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Standardization of Bio-pigment Extraction Techniques from Yellow Flowering Landscape Ornamentals

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ABSTRACT: Bio-pigments obtained from landscape ornamentals plant sources has the potential to be an eco-friendly, non-toxic and cost effective substitute in food and textile industries and can subsequently create entrepreneurship opportunities in rural and urban areas and can reduce the use of synthetic colours and their hazards. Flowering trees and shrubs which are used in landscaping, blooms profusely at certain seasons; however, except for aesthetic gratification the flowers are not utilized in any commercial purpose and referred to as waste material. The present study hypothecates that bio-pigments extracted from the waste flowers can add commercial value to the plants and can convert the flower waste to wealth. Three common yellow flowering landscape plants namely, *Cassis fistula*, *Peltophorum pterocarpum* and *Cassia alata* were selected for extraction of pigment through six different methods and colour, their stability under varying temperature and pH were evaluated. The results of this experiment showed that the eighteen resultant pigments varied significantly with respect to their colour values (ΔE* L*a*b*), anthocyanin and carotenoid contents and colour stability at acidic, alkaline or high temperature condition. The maximum anthocyanin and carotenoid content was observed in Cassia alata with soaking and maceration in acidic solution(58.94mg/l) and *Peltophorum pterocarpum* with soaking and maceration in acidic solution (33.76 µg/g) respectively.

Keywords: Anthocyanin, carotenoid, ΔE* L*a*b*, pigment.

INTRODUCTION

Several uses of colour were noticeable since pre-historical time by all types of societies. From pre-historic times, natural colourants gained much popularity in colouring leather, silk, food, cotton (Alemayehu and Teklemariam 2014). Being non-allergic, non-toxic and eco-friendly, natural dyes gained a place of significant importance to restrict the use of hazardous synthetic dyes. Based on sources, natural dyes are classified as natural dye obtained from plants, animals, insects and minerals (Verma and Gupta 2017). From the beginning of mankind, dyes were used for colouring skin, cloth, dyeing surroundings and for painting. The first evidence of use of dyes by man dated back to 1500-900 BC which was found in the walls of Altamira cave in Spain. The first natural dye, Tyrian purple obtained from molluscs was used in the late Bronze Age and another ancient dye, Indigo was extracted from flowers of *Indigofera tinctoria* through fermentation (Clark *et al.,* 1993). The first commercially produced natural dye from coal tar was Alizarin (1,2,-dihydroxyanthraquinone), derived from anthracene by Perkin's company in 1869. The International market for natural dye is believed to reach USD 4.67 billion by 2023 with a growth rate of 4.8%. UK, Germany, France, Italy and Spain were the leading markets of natural colour (Rymbai *et al.,* 2011). During 2007-11 global food colour market increased by 13% and the share of natural colour and synthetic colour were 39% and 37% respectively. In Europe for food colouring, 85% colours were natural in the year 2009-11 which helped the rise of €155 to €193 million in European natural colours market (EIBI, 2014). A brilliant scarlet natural dye 'eochincal' was introduced to Europe from Mexico in 1518 (Decelles, 1949). Till the end of nineteenth century, main colourants for textile were the natural dyes obtained from plants (roots, stems, leaves, flowers, fruits, seeds and lichens) (Ingamells, 1993). Pioneering synthesis of mauveine, English chemist William Henry Perkin marked the beginning of era of synthetic dyes. From 20th century, use of natural dye was completely replaced by synthetic dye (Welham, 2000). The flowers show a great diversity in their colour due to presence of different pigments which are getting much importance worldwide as a source of natural food colours India, the

forerunners of natural dyeing, is blessed with approximately 4,90,000 plant species of which 450 plants were reported as that could yield dyes. Over 2000 pigments have been reported to be produced from various parts of a plant of which only little more than 150 have been exploited commercially (Siva, 2007). West Bengal, especially North Bengal, has a rich floristic diversity, offering flowers in a panorama of shades in abundance. The state's floristic treasure trove consists of 3580 flowering plant species under 1333 genera under 200 families. *Cassis fistula*, *Peltophorum pterocarpum* and *Cassia alata* are very common yellow flowering plants which are used for landscaping and are found in abundance in natural vegetation, road side plantation and wastelands in Terai region of West Bengal.

