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Biochemical Studies in Several Dye Yielding Plants

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Abstract

Ten natural dye yielding and two mordant plants were biochemically analyzed. Though natural dyes are widely used, information about the active principles responsible for dyeing is hardly available. In the present experiment, total chlorophyll, carotinoids, tannins, phenolics, flavonoids and curcumin were determined among the dye yielding plants, while K, S, P, Ca, Mg, Mn, Zn, Fe, Cu and Co were determined in the case of mordant plants. In *Bixa orellana*, used for yellow dyeing, the carotinoid content was 163.11 mg g^{-1} and in *Clerodendrum chinense* and *Datura stramonium*, which were used for green colouring, total chlorophyll content of 10.29 mg and 11.83 mg g^{-1} was recorded. Curcumin content responsible for orange colouring in *Curcuma domestica* was 27.7 mg g^{-1} while flavonoid content in *Solanum nigrum* and *Terminalia chebula*, which were used for brown, brown to black dyes was 24.89 and 21.73 mg g^{-1}. Among the plants used for dyeing different colours, *Punica granatum* and *Parkia timoriana* were found to contain higher amounts of total phenols and bound phenols by recording 681.2 mg g^{-1} and 287.6 mg g^{-1} total phenols and 151.6 mg g^{-1} and 130.2mg g^{-1} bound phenols, while higher amounts of orthodihydric phenols and tannins were recorded in *Punica granatum* and *Strobilanthes flaccidifolius* by recording 20.11mg g^{-1} and 9.54mg g^{-1} orthodihydric phenols and 675.57mg g^{-1} and 648.12 mg g^{-1} tannins, respectively. In case of the plants used as mordant, higher contents of Ca, Mg, K, Zn, Fe and Mn were detected in *Achyranthes aspera*, while higher amounts of P, Fe and Cu were recorded in *Garcinia xanthochymus*.

Keywords: carotenoids, chlorophyll, curcumin, minerals, natural dyes, phenols, tannins

Introduction

Archaeologists have found evidence of textile dyeing dating back to the Neolithic period. In China, dyeing with plants, barks and insects has been traced back more than 5,000 years. The essential process of dyeing changed little over time. Typically, the dye material is put in a pot of water and then the textiles to be dyed are added to the pot, which is heated and stirred until the color is transferred (Barber, 1991). Vegetable dyes or natural colorants have been used historically throughout the world. Every civilization has its myth and association with colour.

In the early 21st century, the market for natural dyes in the fashion industry is experiencing resurgence. Western consumers have become more concerned about the health and possible environmental impact of synthetic dyes. The European Union, for example, has encouraged Indonesian batik cloth producers to switch to natural dyes to improve their export market in Europe (Bryan and Stella, 2002).

The people of Manipur have been using indigenous dyestuffs from plants and animal sources in handicrafts, handlooms, fine arts etc. since time immemorial. In modern Manipur, natural dyes play an important role in traditional Manipuri fabrics and other household items. They obtain natural dyes from plants and animals by simple, traditional extraction methods (Mutua, 1997; Sharma, 2000).

Natural dyes can be used on most types of material or fibre but the level of success in terms of fastness and clarity of colour varies considerably. Mordant substances are needed to set the colour when using some natural dyes. There are many naturally occurring mordant plants which give different shades and also facilitate the bonding of the dyestuff to the fibre.

The chemical analysis that would definitively identify the dyes used in ancient textiles has rarely been conducted, and even when a dye such as indigo blue is detected, it is impossible to determine which one of the indigo-bearing plants was used (Goodwin, 1982; Hofenk de Graaf, 2004). Nevertheless, in the present experiment, based on the colours of surviving textile fragments and the evidence of actual dyestuffs found in archaeological sites as well as from dyers' house, ten natural dyes yielding plants which have unique uses in the Meitei society of Manipur were analyzed for the biochemical substances responsible for dyeing (Fig. 1) and two other plants which were used as an alkaline and acidic mordant were analyzed for its mineral content (Fig. 2).

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Materials and methods

Plant samples collected from the local dyers of the state were air dried at room temperature and ground with a Remi grinder and sieved (1 mm).The powder samples were stored inside desiccators until analysis. The estimation of chlorophyll was done on the fresh leaves of the experimental material.

Estimation of chlorophyll and carotenoids

Total chlorophyll, chlorophyll a and chlorophyll b content were determined following the method of Jayaraman (1988) and total carotenoid was determined following the method of Jensen (1978).

