A Study on Vegetable Dye from the Seeds of Sinduri (*Bixa orellana*)

S.C. Das[1], S. Akhter[1], M. S. Rahman[1] and K. Ahmed[2]
[1]Bangladesh Forest Research Institute, P.O. Box 273, Chittagong 4000, Bangladesh
[2]Bangladesh Council of Scientific and Industrial Research Laboratories,
Chittagong 4220, Bangladesh

Abstract

Vegetable dye was extracted from the seeds of sinduri {*Bixa orellana* (Linn.)} using water (soaking method) and ethyl acetate (soxhlet and reflux methods) as solvents. Reflux method gave the highest yield (10.1%) while soxhlet method yielded 7.10% of dye. In soaking method, seeds were soaked in water for four different time periods. The highest yield (8.93%) was obtained in 18 hours out of 6,12, 18 and 24 hours soaking periods. Considering the chemicals and processing cost, water soaking method appeared the best although it gave slightly lesser amount of dye compared to other methods. The performance of the extracted dye for its edibility was studied in laboratory against rats. After feeding the dye, mixed with water, the physiological changes of rats were observed. The test showed no adverse effect, and it seemed that the dye is non-toxic to animals. Thus, it can be suggested as a substitute to chemical dye as food colourants.

সারসংক্ষেপ

সিন্দুরী {*Bixa orellana* (Linn.)} গাছের বীজ থেকে পানি (ডুবানো পদ্ধতি) ও ইথাইল এ্যাসিটেড (রিফ্লাক্স ও সক্সলেট পদ্ধতি) সহযোগে ভেষজ রং নিস্কাশন করা হয়। রিফ্লাক্স পদ্ধিতিতে সর্বাধিক ১০.১% এবং সক্সলেট পদ্ধতিতে ৭.১০% রং পাওয়া গেছে। পানিতে ডুবানো পদ্ধতিতে চারটি বিভিন্ন স্থিতিকালে বীজ ডুবানো হয়। ৬, ১২, ১৮ এবং ২৪ ঘন্টা স্থিতিকালে নিস্কাশিত রং এর মধ্যে ১৮ ঘন্টায় সর্বাধিক পরিমান (৮.৯৩%) রং পাওয়া যায়। রাসায়নিক দ্রব্যাদি এবং প্রক্রিয়াজাতকরা খরচের বিবেচনায় পানিতে ডুবানো পদ্ধতিই সবচাইতে ভাল বলে প্রতীয়মান হয়, যদিও এ পদ্ধতিতে উৎপাদিত রং- এর পরিমান অন্যান্য পদ্ধতির তুলনায় কিছুটা কম। খাদ্যোপযোগিতা পরীক্ষার জন্য নিস্কাশিত রং পানিতে মিশিয়ে ল্যাবরেটরীতে ইঁদুরকে খাওয়ানোর পর এদের শারীরিক পরিবর্তন পর্যবেক্ষন করা হয়। পরীক্ষায় বিরূপ কোন প্রতিক্রিয়া পাওয়া যায়নি; এতে প্রতীয়মান হয় যে, নিস্কাশিত রংটি বিষাক্ত নয় এবং এটি রাসায়নিক খাবার রং-এর বিকল্প হিসাবে ব্যবহার করা যেতে পারে।

Key words: Annatto, *Bixa orellana*, edibility test, extraction, non-toxic, rats, vegetable dye

Introduction

Bixa orellana (Linn.), locally known as sinduri, is a kind of shrub native in Central and South America and now widely spread throughout the tropics. Sinduri seed coat contains pigments known as annatto, which is considered a rich source of orange red vegetable dye. Owing to its non-toxic nature, the dye is reported to be used for colouring edible products like butter, cheese, ghee, ice-cream, chocolate, bakery food items and edible oils (Drury 1873, Jondiko and Pattenden 1989). Besides food colouring, the dye is also used in floor wax, brass lacquer, wood stains and hair oils (Singh *et al.* 1983). The dye is valued for its medicinal property and reported to be a good antidote for dysentery and diseases of kidney (Drury 1873). It is also known to have a hyperglycemic effect (Anderson *et al.* 1997).