Cassia fistula. *Cassia fistula* which belongs to the family Fabaceae blooms profusely at the end of February to end of March when it is in leafless condition. As a renewable, eco-friendly natural dye stuff from *Cassia fistula* could be used as commercial natural dyeing of woollen textile material. Kumari and Neelam (2015) extracted natural dye stuff from *Cassia fistula* and applied it on textile wool and acrylic fabrics to evaluate the colour fastness of the dye using different mordants.

Peltophorum pterocarpum. *Peltophorum pterocarpum* belongs to family Fabaceae native to Tropical South-Eastern Asia flowers profusely March to April. Divya and Manonmani (2014) extracted dye from the flowers of *Peltophorum pterocarpum* and utilized it as natural flower dye on silk and cotton using several mordant combinations. Kumar and Prabha (2018) studied the dye yielding potential of *P. pterocarpum* flowers along few other flowering species and evaluated the efficacy of pigment in dyeing cotton, jute and silk fabrics. They designed a quick extraction method involving boiling of the flower samples in de-ionized water for two hours. This resulted in a yellow colour dye. Senoretta and Sumanthy (2016) had done analysis of phytochemicals of crude extract and carotenoid *Peltophorum pterocarpum* flower. They dried the sample and ground into powder. They isolated carotenoid through column chromatography by using silica gel column with 100% hexane and reported that flower of *Peltophorum pterocarpum* contained 0.89µg/g of total carotenoid. As a result, they reported that the presence of maximum phytochemicals was observed in the ethanolic extract.

Cassia alata. Being a medicinal plant *Cassia alata* widely used as ornamental flowering plant which is generally a shrub belongs to the family Fabaceae produces candle like inflorescence profusely at the month of March to May.

MATERIALS

The experiment was conducted at the Uttar Banga Krishi Viswavidyalaya, West Bengal, during 2018 to 2020. Flowers of the three species *Cassis fistula* (Y_1), *Peltophorum pterocarpum* (Y_2) *and Cassia alata* (Y_3) were collected from the road side plantations and were subjected to six methods of pigment extraction *viz.* soaking in cold water (18-20°C) and maceration (M_1), soaking in hot water (80°C) and maceration (M_2), boiling in water at 100°C (M_3), microwave assisted extraction (M_4), soaking and maceration in acidic solution (pH 4.5-5) (M_5) and soaking and maceration in alkaline solution (pH 8.00) (M_6). The experiment was laid in two factor Completely Randomized Design with 18 treatment combinations replicated thrice.

METHODOLOGY

After collection of the flowers, the petals were separated, rinsed with tap water, dried under shade and stored in air-tight containers for extraction of pigments. Flower parts, apart from petals, were considered as wastage and was recorded for each species in percent weight. The dried petals of each species were subjected to six methods of extraction. Two grams of petals was processed in 100ml of double distilled water/ solution in all the methods. In first and second methods, soaking was carried out in cold (18-20°C) and hot water (80°C), respectively for 1 hour followed by maceration in electric grinder for 5 minutes. For the third method (M_3), the petals were boiled in a water-bath until most of the pigment was extracted. The final volume of the pigment extract was made up to 100 ml for further analysis. Microwave assisted extraction (M_4) was carried out in a microwave oven at high power for 5 minutes. For the fifth and sixth methods acidic solution (pH-4.5) and alkaline solution (pH-8.00) were prepared using citric acid (500ppm) and sodium hydroxide (500ppm), respectively. The petals were soaked for 15 minutes in the solutions and macerated in a grinder. After extraction, the extracts were first strained through a fine mesh strainer to separate out the petal mass followed by filtration through Whatman-1 filter paper to obtain clear pigment extracts which were subjected to further analysis.