Estimation of tannins, phenols and flavonoids

The tannin content was determined by the method of Schanderi (1970). Total phenols, bound phenols and ortho-dihydric phenols were determined as per the methods of Bray and Thorpe (1954) and Mahadevan and Sridhar (1986). Flavonoid was determined following the method of Chang *et al.* (2002).

Estimation of curcumin

The curcumin content was estimated as per the procedures described by Sadasivam and Manickam (1992).

Estimation of minerals

Wet digestion method of Capar *et al.* (1978) was followed for the analysis of different minerals. K was estimated in a systronics-105 flame photometer. Sulphur and phosphorus were determined in a UV-VIS double beam Spectrophotometer following the procedures described by Murthy (2006) and Gupta (2006). Ca, Mg, Mn, Zn, Fe, Cu and Co were analyzed in a Perkin Elmer Atomic Absorption Spectrophotometer, Analyst AA-200.

Statistical analysis

The data obtained was statistically analyzed by using proper statistical methods and reported as means ± S.Em.

Results

The ten plants taken for the experiment and their parts used are indicated in Tab. 1 and 2. The quantitative analysis of the ten selected plants with their respective secondary metabolites or pigments responsible for dyeing is given in Tab. 3. *Bixa orellana* recorded 163.11 mg g^{-1} of carotenoid pigment responsible for dyeing while curcumin content in *Curcuma domestica*, which is the pigment responsible for yellow to orange colour dye was found to be 27.7 mg g^{-1}. Total chlorophyll content of *Clerodendrum chinense* and *Datura stramonium* which is used to dye green colour was 10.29 mg g^{-1} and 11.83 mg g^{-1}, while that of chlorophyll a

Fig. 1. Photographs of the dye yielding plants. (A) *Bixa orellana* , (B) *Clerodendrum chinense*, (C) *Curcuma domestica*, (D) *Datura stramonium*, (E) *Parkia timoriana*, (F) *Lithocarpus pachyphylla*, (G) *Punica granatum*, (H) *Solanum nigrum*, (I) *Strobilanthes flaccidifolius* and (J) *Terminalia chebula*

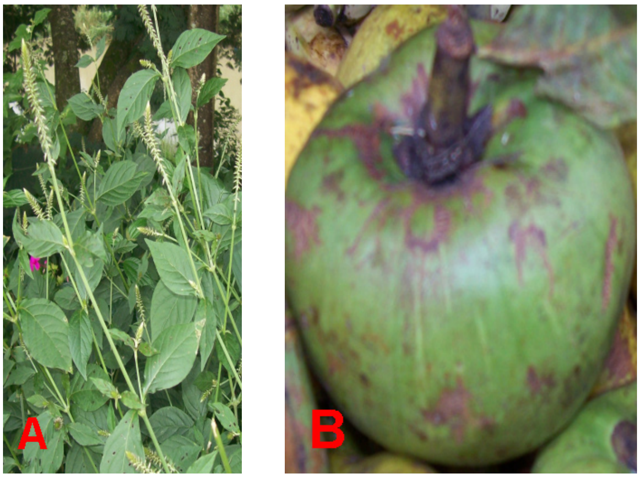

Fig. 2. Plants used as mordants, (A) *Achyranthes aspera* and (B) *Garcinia xanthochymus*

and chlorophyll b recorded were 7.43 mg g^{-1} and 2.87 mg g^{-1} in *Clerondendrum chinense* and 8.72 mg g^{-1} and 3.11 mg g^{-1} in *Datura stramonium,* respectively. *Punica granatum* and *Parkia timoriana* indicated higher amounts of total phenols (681.2 mg g^{-1} and 287.6 mg g^{-1}), bound phenols (151.6 mg^{-1} and 130.2 mg g^{-1}) and orthodihydric phenols (20.11 mg g^{-1} and 7.29 mg g^{-1}). Considerably higher amounts of tannins, which is responsible for brown colour of the dye was also recorded in *P. granatum* (657.57 mg g^{-1}), *S. flaccidifolia* (648.12 mg g^{-1}), *P. timoriana* (353.54 mg g^{-1}) and *T. chebula* (292.24 mg g^{-1}), while that of flavonoids recorded in *Solanum nigrum* and *Terminalia chebula* were 24.89 mg g^{-1} and 21.73 mg g^{-1} respectively.

Tab. 4 indicated mineral contents in two mordant plants, *Achyranthes aspera* and *Garcinia xanthochymus.* Higher contents of Ca, Mg, K, Zn, Fe and Mn were detected by recording 6.24, 6.04, 21.36, 0.28, 1.0 and 0.22 mg g^{-1} in *A. aspera*, while higher contents of K(15.17 mg g^{-1}), P (3.27 mg g^{-1}), Fe (0.99 mg g^{-1}) and Cu (0.24 mg g^{-1}) were recorded in *G. xanthochymus*.