The major producers of annatto are Peru, Brazil and Kenya (Mercadante and Pfander 1998). In Brazil, annatto ranks second in economic importance among the naturally occurring colourants. It is largely cultivated in India for extraction of dye but the yield and quality are very low (Aparnathi *et al.* 1990). In Bangladesh, it is cultivated in gardens and roadsides as a hedge and fire break (Ara *et al.* 1997). But there is no information of using bixa seeds as a source of vegetable dye in the country. However, due to the medicinal importance of the dye, the plant has drawn attention as a valuable medicinal plant in the country (Ara *et al.* 1997). Considering the demand, the plant could be exploited as a source of eco-friendly vegetable dye in the country. The present study attempts to ascertain a suitable extraction technique of the dye and its possibility to use as a substitute to imported chemical dye for colouring food products.

Materials and methods

B. orellana fruits were collected from Kumira, Chittagong in February 1996. Fruits were dried in the sun for three days until the outer shells of the fruits cracked. The shells were removed and seeds were separated. The seeds were then dried in the sun for two days and screened on a wire net to remove dirt. The seed samples were kept in a polybag and stored in a refrigerator.

Extraction of the dye

In traditional method, water was used as the common solvent to extract the dye. Extraction technique was later improved and chromatographic technique was introduced in the extraction processes (Bhal *et al.* 1971, Jondiko and Pattenden 1989). To avoid thermal degradation supercritical carbon dioxide was also used in extracting the dye (Chao *et al.* 1991, Anderson *et al.* 1997). However considering the present laboratory facilities, the following three methods were followed for the extraction of dye.

(a) Extraction with water

Dried seed samples were soaked in water at room temperature (1:10 seed and water ratio, w/v) for 6, 12, 18 and 24 hours with 3 replications in each period with occasional stirring. At the end of each period the dye was separated from the seeds using wire net, and the filtrates were stored separately. Water extraction brought in many impurities in addition to dye. Treatment with vinegar was considered to be a good method of separating the dye from other water-soluble materials (Watt 1972). Required quantities of vinegar solution (5% of acetic acid) were added to the filtrates (v/v) of each soaking period so that the filtrates appeared to be of 2.50%, 5.00%, 7.50% and 10.0% vinegar concentrations individually and boiled on water baths under reflux until the dye precipitated. After cooling at room temperature the dye was filtered on previously weighed filter papers. Filter papers containing the dye were dried at 105 ± 3^{0}C in an oven for 24 hours to dryness, cooled in a desiccator and weighed. The percentage of dye was calculated on oven dry basis of seed samples.

(b) Extraction with organic solvent

Solvent like hexane, chloroform, ethyl acetate, acetone and alcohol are considered suitable for extraction of the dye. Considering the availability and cost of solvents, ethyl acetate was used to extract the dye (1:10 seed solvent ratio, w/v). Two different extraction methods, *viz.*, soxhlet and reflux were followed and seeds were extracted for 12 hours. The extracted dye was collected in petridishes and evaporated the excess ethyl acetate to dryness. The petridishes were placed in an oven at 105 ± 3^{0}C and kept for 24 hours to get constant weight. The petridishes were then cooled in a desiccator and weighed. The percentage of dye was calculated on oven dry basis of seed samples.

Edibility test of the extracted dye

Edibility test of the dye was conducted in order to ascertain its feasibility to use as food

colourants. The test animal, rats (*Rattus norvegicus*) were fed the dye extracted both with water and ethyl acetate mixed with water orally using feeding needles to maintain the dose level. The experiment was carried out at BCSIR laboratories, Chittagong. Twenty rats (weighing 130-140 gm.) were taken for the experiment and divided equally into two groups, one control and another treated. Each group had five males and five females. The treated group was fed the dyes separately in addition to normal diets. The doses of the dye were 1 gm/kg average body weight of the rats. The control group was fed only normal diets. Their physiological change e.g. body weight, food intake, number and condition of fecal pellets, skin and fur colour and gross general appearance were observed daily and compared with those of control ones. The experiment was continued for one month.