RESULTS AND DISCUSSIONS

Colour of fresh petal. The colours of fresh petals of *Cassia fistula* (Y_1), *Peltophorum pterocarpum* (Y_2) and *Cassia alata*(Y_3) were recorded with the help of RHS colour chart. *Peltophorum pterocarpum* (Y_2) and *Cassis fistula* (Y_1) showed yellow group of colour which are yellow group 5-C and yellow group, 7-A respectively while *Cassia alata* (Y_3) showed yellow orange group of colour which is yellow orange group 14-B.

Fresh weight of flowers, petals and wastage percentage. For each species, the fresh weight of twenty flowers as well as petals obtained from them were recorded and wastage percentage was calculated (Table 1). Wastage is calculated by using following equation and represented as percentage.

$$\text{Wastage (\%)} = \frac{\text{Fresh weight of flower} - \text{Fresh weight of petals of flowers}}{\text{Fresh weight of flowers}}$$

These parameters of harvested fresh mass and useable fresh mass could be helpful as indices for economic estimation of the final products. Significant variation in all the three parameters was noted among the species. Maximum fresh weight of twenty flowers 6.45g was recorded in *Cassia fistula* (Y_1) whereas the minimum

fresh weight of twenty flowers 4.58g was recorded in *Cassia alata* (Y_3). Individual flowers of *Cassia fistula* (Y_1) are larger than the two flowers in the group having an average length of 5.5-6.7cm whereas the average length of *Cassia alata* (Y_3) varies 4.5-5.5cm. Difference in size, length, thickness, moisture content in different parts of the flower contributed the variation of weight of fresh twenty flowers among the three species. The maximum weight of fresh petals from twenty flowers (4.05g) were obtained in *Cassia alata* (Y_3) which has the minimum weight of fresh twenty flowers discussed earlier and the minimum weight of fresh petals from twenty flowers (1.67g) were recorded in *Cassia fistula* (Y_1) which got the maximum weight of fresh twenty flowers. Size of petals, petal thickness, moisture content present in fresh petals are the main reason of the variation of the fresh weight of petals of twenty flowers. The maximum wastage of 74.18% was observed in *Cassia fistula* (Y_1) followed by 36.83% in *Peltophorum pterocarpum* (Y_2) and 11.69% in *Cassia alata* (Y_3). This wastage percentage of non-useable parts of flower indicates that the less amount of wastage or the less percentage of wastage needs fewer amounts of flowers for extraction of pigments whereas in more wastage percentage more flowers are needed for extraction. Weight, size, thickness and numbers of sepals, and roecium and gynoecium contributed to this variation.

Dry weight of petals of twenty flowers (g). For comparative study of dry mass content, petals of twenty flowers of each species were subjected to shade drying. Significant variation was evident in the data (Table 1). Maximum dry weight of petals of twenty flowers (1.73g) recorded in *Cassia alata* (Y_3) and minimum (0.13g) in *Cassia fistula* (Y_1).

Rate of drying. Before extraction of pigments, fresh petals were dried in shade. The rate of drying, which indicates the percent moisture loss per hour, was calculated using the following equation.

$$\text{Rate of drying (mg/hour)} = \frac{\text{Initial weight of fresh flower} - \text{Dry weight of flowers}}{\text{Initial weight of fresh flower} \times \text{Time required for drying (hours)}} \times 100$$

Among the three species *Cassia fistula* (Y_1) showed the maximum rate of drying (3.68%/hour) followed by *Cassia alata* (Y_3) (1.23%/hour) and the minimum rate of drying is observed in *Peltophorum pterocarpum* (Y_2) (1.23%/hour). This variation might be due to the variation in petal thickness and moisture content. Higher rate of drying can expedite the total process.

Colour of pigments extracted. The colour of extracted pigments from the three species and by six extraction methods were measured by Hunter Colour Meter and corresponding L* a* b* values are presented in Table 2.