Discussion

The natural organic dyes and pigments cover a wide range of chemical classes viz., quinone, benzoquinone, naphthaquinone, anthraquinone, flavone, flavonone, indigoid, chalcone, aurone, carotenoid, diaroyl methane, alkaloid, chlorophyll etc. (Daniel *et al*., 2006). In Tab. 3, the seeds of *B. orellana* were found to contain higher concentration of carotinoids. *B. orellana* seeds were used to dye local made bathing towels known as 'phadi' (Fig. 3A to D). The data agrees with the reports of Gokhale *et al.* (2004) that a carotenoid, bixin occurring in *B. orellana* is responsible for yellow to red dyeing property of the plant. The yellow colour dye of *C. domestica* and green colour dye of *C. chinense* and *D. stramonium* were used to dye cotton clothes for making small religious flags used for offering to deities (Fig. 3E and F).

Curcumin and other related curcuminoids were also reported for yellow colour of the dye in *C. domestica* (Gulrajani *et al*., 1992). Ruby *et al.* (1995) reported 90% of the yellow dye in *C. domestica* is due to curcumin, the remaining comes from demethoxycurcumin and bisdemethoxycurcumin. Higher content of curcumin (27.7 mg g^{-1}), in *C. domestica* correlates with its yellow dyeing property.

Higher phenol concentration in plants like *P. timoriana*, *L. pachyphylla*, *P. granatum* and *S. flaccidifolius* indicated their properties of dyeing as the presence of phenolic compounds was found to be inhibiting decolorization at a concentration of 1mM (Tony and Risky, 2012). Phenolics were reported to be responsible for dyes in various plants (Aviram and Domfeld, 2001; Salah *et al*., 2002). *S. flaccidifolius* dye is used for applying black and indigo colour to loin loom 'phanek' (Fig. 3G and 3H), a Meitei women's formal folk dress. The fruit peel of *P. timoriana* is used for dyeing the local fishing net (Fig. 3I). Similarly, fruits of *T. chebula*, *L. pachyphylla*, and *P. granatum* are used in dyeing 'chaddars' worn by the Meitei women (Fig. 3J). Higher contents of tannins in *P. timoriana* (353.54 mg g^{-1}), *P. gra-*

Tab. 1. List of ten selected natural dye-yielding plants for the biochemical analysis

Sl. no.	Botanical Name	Local Name	Family	Part Used	Colour of the dye
1.	*Bixa orellana* L.	Ureirom	Bixaceae	Seed	Red
2.	*Clerodendrum chinense* (Osbeck) Labb.	Kuthap	Lamiaceae	Leave	Green
3.	*Curcuma domestica* Valeton.	Yaingang	Zingerberaceae	Rhizome	Yellow
4.	*Datura stramonium* L.	Sagol-hidak	Solanaceae	Leave	Green
5.	*Parkia timoriana* (A.DC.) Merr.	Yongchak	Fabaceae	Fruit peel	Brown
6.	*Lithocarpus pachyphylla* (Kurz) Rehd.	Kuhi	Fagaceae	Bark	Black
7.	*Punica granatum* L.	Kaphoi	Punicaceae	Fruit peel	Brown to black
8.	*Solanum nigrum* L.	Khamu	Solanaceae	Fruit	Brown
9.	*Strobilanthes flaccidifolius* Nees	Kum	Acanthaceae	Leave	Brown
10	*Terminalia chebula* Retz.	Manahei	Combretaceae	Fruit	Black

Tab. 2. List of two selected plants used as alkaline and acidic mordants for mineral analysis

Sl. no.	Botanical Name	Local Name	Family	Part Used
1.	*Achyranthes aspera* L.	Khujum pere	Amaranthaceae	Whole plants
2.	*Garcinia xanthochymus* Hook.	Heibung	Guttiferae	Fruit

Fig. 3. Different shades and designs of clothes dyed from the ten dye yielding plants

natum (675.57 mg g^{-1}), *S. flaccifolius* (648.12 mg g^{-1}) and *T. chebula* (292.24 mg g^{-1}) indicated its use as dyes as tannins are reported to be responsible for various dyes (Shahid *et al*., 2009). The fruits of *Solanum nigrum* were used to dye a cloth worn by the royals in early days known as 'Khamen chatpa' (Fig. 3K). Appreciable quantity of flavonoids in *S. nigrum* (24.89 mg g^{-1}) and *T. chebula* (21.72 mg g^{-1}) correlates their dyeing property as flavonoids are also reported to be used in dyeing brown and black hues (Jondiko and Pattenden, 1989).

