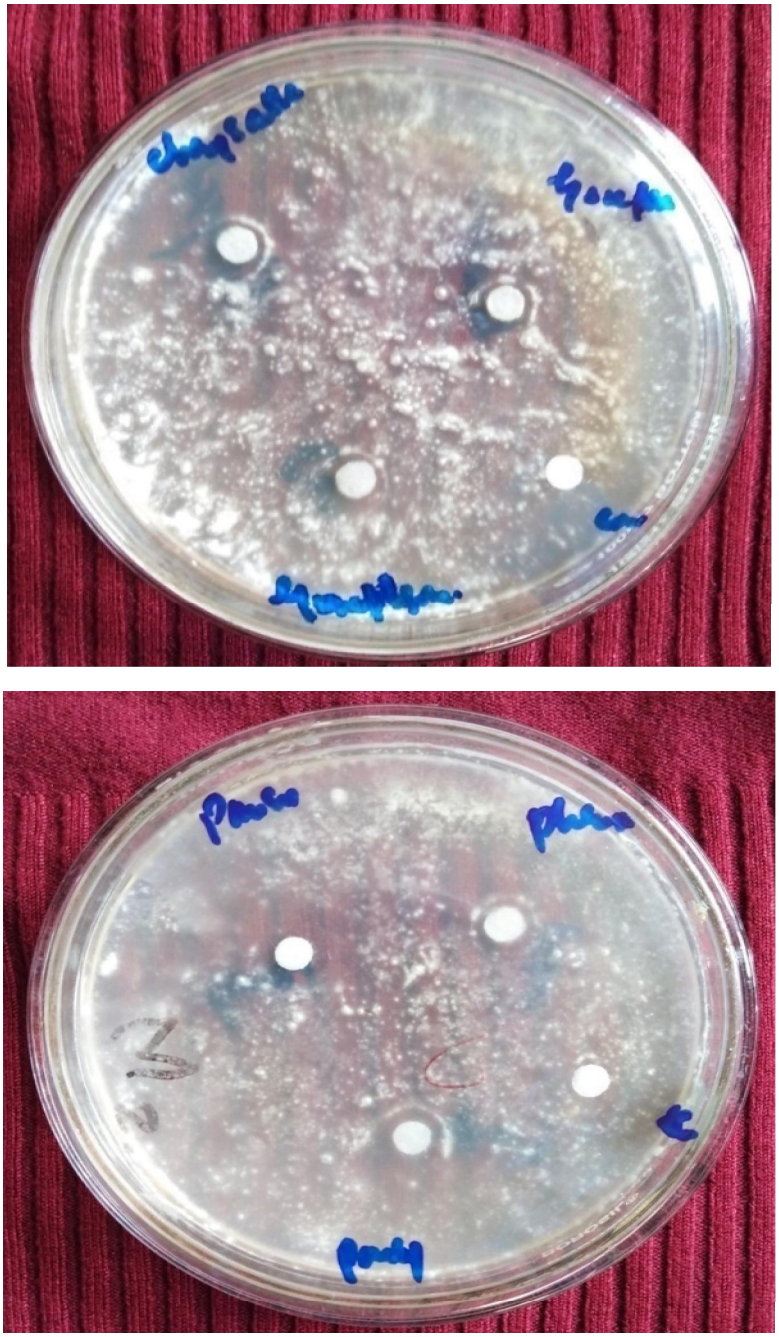

Plate 6: Zone of inhibition in *Aspergillus niger*

Chapter 5

DISCUSSION

The present investigation entitled "Extraction and Stability of Natural dyes from Selected Ornamental Plant Species" was carried out during 2017-2018 at Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar (J&K) and is discussed as under:

5.1 Anthocyanins

5.1.1 Quantification

Anthocyanins are water soluble vacuolar pigments of higher plants. Anthocyanins are glycosides of anthocyanidins and give rise to the blue, purple, red, orange colour to flowers and fruits of many plants. They take part in antioxidation, enzyme inhibition, viral resistance and inhibition of microbial growth and respiration (Hrazdina, 1982; Jackman and Smith, 1996; McClure 1975). Anthocyanin synthesis in vegetative organs is induced by different environmental factors (Chalker, 1999) and anthocyanin concentration increases during senescence (Chang *et al.,* 1989).