L*. The L* value measures the darkness vs lightness of a colour in a range of 0 to100 where lower values represent darker shades. The results indicate that L* values of the pigments varied significantly with species, methods of extraction and interaction of the two factors (Table 2). Among the three species maximum value of L* (24.86) was observed in *Cassia fistula* (Y_1) followed by *Peltophorum pterocarpum* (Y_2) (23.65) and the minimum (23.08) was observed in *Cassia alata* (Y_3). Among the methods of extraction the maximum value of L* (27.53) was obtained with cold water soaking and maceration (M_1) which denotes the lightest colour and the minimum value (19.97) was observed with soaking and maceration in acidic solution (M_5) which denotes the darkest colour among the all extraction methods. Among the interaction maximum value of L* (29.23) was observed in *Cassia fistula* with cold water soaking and maceration (Y_1M_1) which is the lightest coloured pigment whereas the minimum value (18.46) was observed with *Cassia alata* in soaking and maceration in acidic solution (Y_3M_5) which is the darkest coloured pigment among the eighteen treatments in this experiment.

a*. The a* value of a colour denotes its redness *vs.* greenness, where an increasing positive value refer to increasing redness and increasing negative value indicates increasing greenness. As the present study involved red coloured flowers only, the a* values for all the extracts were eventually positive, however, species, methods of extraction and their interaction showed highly significant impact on this index (Table 2). Among the three species maximum value of a* (1.94) was observed in *Cassia fistula* (Y_1) followed by *Cassia alata* (Y_3) (1.34) and the minimum (0.66) was observed in *Peltophorum pterocarpum* (Y_2). Methods of extraction also significantly affected the a* value whereas among the methods of extraction maximum value of a* (2.24) was obtained with cold water soaking and maceration (M_1) and the minimum value (0.58) was observed with hot water soaking and maceration (M_2). The maximum value of a* (4.77) was observed in *Cassia fistula* with cold water soaking and maceration (Y_1M_1) whereas the minimum value (0.22) was observed with *Peltophorum pterocarpum* in soaking and maceration in alkaline solution (Y_2M_6) at per with Y_3M_2 (0.26), Y_2M_5 (0.28) and Y_2M_2 (0.32), respectively.

b*. The b* value denotes the yellowness *vs.* blueness of a colour, where an increasing positive value refer to increasing yellowness while increasing negative value indicates increasing blueness. All the extracted pigments showed positive value of b* which indicates yellowness (Table 2). Among the three species maximum value of b* (4.65) was observed in *Cassia fistula* (Y_1) followed by *Cassia alata* (Y_3) (1.79) and the minimum (1.56) was observed in *Peltophorum pterocarpum* (Y_2) whereas among the methods of extraction the maximum value of b* (4.43) was obtained with soaking and maceration in alkaline solution (M_6) and the minimum value (1.39) was observed with microwave assisted extraction (M_4). The maximum value of b* (7.27) was observed in *Cassia fistula* with soaking and maceration in alkaline solution (Y_1M_6) whereas the minimum value (0.44) was observed with *Peltophorum pterocarpum* in hot water soaking and maceration (Y_2M_2).

pH of extracted pigments. The pH of the extracted pigments was recorded (Table 3), since, acid reaction is an important factor guiding selection of pigments for application in food, cosmetics and fabric. *Cassia fistula* (Y_1) showed the maximum pH among the three species with an average pH of 7.33 followed by *Peltophorum pterocarpum* (Y_2) (7.12) and *Cassia alata* (Y_3) (6.63). Methods of extraction also significantly affected the pH of pigments which ranges from 3.99 to 10.17. The maximum pH was observed with soaking and maceration in alkaline solution (M_6) with an average pH of 10.17 and minimum pH was observed in soaking and maceration in acidic solution (M_5) with an average pH of 3.99 which were obvious. Among the other four aqueous extraction methods maximum pH was observed with cold water soaking and maceration (M_1) (7.15) and the minimum pH was observed with boiling in water (M_3) with an average pH of 6.78 whereas among the interaction maximum (10.27) and minimum (3.94) pH was occurred in *Cassia alata* with soaking and maceration in alkaline solution (Y_3M_6) and *Peltophorum pterocarpum* with soaking and maceration in acidic solution (Y_2M_5) respectively.