Among the two mordant plants, higher contents (mg g^{-1}) of Ca (6.24), Mg (6.04), K (21.36), Zn (0.28), Fe (1.0) and Mn (0.22) were detected in *A. aspera*, while appreciable amounts of K (15.17 mg g^{-1}), P (3.27 mg g^{-1}), Fe (0.99

Tab. 3. Pigments responsible for dyeing in ten selected natural dye yielding plants of Manipur (mg/g) mean ± S. Em

Sl no	Botanical Name	Total Phenols	Phenol Bound	Ortho	Tannin	Flavonoid	Total Chlorophyll	Chlorophyll 'a'	Chlorophyll 'b'	Carotenoid	Curcumin
1.	*Bixa orellana* L.	-	-	-	-	-	-	-	-	163.11±0.73	-
2.	*Clerodendrum chinense* (Osbeck) Labb.	-	-	-	-	-	10.29±0.001	7.43±0.04	2.87±0.06	-	-
3.	*Curcuma domestica* Valeton.	-	-	-	-	-	-	-	-	-	27.7±2.19
4.	*Datura stramonium* L.	-	-	-	-	-	11.83±0.06	8.72±0.1	3.11±0.001	-	-
5.	*Parkia timoriana* (A.DC.) Merr.	287.6±0.32	130.2±0.69	7.29±0.04	353.54±0.001	-	-	-	-	-	-
6.	*Lithocarpus pachyphylla* (Kurz) Rehd.	77.92±2.46	34.34±0.5	5.09±0.07	106.41±2.34	-	-	-	-	-	-
7.	*Punica granatum* L.	681.2±24.9	151.6±0.79	20.11±0.61	675.57±0.001	-	-	-	-	-	-
8.	*Solanum nigrum* L.	14.19±2.81	5.69±0.13	1.73±0.04	37.09±0.35	24.89±0.12	-	-	-	-	-
9.	*Strobilanthes flaccidifolius* Nees	65.75±0.01	20.26±0.17	9.54±0.45	648.12±8.64	-	-	-	-	-	-
10	*Terminalia chebula* Retz.	33.87±1.14	13.55±0.41	5.26±0.28	292.24±3.63	21.72±0.42	-	-	-	-	-

Tab. 4. Micro- and micro-element contents (mg/g) of the two plants used as mordant (Mean± S. Em)

Botanical Name	Ca	Mg	K	P	S	Zn	Fe	Mn	Cu	Co
Achyranthes aspera	6.24±0.16	6.04±0.06	21.36±1.15	1.23±0.02	1.08±0.31	0.28±0.001	1.00±0.01	0.22±0.006	0.15±0.001	0.03±0.001
Garcinia anomala	2.21±0.17	1.16±0.11	15.17±0.09	3.27±2.19	1.06±0.07	0.12±0.001	0.94±0.01	0.07±0.001	0.24±0.02	0.04±0.001

mg g^{-1}) and Cu (0.24 mg g^{-1}) were recorded in *G. xanthochymus* (Tab. 3). Higher concentration of potassium, iron and copper shows the property of using as mordants as the metal salts of iron, copper, aluminium etc. were reported to be used as metallic mordants (Gulrajani, 1999). Alum, chrome, stannous chloride, copper sulphate, ferrous sulphate etc. were also reported to be the commonly used mordants (Siva, 2007; Mahangade *et al*., 2009; Samanta and Agarwal, 2009). Metal ions of mordants act as electron acceptors for electron donors to form co-ordination bonds with the dye molecule, making them insoluble in water (Mongkholrattansit *et al*., 2011). Use of *A. aspera* and *G. xanthochymus* as mordants agrees with the above reports.

Conclusions

Natural dyes are nowadays in demand not only in textile industry but in cosmetics, leather, food and pharmaceuticals. Natural dyes can offer not only a rich and varied source of dyestuff, but also the possibility of an income through sustainable harvest and sale of the dye plants. In areas, where synthetic dyes and mordants are imported (therefore relatively expensive), natural dye yielding and mordant plants like *B. orellana, C. domestica, P. granatum, P. timoriana*, *S. nigrum* and *A. aspera* can offer an attractive alternative.

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References

Aviram M, Domfield L (2001). Pomegranate juice consumption inhibits serum angiotension converting enzyme activity and reduces systolic blood pressure. Elsevier, Atherosclerosis 158:195-198.

Barber EJW (1991). Prehistoric textiles: The development of cloth in the Neolithic and bronze ages with special reference to the Aegean.Princeton University Press, Princeton, 508 p.