In the present study, total anthocyanin content ranged between 5.74 to 471.08 mg/100g among all the selected plant species. The highest total anthocyanin content (471.08 mg/100g) was found in Pansy (*Voila tricolour* var. *hortensis* DC.) followed by Phlox (398.45 mg/100g) and Globe Amaranth (110.74 mg/100g) while as lowest anthocyanin content (5.74mg/100g) was recorded in Chrysanthemum (*Chrysanthemum morifolium* L.).

5.1.2 Storage impact on anthocyanin stability

In Pansy (*Voila tricolour* var. *hortensis* DC.) anthocyanin content of 150.38 mg/100g was recorded even after 5th month of storage interval under ambient condition. In phlox (*Phlox paniculata* L.), under ambient condition after 5th month of storage 10.28 mg/100g of anthocyanin content was recorded. The

amount of total anthocyanin content decreased with increase in storage time in Globe Amaranth (*Gomphrena globosa* L.) as well. After 5th month of storage 9.71 mg/100g of anthocyanin content was detected. In Chrysanthemum (*Chrysanthemum morifolium* L.), 0.19 mg/100g was detected after 3rd month of storage while as no anthocyanin content was recorded after 4th month of storage. Anthocyanin molecules are unstable and very sensitive to the technological processing particularly when heat is involved. Waskar and Khurdiya (1987) have also reported degradation of anthocyanin in phalsa nector, concentrate, squash and crush during entire period of storage.

The drastic decrease of anthocyanin content is due to rapid degradation. Forni *et al.* (1993) reported loss of anthocyanin content in thermal processing and storage in osmodehydrated and pasteurized cherries. Similar findings were reported by Siddiq *et al.* (1994) in plum juice, in cherry nectar (Uygan and Acar, 1995), in black currant nectar (Iversen, 1999), in cherry, plum and raspberry (Gimenez *et al.,* 2001) Present results are in well agreement with the study conducted by Qazi (2013).

5.2 Carotenoids

5.2.1 Quantification

Carotenoids are lipid soluble, yellow, orange and red pigments found in all higher plants. Carotenoids are also called as tetraterpenoids and are brightly coloured natural organic pigments found in the chloroplast and chromoplast nearly in all plants. Carotenoids are required for the correct assembly of photosynthesis (Pogson *et al.*, 2005; Li and Guthrie, 2009).

The total carotenoid content was found in between the range of 0.019 to 149.20 mg/100g. The highest carotenoid content (149.20 mg/100g) was recorded in Chrysanthemum followed by Phlox (0.56mg/100g) and Pansy (0.042 mg/100g) while as the lowest carotenoid content (0.019 mg/100g) was recorded in Globe

Amaranth (*Gomphrena globosa* L.). Qazi (2013) reported the highest carotenoid content in between the range of 170.00 to 288.33 mg/100g in pot marigold.

5.2.1 Storage impact on carotenoid stability

In Chrysanthemum (*Chrysanthemum morifolium*L.), carotenoid content of 49.11 mg/100g was recorded even after 5th month of storage interval under ambient condition. In Phlox (*Phlox paniculata* L.) 0.084 mg/100g of carotenoid content was recorded after 5th month of storage. In Pansy (*Voila tricolour* var. *hortensis* DC.), 0.0003 mg/100g of total carotenoid content was detected after 4th month of storage and no carotenoid content was detected after 5th month of storage interval. In Globe Amaranth (*Gomphrena globosa* L.), 0.0003 mg/100g was recorded after 3rd month of storage while as no carotenoid content was recorded after 4th month of storage interval. The decrease in carotenoid content during storage may be attributed to degradation of carotenoids. The loss of carotenoids may be due to increased temperature during storage. The above findings regarding the decrease in total carotenoid content during storage are in conformity with that of Roa (1970) in mango juice, Sadhu and Bose (1976) in mango cultivars and (Krishnaveni *et al.,* 2001) in jackfruit RTS beverages. Similar results have been observed by Szymezak and Plocharski (2000), Peggy and James (2003), Raynal *et al.* (1989) and Maria *et al.* (2002). The decreasing trend of carotenoids was observed by Srivastava *et al.* (1985) in blending of mango varieties for juice preparation during storage, (Sahani *et al.,* 1994) in carotene profile of mango and (Deka *et al.,* 2005) in spiced mango, pineapple beverages and Punchok (2005) in apricot and seabuck thorn squash. Data further revealed that highest carotenoid content was found during 0 days of storage in all plant species.