Stability of colour under varying pH condition. In order to determine the range of acid reactions in which the pigment keeps its original colour, the stability of the pigments was assessed in low and high pH conditions. This is important because when pigments are used in food matrix, cosmetics, or textiles, they are frequently exposed to a wide pH range. The pH of the pigments was lowered for the stability test by gradually adding citric acid at a rate of 0.5%, and the pH was gradually increased by gradually adding sodium hydroxide at a rate of 0.5%. As soon as any colour change was seen, the pH of the solution was recorded. The information is shown in Table 3. The maximum stability was observed in *Cassia fistula* with soaking and maceration in acidic solution (Y_1M_5) whereas the minimum stability was observed in *Cassia fistula* with soaking and maceration in alkaline solution (Y_1M_6).

Stability at higher temperature (°C). In the process of dyeing of textile or application in food, the pigments are often subjected to high temperature nearing 100°C. Hence, stability of pigment's colour at higher temperatures is an essential requirement for their commercial application. The extracted pigments were heated over water bath and the temperature, where any change in colour was visible was recorded and presented in Table 4. Significant variation in temperature stability was noted among the species, methods of application and interactions as well. Maximum stability of colour at higher temperature was observed in *Cassia alata* (Y_3) with an average temperature of 100.68°C followed by *Cassia fistula* (Y_1) (92.52°C) whereas *Peltophorum pterocarpum* (Y_2) shows the lowest stability in higher temperature. Colour of the pigments of *Peltophorum pterocarpum* (Y_2) started changing its colour above 90.68°C. Methods of extraction significantly affected the higher temperature stability of pigments. The pigment extracted with microwave assisted extraction (M_4) showed the maximum stability of colour at higher temperature (100.11°C) followed by soaking and maceration in acidic solution (M_5) (98.79°C) whereas minimum stability (88.00°C) was recoded with cold water soaking and maceration (M_1). Pigment extracted from *Cassia alata* with boiling in water (Y_3M_3) showed temperature stability up to a peak temperature of 105.50°C whereas pigment from *Peltophorum pterocarpum* with cold water soaking and maceration (Y_2M_1) up to a temperature of 82.00°C.

Anthocyanin content. Anthocyanins are a major group of water-soluble pigments which impart pink, cyan, blue and purple colours to flowers. For determination of total anthocyanin content, absorbance of light by the pigment solution was read at 520nm wavelength by spectrophotometer and the concentration was calculated by using following formula and expressed as cyanidine-3-glucoside equivalent:

$$\text{Total Anthocyanin content (mg/l)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1}$$

Where,

A= absorbance at 520nm wavelength, MW (Molecular weight) of cyanidine-3-glucoside = 449.2 g/mol, DF=dilution factor, ε = molar extinction coefficient of cyanidine-3-glucoside *i.e.* 26900 L/mol/cm, 10^3= factor for conversion from g to mg (Lee, 2005).

Anthocyanin content in the pigments was distinctly influenced by species, extraction methods and their interaction (Table 4). Among the three species, pigment extracted from *Cassia alata* (Y_3) showed the maximum anthocyanin content with a mean value of 47.41 mg/l followed by *Cassia fistula* (Y_1) (39.70 mg/l) and the lowest amount of anthocyanin (24.76 mg/l) is observed in the pigment extracted from *Peltophorum pterocarpum* (Y_2). Among the methods of extraction maximum anthocyanin content (52.42 mg/l) was observed in soaking and maceration in alkaline solution (M_6) whereas minimum anthocyanin content (26.35 mg/l) was observed in microwave assisted extraction (M_4). The effect of interaction of species and methods of extraction showed statistically significant variation in the total anthocyanin content. The maximum value (58.94mg/l or 589mg/100g dried sample) was observed in *Cassia alata* with soaking and maceration in acidic solution (Y_3M_5) which is comparable to black grapes (181.2-716.4mg/100g fresh weight) (Nile *et al.*, 2015). Least anthocyanin (10.15mg/l) was recovered from *Peltophorum pterocarpum* with soaking and maceration in acidic solution (Y_2M_5).