Bray HG, Thorpe WV (1954). Analysis of phenolic compounds of interest in metabolism. Meth Biochem Anal 1:27-52.

Bryan NG, Stella Y (2002). Navajo natives dyes: Their preparation and use. Dover Publications, Mineola.

Capar SG, Tanner JT, Freidman MH, Boyer KW (1978). Multielement analysis of animal feed, animal waste and sewage sludge. Environ Sci Technol 12:785-790.

Chang C, Yang M, Wen H, Chern J (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 10:178-182.

Daniel M, Bhattacharya SD, Arun A, Vinay MR (2006). Natural dyes: Scope and challenges. 3rd Ed. Scientific Publishers, Jodhpur, India, 463 p.

Gokhale SB, Tatiya AU, Bakliwal SR, Fursule RA (2004). Natural dye yielding plants in India. Nat Prod Radiance 3(4):228-234.

Goodwin J (1982). A Dyer's manual. Pelham Books, London, 231 p.

Gulrajani ML, Gupta DB, Agarwal V, Jain M (1992). Some studies on yellow dyes, Part I: CI Natural Yellow 3: Turmeric. The Indian Textile J 102(10): 50-56.

Gulrajani ML (1999). Natural dyes- Part I- Present status of natural dyes. Colourage 7:19-28.

Gupta PK (2006). Soil, plant, water and fertilizer analysis. Agrobios Agro House, Chopsail Road, Jodhpur, India, 241 p.

Hofenk de Graaf J (2004). The colourful past: Origins, chemistry and identification of natural dyestuffs. Abegg-Stiftung and Archetype Publications, 396 p.

Jayaraman J (1988). Laboratory manual in biochemistry. 3rd Ed. Wiley Eastern Ltd, New Delhi, India, 171 p.

Jensen A (1978). Chlorophylls and carotenoids. In: Hellebust A, Cragie JS (Eds.). Handbook of phytological methods. Cambridge Univ. Press, London.

Jondiko IJO, Pattenden G (1989). Terpenoids and an apocarotenoid from seeds of *Bixa orellana*. Phytochemistry 28:3159-3162.

Mahadevan A, Sridhar R (1986). Methods in physiological plant Pathology. 3rd Ed. Sivakami Publications, Chennai, 183 p.

Mahangade RR, Varadarajan PV, Verma JK, Bosco H (2009). New dyeing techniques for enhancing color strength and fastness properties of cotton fabric dyed with natural dyes. IJFTR 34:279-282.

Mongkholrattansit R, Krystufek J, Wiener J, Vikova M (2011). Dyeing, fastness and UV protection properties of silk and wool fabrics dyed with eucalyptus leaf extract by the exhaustion process. Fibres and Textiles 86(3):94-99.

Murthy YLN (2006). Estimation of secondary and micronutrients in soil and plant samples. Procof the New Dimensions in Integrated Nutrient Management of Major Field Crops for the Sustainable Crop Production. Directorate of Oilseeds Research, Hyderabad, India, 85 p.

Mutua B (1997). Traditional textiles of Manipur. Mutua Museum, Keisampat, Imphal, India, 104 p.

Ruby J, Kuttan G, Babu KD, Rajashekharan KN, Kuttan R (1995). Antitumor and oxidant activity of natural curcuminoids. Cancer Lett 94:79-83.

Sadasivam S, Manickam A (1992). Biochemical methods for agricultural sciences. 2nd Ed. Wiley Eastern Ltd., New Delhi, India, 250 p.

Salah A, Al-Maiman, Dilshad A (2002). Changes in physical and chemical properties during pomegranate (*Punica granatum* L.) fruit maturation. J Food Chemistry 76:437-441.

Samanta AK, Agarwal P (2009). Application of natural dyes on textiles. IJFTR 34:384-399.

Schanderi SH (1970). Method in food analysis. Academic Press, New York, 709 p.

Shahid A, Saukat A, Ijaz AB, Ferenc Z (2009). Dyeing of cotton fabric using pomegranate (*Punica granatum*) aqueous extract. Asian J Chemistry 21(5):3493-3499.

Sharma BK (2000). Meiteigi Machugi Manat (in Manipuri). B. Roshan Manuscript Library, Imphal, Manipur, 207 p.

Siva R (2007). Status of natural dyes and dye-yielding plants in India. Current Sci 92(7):916-925.

Tony H, Risky AK (2012). Effect of environmental factors in the colorization of remazol brilliant blue R by *Polyporus* S133. J of the Chil Chem Soc 57(2):115-119.