5.3 L*, a*, b*, Chroma(C*), Hue angle (H°), ΔE

L* is the lightness or darkness ranging from 0-100 i.e. black to white (L*= 0 yields black colour and L*= 100 indicates white). Positive values of a* are in

the direction of redness and negative in the direction of green. Positive values of b* are in the direction of yellowness and negative in the direction of blueness. The asterisk (*) after L*, a*, b*are pronounced star. Hue angle is defined as a colour wheel. The units are in the form of degrees ^{o} (or angles) ranging from 0 $^{\circ}$ (red) through 90 $^{\circ}$ (yellow), 180 $^{\circ}$ (green), 270 $^{\circ}$ (blue) and black to 0. C* represents chrome, which is a measure of intensity and represents colour saturation from dull (low value) to vivid colour (high value). This ranges from 0 at the centre of the circle, which is completely unsaturated (i.e. a neutral grey, black or white) to 100 or more at the edge of the circle for very high chroma (saturation) or "colour purity". ΔE* represents total colour change.

The L* value of pansy (*Voila tricolour* var. *hortensis* DC.) ranged between 16.92 to 45.25.The L* value showed the increasing trend with increase in storage time under ambient conditions, showing lightening of colour with storage. The a* value ranged between 32.25 to 47.04. The a* value showed the decreasing trend with increase in time of storage, showing decrease in redness with storage. Our values are almost same than those reported by Patras *et al.* (2009) who reported a* value of 32.24 in the unprocessed purees of strawberry and blackberry. In addition to simple thermal degradation of anthocyanins, the colour loss has also been attributed to increased rates of enzyme mediated losses via enzymes such as peroxidases, polyphenol oxidase and glucosidase (Chang and Breen, 1991). The b* value ranged between 42.35 to 54.16. The b* value showed the decreasing trend with increase in time of storage. Further the C* ranged from 53.23 to 71.52 showing the decreasing trend reflecting the transformation of the more vivid colour to dull one. The H $^{\circ}$ ranged from 48.22 to 53.54 $^{\circ}$ it showed a decreasing trend. ΔE* represents total colour difference ranged between 82.52 to 87.57, it also showed decreasing trend with the storage. Same decrease in ΔE* was reported by Patras *et al.* (2009) in thermally processed purees of straw berry and black berry.

In Phlox (*Phlox paniculata* L.) the L* value ranged between 31.92 to

51.55. L* value designating lightness of colour increased gradually as the storage period increased. The a* value ranged between 48.27 to 59.71. The a* value showed the decreasing trend with storage under ambient condition. The decline in a* value is due to anthocyanin degradation because of enhancement of the activity of PAL (Phenylanaline ammonia- Lyase) and flavonoid glucosyl tranferase (GT). Same findings were reported by Given *et al.* (1988). The b* value ranged between 13.12 to 20.86. The b* value showed the decreasing trend with increase in the time of storage. The C* ranged between 50.27 to 63.24 showing the decreasing trend. The H° ranged between 15.11 to 19.25° it also showed a decreasing trend. ΔE* represents total colour change ranged between 72.33 to 90.28, again it showed the decreasing trend.