Carotenoid content. The total carotenoid content of the pigments was estimated through spectrophotometric method by using the following formula (Carvalho *et al.*, 2012; Surendran *et al.*, 2022).

$$\text{Total carotenoid content (µg/g)} = \frac{A \times V \times 10^4}{A^{1\%}_{1\,cm} \times P}$$

Where,

A= Absorbance of light at 450nm; V= Total extracted volume in ml; P= Sample weight in gram; $A^{1\%}_{1\,cm}$ = 2592 *i.e.* β-carotene extinction coefficient in petroleum ether.

Carotenoid content in the pigments varied significantly with species, method of extraction and their interaction

(Table 4). Among the three species, pigment extracted from *Cassia alata* (Y_3) showed the maximum value for total carotenoid content (15.58µg/g) followed by *Peltophorum pterocarpum* (Y_2)(9.53µg/g) and the lowest amount of total carotenoid (6.86µg/g) was observed in the pigment extracted from *Cassia fistula* (Y_1).Pigments extracted with soaking and maceration in acidic solution (M_5) recorded the maximum carotenoid content (22.72µg/g) whereas minimum carotenoid content (4.18µg/g) was observed with soaking and maceration in alkaline solution (M_6). Carotenoid content in pigments extracted from *Peltophorum pterocarpum* by soaking and maceration in acidic solution was as high as 33.76µg/g whereas, β-carotene content of carrots ranges from 41.62µg/g to 71.20µg/g (Bozalan and Karadeniz 2011).

DISCUSSION

Bahorun *et al.* (2005) studied pigment composition of *Cassia fistula* flowers and reported total pro-anthocyanidins content to be 14mg cyaniding chloride equivalent/g of dry weight whereas, in flower bud the amount was 20mg quercetin equivalent/g of dry weight. Total flavonoids content in flower buds and flowers were 8mg quercetin equivalent/g of dry weight. In *Peltophorum pterocarpum* flavonoid content was found to be 1.44 ± 0.01mg quercetin equivalent/g of plant tissue (Muthukumaran *et al.*, 2016). Total carotenoid content in flowers of *Peltophorum pterocarpum* was reported to be 0.89µg/g (Senoretta and Sumathy 2016). However as per our study the carotenoid content of flowers of this species is 9.53µg/g, this variation might be due to use of fresh flowers in the previous study. Abdulwaliyu *et al.* (2013) reported that *Cassia alata* flowers are a rich source of β-carotene. As per the methods of extraction are concerned, they exerted significant effect on all the parameters under study and the results corroborate with the present experiment.

Table 1: Fresh weight of flowers, fresh and dry weight of petals, rate of drying and wastage from non-useable parts of flowers of *Cassia fistula* (Y_1), *Peltophorum pterocarpum* (Y_2) *and Cassia alata* (Y_3).

Treatment	Fresh weight of 20 flowers (g)		Fresh weight of petals of twenty flowers (g)		Wastage from non-useable parts of flower (%)		Dry weight of petals of twenty flowers (g)		Rate of drying (%moisture loss/hour)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Y_1	6.45	0.44	1.67	0.24	74.18	2.30	0.13	0.017	3.683	0.04
Y_2	5.39	0.21	3.41	0.17	36.83	1.08	1.53	0.076	1.227	0.09
Y_3	4.58	0.24	4.05	0.34	11.69	3.15	1.73	0.164	1.233	0.02

Table 2: Colours of *Cassia fistula* (Y_1), *Peltophorum pterocarpum* (Y_2) *and Cassia alata* (Y_3) pigments extracted through different methods as represented by L*, a* and b* values.