Results and discussion

In the study, the traditional water extraction method was improved by treatment with vinegar to separate the dye from other impurities. The percentages of the dye in water extraction method are presented in Table 1.

It is seen that filtrates containing 7.50% of vinegar yielded the maximum percentage of dye (Table 1); in the cases of 18 and 24 hours soaking periods, the yields were 8.93% and 8.89% respectively. As 18 hours treatment gave the maximum yield, it appears to be the best for extraction of the dye. The percentages of the dye extracted with ethyl acetate for 12 hours are presented in Table 2. In reflux method the yield was found to be 10.1% (Table 2). In this method the solvent extracted the dye at higher temperature so that some components other than the dye might have contributed to the yield. In addition, thermal degradation might produce other components that gave higher yield.

In soxhlet method the yield was found to be 7.10% (Table 2). In this method the solvent extracted the dye at lower temperature compared with reflux method, and there was less chance of coming out other components in association to the dye. Probably due to this, the yield here was lower.

ANOVA (Balanced design) result shows that the percentage of dye in different soaking hours and vinegar concentrations were significantly different ($P<0.001$). Lower soaking time extracted minimum percentage of dye, which were significantly different from those of other soaking periods. There was no significant difference between 12, 18 and 24-hours treatments. However, 7.50% vinegar treatment yielded the maximum amount of dye. Thus 18 hours soaking period with 7.50% vinegar treatment appeared to be an appropriate method of extracting the dye. In addition, as the extraction method was carried out at room temperature there was less chance of thermal degradation. As the method was easy it could be used by the small entrepreneurs.

Statistical analysis of the results (One way ANOVA) shows that all the three extraction methods (18 hours soaking, reflux, soxhlet) are significantly different from each other ($P<0.001$). Although the reflux method yielded the highest percentage, colour and odour of the extracted dye was physically seen inferior as compared to that of the other two methods. In addition solvent extraction method needed expensive chemicals. As a result this extraction method could not be considered as a compatible method of extraction of dye. Between 18 hours soaking period and soxhlet extraction methods, the former method yielded higher amount of dye, and thus could be suggested for extraction dye form *B. orellana* seeds.

Edibility test of the extracted dye showed no sharp difference in physiological changes of the rats between control and treated groups (Table 3).

Table 1. Average yield of dye (%) from *B. orellana* seeds in soaking method.

Vinegar concentration (%)	Yield of dye (% ±sd) in different soaking hours			
	6 hours	12 hours	18 hours	24 hours
2.50	5.69 ± 0.41	6.94 ±0.82	6.93 ±0.54	6.91 ±0.19
5.00	5.54±0.22	7.13±1.11	7.78±0.66	7.81±0.55
7.50	7.33±1.23	8.28±0.84	8.93±0.16	8.89±0.40
10.0	7.16±0.14	7.20±0.33	7.53±0.28	7.33±0.21

Table 2. Yield of dye (%) from *B. orellana* seeds (reflux and soxhlet methods).

Method	Yield of dye (%)
Soxhlet	7.10
Reflux	10.1

Table 3. Results of one month feeding of *B. orellana* dye (1 gm/kg body weight) extracted with water and ethyl acetate against rats.