The L* value of Globe Amaranth (*Gomphrena globosa* L.) ranged between 42.34 to 50.61. The L* value showed the increasing trend with increase in storage time, showing lightening of colour with storage. These values were lower when compared with the values (83.2 to 85.3) reported by Kishimoto *et al.* (2007) in yellow flowered varieties of *Calendula officinalis* L. The a* value ranged between 22.58 to 28.63. The a* value showed the decreasing trend with increase in time of storage, showing decrease in redness with storage. In addition to simple thermal degradation of anthocyanins, the loss of colour has been attributed to increased rates of enzyme mediated losses via enzymes such as peroxidases, polyphenol oxidase and glucosidase (Cheng and Breen, 1991). The b* value ranged between 6.42 to 12.62. The b* value showed the decreasing trend with increase in the storage time. The C* ranged between 23.44 to 31.29, showing decreasing trend. The H° ranged between 15.93 to 23.78° it also showed decreasing trend. ΔE* represents total colour change ranged between 104.52 to 109.79, it showed the decreasing trend with storage. Same decrease in ΔE* was reported by Patras *et al.* (2009) in thermally processed purees of strawberry and blackberry.

In Chrysanthemum (*Chrysanthemum morfolium* L.) the L* value ranged between 41.58 to 52.75. The L* value showed the decreasing trend with increase

in storage time, showing darker colour with storage. The a* value ranged between 22.12 to 31.89. Our values were less than those reported by Kishimioto *et al.* (2007) who reported a* values of 65 in orange flowered cultivars of *Calendula officinalis* L. The b* value ranged between 25.94 to 53.98. The b* value showed the decreasing trend with increase in storage time. The C* ranged between 41.11 to 58.34, showing the decreasing trend reflecting colour change of sample from more vivid colour to dull one. The H° ranged between 39.37 to 67.71, it showed decreasing trend reflecting the colour changes from dark yellow to light yellow. ΔE* represents total colour difference of ranged between 80.10 to 94.30, it showed the decreasing trend with storage time. Colour change is widely used as a visual maturity index in many fruits (Ried, 2002). Colour intensity and uniformity affect fruit quality (Camelo, 2003). Since in many fruits these involve loss of chlorophyll, synthesis of new pigments such s carotenoids and unmasking of other pigments previously formed during fruit development (Aked, 2000; Ferrer *et al.,* 2005).

5.4 Antimicrobial activity

Escherichia coli is a gram negative, facultative anaerobic rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms. Most *E.coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination. The harmless strains are part of the normal microbiota of the gut, and can benefit their hosts by producing vitamin K_2, and preventing colonization of the intestine with pathogenic bacteria, having a symbiotic relationship.

Aspergillus niger is a fungal species which causes a disease called black mold on certain fruits and vegetables. It is ubiquitous in soil and is commonly reported from indoor environments. *Aspergillus niger* is less likely to cause human disease than some other *Aspergillus* species. In extremely rare instances,

humans may become ill, but this is due to serious lung disease, aspergillosis, that can occur.

Pathogens are a major concern for a variety of industries. Foodborne illnesses are a continuous threat to public health. 31 major foodborne pathogens account for sickness of nearly 9.4 million people, more than 55,961 hospitalizations, and 1,351 deaths (Scallan *et al.*, 2011). Therefore, the use of natural plant derived products as antimicrobials are increasing significantly (Gupta and Birdi, 2017).

In the present study, the zones of inhibition for *E. coli* and *Aspergillus niger* due to flower extracts of selected species ranged between 7.40 to 10.80 mm. The highest antibacterial activity (9.60 mm) was recorded due to Chrysanthemum (*Chrysanthemum morifolium* L.) followed by Globe Amaranth (9.00 mm) and Phlox (8.40 mm) while as lowest antibacterial activity (7.90 mm) was recorded due to Pansy (*Voila tricolour* var. *hortensis* DC.). Highest antifungal activity (10.80 mm) was recorded against Chrysanthemum (*Chrysanthemum morifolium* L.) followed by Phlox (10.00 mm) and Globe Amaranth (7.80 mm) while as lowest antifungal activity (7.40 mm) was recorded due to Pansy (*Voila tricolour* var. *hortensis* DC.).