Method of extraction	L*				a*				b*			
	Y_1	Y_2	Y_3	Mean	Y_1	Y_2	Y_3	Mean	Y_1	Y_2	Y_3	Mean
M_1	29.23	27.59	25.76	**27.53**	4.77	0.63	1.32	**2.24**	7.12	2.33	3.01	**4.15**
M_2	24.14	23.95	24.47	**24.19**	1.17	0.32	0.26	**0.58**	2.85	0.44	0.91	**1.40**
M_3	22.91	24.22	23.73	**23.62**	0.36	1.94	1.87	**1.39**	6.99	1.57	0.52	**3.03**
M_4	23.49	24.26	24.30	**24.02**	2.26	0.54	1.99	**1.60**	1.73	0.91	1.53	**1.39**
M_5	22.62	18.83	18.46	**19.97**	0.91	0.28	1.08	**0.76**	1.91	1.48	1.43	**1.61**
M_6	26.75	23.05	21.75	**23.85**	2.17	0.22	1.54	**1.31**	7.27	2.65	3.36	**4.43**
Mean	**24.86**	**23.65**	**23.08**		**1.94**	**0.66**	**1.34**		**4.65**	**1.56**	**1.79**	
Factor	C.D. at 1%		SE(m) ±		C.D. at 1%		SE(m) ±		C.D. at 1%		SE(m) ±	
Species	0.024		0.008		0.056		0.019		0.084		0.029	
Method	0.033		0.012		0.079		0.027		0.119		0.041	
Species × Method	0.058		0.020		0.137		0.047		0.206		0.072	

Table 3: Effect of species and method of extraction on pH of the pigments, minimum and maximum pH for colour stability of *Cassia fistula* (Y_1), *Peltophorum pterocarpum* (Y_2) *and Cassia alata* (Y_3) flowers.

Method of extraction	Normal pH				Minimum pH for colour stability				Maximum pH for colour stability			
	Species				Species				Species			
	Y_1	Y_2	Y_3	Mean	Y_1	Y_2	Y_3	Mean	Y_1	Y_2	Y_3	Mean
M_1	7.52	7.41	6.52	**7.15**	5.02	4.91	4.44	**4.79**	11.35	11.87	11.33	**11.52**
M_2	7.34	7.22	6.42	**6.99**	5.12	4.38	4.41	**4.64**	11.61	11.87	11.49	**11.66**
M_3	7.23	7.09	6.02	**6.78**	4.22	4.19	4.49	**4.30**	12.15	11.69	11.59	**11.81**
M_4	7.66	7.03	6.54	**7.08**	3.18	4.10	3.03	**3.44**	11.78	11.77	11.81	**11.79**
M_5	4.05	3.94	3.99	**3.99**	2.92	2.73	2.03	**2.56**	11.54	6.99	6.32	**8.28**
M_6	10.20	10.03	10.27	**10.17**	8.89	7.09	7.09	**7.69**	12.56	12.76	13.48	**12.93**
Mean	**7.33**	**7.12**	**6.63**		**4.31**	**4.89**	**4.57**		**11.83**	**11.66**	**11.83**	
Factor	C.D. at 1%		SE(m) ±		C.D. at 1%		SE(m) ±		C.D. at 1%		SE(m) ±	
Species	0.017		0.006		0.015		0.005		0.033		0.011	
Method	0.024		0.008		0.021		0.007		0.046		0.016	
Species × Method	0.042		0.015		0.037		0.013		0.080		0.028	

Table 4: Effect of species and methods of extraction on colour stability of pigments of *Cassia fistula* (Y_1), *Peltophorum pterocarpum* (Y_2) *and Cassia alata* (Y_3) flower pigments under high temperature condition.