Observation (10 animals per group)		Control	Treated (water extracted dye)	Control	Treated (ethyl acetate extracted dye)
Average body weight (per day per rat) (gm)	Initial	133.5	146.0	136.0	142.5
	Final*	145.6	161.1	147.8	156.3
Average food intake (per day per rat) (gm.)		10.3	13.3	9.64	9.76
Average nos. of fecal pellets (per day per rat)		34.8	36.8	21.8	22.4
Average nos. of soft pellets (per day per rat)		3.7	2.7	1.4	3.4

*Average of one month

After one month feeding of the dye, average body weights of the rats were increased by 10.3% with water extracted dye and 9.68% with ethyl acetate extracted dye whereas those of the control groups by 9.0-6% and 8.68% based on initial weights (Table 3). Treated groups took slight higher food than that of control group. Other observations like skin and fur colour and overall gross general appearance were comparable to both the groups. It reveals that there is no adverse effect of physiological changes between the treated and control groups. Thus the test indicates that the dye is non-toxic to the experimental animals at a dose level of one gm/kg body weight.

The findings of the edibility test agree with the previous reports of using the dye as food colourants (Drury 1873, Jondiko and Pattenden 1989, Aparnathi *et al.* 1990). Thus based on the results and the literature reports (Bhal *et al.* 1971, Degnan *et al.* 1991, Manalo *et al.* 1989) the dye could meet the partial demand of vegetable dye in the country.

Conclusion

Reflux and soxhlet methods involve with high energy cost, solvent disposal and environmental pollution problems. On the other hand, soaking method avoids these problems and gives higher yield. So, the latter method is considered beneficial and convenient for extraction of the dye. As the method is easy, small cottage industries could be benefited by using this technology.

References

Anderson, S.G.; Nair, M.G.; Amitabh, C.; Morisson, E. and Chandra, A. 1997. Supercritical fluid carbon dioxide extraction of annatto seeds and quantification of trans-bixin by high pressure liquid chromotography. *Phytochemical Analysis* 8(5): 247-249.

Aparnathi, K.D.; Lata, R. and Sharma, R.S. 1990. Annatto (*Bixa orellana* L.)-Its cultivation, preparation and usage. *International Journal of Tropical Agriculture* 8(1): 80-88.

Ara, R.; Merry, S.R. and Siddiqi, N.A. 1997. *Cultivation and Uses of Twelve Medicinal Plants of Bangladesh.* Bulletin 7, Minor Forest Products Series, Bangladesh Forest Research Institute, Chittagong. 7-9 pp.

Bhal, C. P.; Seshadri, T. R. and Vedantham, T. N. C. 1971. Preparation of bixin and methyl bixin from Indian seeds of *Bixa orellana*. *Current Science* 40(2): 27-28

Chao, R.R.; Mulvaney, S. J.; Sanson, D. R.; Hsieh F.H. and Tempesta, M. S.1991. Supercritical CO_2 extraction of annatto (*Bixa orellana*) pigments and some characteristics of the color extracts. *Journal of Food Science* 56(1): 80-83.

Degnan, A.J.; Elbe, J.H. and Hartel, R.W. 1991. Extraction of annatto (*Bixa orellana*) seed pigment by supercritical carbon dioxide. *Journal of Food Science* 56(6): 1655-1659.

Drury, C.H. 1873. *The Useful Plants of India*. Periodical Experts Book Agency, Delhi. 79-80 pp.

Jondiko, I. J. O. and Pattenden, G. 1989. Terpenoids and an apocarotenoid from seeds of *Bixa orellana*. *Phytochemistry* 28(11): 3159-3162.

Manalo, J. B.; Anzaldo, F. E. ; Coronel, V. Q. ; Torres, R. C. and Briones, A. V. 1989. The extraction and application of annatto extract (carotenoid) a local natural organic pigment (from *Bixa orellana*) *Philippines Journal of Science* 118 (2): 101-122.

Mercadante, A. Z. and Pfander, H. 1998. Carotenoids from annatto: a review. *Recent Research Developments in Agricultural and Food Chemistry* 2(1): 79-91.

Singh, U.; Wadhwani, A.M. and Johri, B.M. 1983. *Dictionary of the Economic Plants in India.* 2nd edition. Indian Council of Agricultural Research. New Delhi. 30 pp.

Watt, G. 1972. *A Dictionary of the Economic Products of India.* Vol.1, Periodical Experts, Delhi. 454-457 pp.