The antimicrobial activity of selected plant extracts is believed to be due to presence of flavonoids, steroids, saponins, tannins, carbohydrates, proteins, alkaloids, essential oils etc (Murugan *et al.,* 2013). Flavonoids and volatiles in *Chrysanthemum morifolium* Ramat flower were identified by HPLC and GC/MS, respectively. Eight flavonoids and fifty eight volatiles were identified. Luteolin-7-glucoside and quercitrin were the most abundant flavonoids amounted for 85.7% of total detected flavonoids. B-Humulene was the most abundant volatile and Iedene oxide-(ı) the next abundant one and the two volatiles were 16.3 and 9.0% of total volatiles, respectively (Sun *et al.,* 2010).

Sassi *et al.* (2014) reported that essential oils from the leaves, stems and roots of *Chrysanthemum trifurcatum* (Desf.) Batt. contained many compounds such as limonene, γ-terpinene, α-pinene and α- terpenyl acetate. These compounds of *Chrysanthemum trifureatum* showed great potential of antibacterial effect against *Bacillus spp.* and *Staphylococcus species*. Sassi *et al*. (2008) also reported the medicinal activity of some Chrysanthemum species. Different parts of four Tunisian Chrysanthemum species were extracted with solvents and these extracts were subjected to test their antimicrobial activity against different bacteria and fungus. The findings showed that Chrysanthemum extracts exhibited good microbial resistance. Derouiche *et al.* (2014), Nowrid (2017) and Voon *et al.* (2012) also showed similar results.

Chapter 6

SUMMARY AND CONCLUSION

The present investigation entitled "Extraction and Stability of Natural dyes from Selected Ornamental Plant Species" was carried out during 2017-18 at Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Shalimar, Srinagar (J&K). The findings of the present study are summarized below:

- Highest value of total anthocyanins (471.08 mg/100g) was recorded in Pansy (*Voila tricolour* var. *hortensis* DC.) and lowest value (5.74 mg/100g) was recorded in Chrysanthemum (*Chrysanthemum morifolium* L.).
- Highest value of total carotenoid content (149.20 mg/100g) was recorded in Chrysanthemum (*Chrysanthemum morifolium* L.) and lowest content (0.019 mg/100g) was recorded in Globe Amaranth (*Gomphrena globosa* L.).
- Highest pigment content was recorded during 0 days of storage in all plant species.
- With increase in days of storage, pigment content decreased in selected species.
- Colour quality of the plant species changed with increase in days of storage.
- Highest antibacterial activity (9.60 mm) was recorded due to Chrysanthemum (*Chrysanthemum morifolium* L.) followed by Globe Amaranth (9.00 mm) and Phlox (8.40 mm) while as lowest antibacterial activity (7.90 mm) was recorded due to Pansy (*Voila tricolour* var. *hortensis* DC.).

- Highest antifungal activity (10.80 mm) was recorded due to Chrysanthemum (*Chrysanthemum morifolium* L.) followed by Phlox (10.00 mm) and Globe Amaranth (7.80 mm) while as lowest antifungal activity (7.40 mm) was recorded due to Pansy (*Voila tricolour* var. *hortensis* DC.).

CONCLUSION

It is concluded from the study that among all species Pansy (*Voila tricolour* var. *hortensis* DC.) and Chrysanthemum (*Chrysanthemum morifolium* L.) had highest level of total anthocyanin and total carotenoids respectively, hence they can be used as source of natural dye. The pigment content decreased with increase in days of storage in all selected plant species as highest pigment content was recorded on 0 days of storage. Colour quality of the selected plant species also changed with increased days of storage. The selected plants have anti-microbial activity, with highest antimicrobial activity in chrysanthemum. Pansy (*Voila tricolour* var. *hortensis* DC.) and Chrysanthemum (*Chrysanthemum morifolium* L.) can be utilized as an eco-friendly dye after working out other relevant parameters.

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Dr. Manzoor Ahmad Shah, Associate Professor, Department of Botany, University of Kashmir during Viva-Voce examination held on 04-11-2019 have been incorporated in the manuscript entitled **"Extraction and Stability of Natural dyes from Selected Ornamental Plant Species"** submitted by **Ms. Mubeena Manzoor (Regd. No. 2016-ES-26-M).**

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