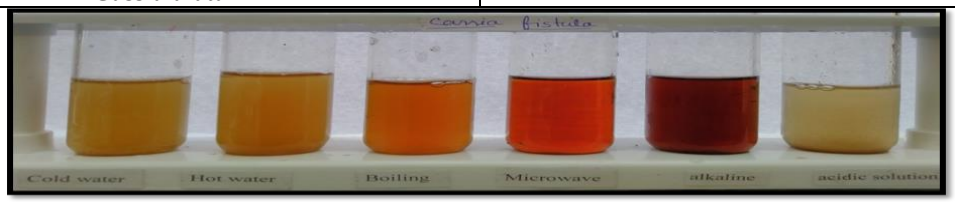
Method of extraction	Anthocyanin content (mg/l)				Carotenoid content (µg/g)				Maximum temperature for colour stability (°C)			
	Species				Species				Species			
	Y_1	Y_2	Y_3	Mean	Y_1	Y_2	Y_3	Mean	Y_1	Y_2	Y_3	Mean
M_1	39.21	23.62	53.73	**38.85**	7.78	5.72	16.13	**9.88**	83.80	82.00	98.20	**88.00**
M_2	43.26	26.64	58.24	**42.71**	10.54	8.62	23.34	**14.17**	89.30	84.20	101.13	**91.54**
M_3	41.36	16.36	30.48	**29.40**	6.69	4.44	12.99	**8.04**	95.83	88.23	105.50	**96.52**
M_4	29.61	16.32	33.13	**26.35**	2.96	2.45	9.45	**4.95**	98.50	97.53	104.30	**100.11**
M_5	32.88	10.15	58.94	**33.99**	5.46	33.76	28.94	**22.72**	103.0	88.23	105.13	**98.79**
M_6	51.88	55.44	49.93	**52.42**	7.72	2.18	2.63	**4.18**	84.70	103.9	89.80	**92.80**
Mean	**39.70**	**24.76**	**47.41**		**6.86**	**9.53**	**15.58**		**92.52**	**90.68**	**100.68**	
Factor	C.D. at 1%	SE(m) ±			C.D. at 1%		SE(m) ±		C.D. at 1%		SE(m) ±	
Species	0.080	0.028			0.351		0.122		0.377		0.131	
Method	0.113	0.039			0.496		0.172		0.533		0.185	
Species × Method	0.195	0.068			0.859		0.298		0.923		0.320	

Cassia fistula

Peltophorum pterocarpum

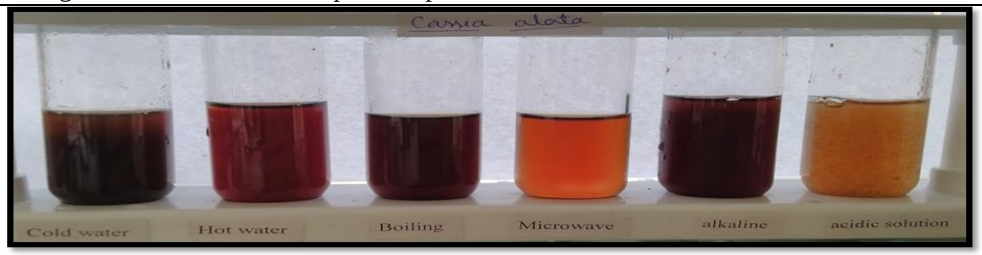
Cassia alata

Cassia alata flower



Pigments extracted from *C. fistula* flowers with different methods of extraction

Pigments extracted from *P. pterocarpum* flowers with different methods of extraction



Pigments extracted from *C. alata* flowers with different methods of extraction

CONCLUSIONS

The colour of a pigment is dictated by the co-pigmentation of anthocyanins and flavonols which vary with species, methods of extraction, pH and temperature. These landscape ornamental species can be effectively exploited for extracting pigments of different colours and shades by manipulating the methods of extraction. The flowers especially, *Cassia alata* and *Peltophorum pterocarpum* was noted to be rich source of anthocyanin and carotenoids. The pigments also showed good stability under wide range of pH and temperature, hence further study on their applicability on food, textile and cosmetics can lead to commercialization of these pigments.

FUTURE SCOPE

It is a preliminary study in this aspect which opens the scope of investigating the applicability of these pigments in textile as well as food and cosmetics industry in future. Standardization of methods of extraction of these pigments can ease the extraction procedure and help to obtain the particular shade of pigment. Due to lack of information about the natural colours and availability of the precise technology on the extraction methods, application, storage and their effect on food matrix, natural pigments are yet to get the desired success in commercial food industry. Hence, in future there is a scope of examining the applicability of these pigments on food through toxicity analysis, analysis of bio-chemical components and study of their behaviour in food matrix.

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