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Isolation and Characterization of Anthraquinones from The Bark of Two *Cassia* Species and Optimization of Dyeing Process on Wool by Their Bark Extracts

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Wool fabric has been dyed with aqueous extracts of powder bark of *Cassia fistula* and *Cassia siamea*. The dyeing characteristics of the colouring matter on wool have also been studied with or without mordants. Optimization of dyeing process for wool was done by bark extracts of both the plants. Three anthraquinones 1,8-dihydroxy-6-methoxy-3-methyl anthraquinone from the bark of *Cassia fistula* and 1, 8-dihydroxy-3-methyl anthraquinone and bianthraquinone cassiamin from *Cassia siamea* have been isolated and characterized with help of chromatographic and spectroscopic techniques. These anthraquinones are responsible for dyeing properties in both the plants.

Key Words: *Cassia fistula*, *Cassia siamea*, Dyeing, Mordants, Natural dye, Wool.

INTRODUCTION

An increased environmental awareness and health hazard associated with the use of synthetic dyes has led to the revival of natural dyes. Synthetic dyes production requires strong chemicals like strong alkalis, acids, metal catalyst, which are not only toxic but also their prolonged exposure, can cause skin cancer. Natural dyes/colourants[1] derived from flora and fauna are believed to be safe because of their non-toxic, non-carcinogenic and biodegradable nature.

Cassia (family: Leguminous; subfamily: Caesal piniaceae) is a large genus with about 580 species of herbs, shrubs and trees[2-4]. Two *Cassia* species *i.e. Cassia fistula* and *Cassia siamea* were selected for present investigation. The present investigation was undertaken to isolate and characterize colour components *i.e.* anthraquinones which are main source of natural dyes of both the species. Further, the dyeing characteristics of the colouring matter on wool have also been studied with or without the use of mordants and the properties of the dyed fibers valuated. Optimization of dyeing process for wool by their bark extracts was also undertaken.

EXPERIMENTAL

Fresh bark (1 kg/plant) of the two plants species, namely *Cassia fistula* and *Cassia siamea*[5-9] were collected from local forest during winter season. Bark was dried under shade and pulverized in Wiley mill to powder form and then stored at ambient temperature in sealed plastic bags. Australian Marino wool was purchased from Gandhinagar. C.D.H. and Qualigens made mordants such as potassium dichromate (chrome), potassium aluminium sulphate (alum), strontium nitrate, nickel sulphate, barium chloride, copper sulphate, chromium nitrate and manganese acetate of LR/AR grades were used. Solvents like *n*-hexane, petroleum ether, ethyl acetate, chloroform, diethyl ether, methanol and ethanol were used for the extraction and eluents. The main component of dye was separated by chromatography using silica gel obtained from Qualigens, India. Distilled water was used for the extraction of colour components and for the preparation of all chemical solution. Deionized water was used for dyeing purpose.

Optimization of dyeing process[10-12]: The skins of wool were soaked in tap water for 0.5 h. The dye liquor was prepared by taking different concentrations (2, 4, 6, 8 and 10 g) of dry bark powder in 100 mL of water. These were extracted for 1 h and filtered. Two milliliter of aliquot was taken as a sample and diluted to 5 times to record optical density. Wool samples weighing 1 g each were dyed in the dye solution for 1 h at 100 ºC. The optical density was measured by Elico SL-159 spectro photometer integrated with Zenith PC at 380 mm. The optical density of left over dye solution was recorded (Table-1).

TABLE-1
PERCENTAGE ABSORPTION FOR DIFFERENT CONCENTRATION OF *Cassia fistula* AND *Cassia siamea* BARK POWDER; WAVELENGTH: 380 nm

Cassia fistula			
Concentration of bark powder 1 g/100 mL	O.D. before dyeing	O.D. after dyeing	Percentage absorption
2	0.06	0.05	16.60
4	0.18	0.14	22.22
6	0.25	0.17	32.00
8	0.50	0.35	30.00
10	0.95	0.72	24.31
Cassia siamea			
Concentration of bark powder 1 g/100 mL	O.D. before dyeing	O.D. after dyeing	Percentage absorption
2	0.08	0.07	12.50
4	0.15	0.13	13.33
6	0.20	0.15	25.00
8	0.44	0.40	9.09
10	0.85	0.70	17.64

Optimum time for extraction of dye, amount of different mordants and time for mordanting were selected based on maximum absorption of dye by wool samples. The optical density of dye liquor was measured before and after dyeing of wool. Three methods *i.e.* (a) pre-mordanting: mordanting followed by dyeing (b) simultaneous mordanting and dyeing: dyeing and mordanting were carried out at the same time and (c) post-mordanting: the sample was first dyed and then mordanted. On the basis of the depth of colour, evenness of dye and brightness of the shade, pre-mordanting was found to be the best method. Thus this method was selected for further experiments.

The fix percentage of each mordant was dissolved in 10 mL boiling water. The solution was transferred to a beaker containing 90 mL of warm water. The soaked samples were added to the solution and temperature was raised at 100 ºC. The solution was stirred occasionally to bring an even distribution of mordant over the wool fiber. Mordanting was continued for 1 h and then the samples were allowed to cool in the mordant solution and dried under shade. The samples mordanted with chrome, ferrous sulphate, copper sulphate and stannous chloride were dried immediately after mordanting, whereas the samples mordanted with alum were left overnight to give best shades. In case of mordanting with chrome the dye bath was properly covered because chrome is very sensitive to light.

In order to find out the optimum concentration of the various metallic mordants, different concentration of each mordant, namely 2 % conc. of alum, barium chloride, manganese acetate, 1 % of potassium dichromate, strontium nitrate, nickel sulphate, copper sulphate and chromium nitrate and 1.5 % of ferrous sulphate were used to dye the wool samples.

Colour fastness of dyed sample towards light was measured by Fed = 0 = meter while launder = 0 = meter was used to check the colour fastness to washing and staining.

Isolation of anthraquinones from *Cassia fistula* and *Cassia siamea* Bark

***Cassia fistula*:** The air dried, crushed and defatted stem bark of *Cassia fistula* was repeatedly extracted with boiling ethanol, concentrated under reduced pressure in a rotavapour. The extract was then pored into ice cold water to get water soluble and water insoluble portions. The water soluble portion was successively extracted with ether and ethyl acetate. The ether extract had no colour residue while ethyl acetate extract on concentration afforded a brown colour pigment. The brown colour pigment was subjected to thin layer chromatography by silica gel coated glass plate using chloroform:ethyl acetate:methanol (4:6:10 v/v/v) solvent system. It is found to be homogenous. It responded positively with methanolic magnesium acetate and hydroxide for an anthraquinone. This anthraquinone was identified on the basis of colour reactions and spectral data.

***Cassia siamea*:** The air dried and powdered bark was successively extracted in a soxhlet extractor with hexane, chloroform and ethanol. The total percentage was found to be 28.6 %. The ethanol extract (23.71 %) was concentrated under reduced

pressure. The concentrated mass of ethanolic fraction on chromatograph over silica gel using benzene:chloroform (4:6 v/v) afforded yellow colour compound **1** (300 mg). Further elution with chloroform:methanol (6:4 v/v) afforded an orange colour compound **2** (700 mg). Both the compounds gave positive colour with methanolic magnesium acetate and methanolic sodium hydroxide and identified on the basis of spectral data.

RESULTS AND DISCUSSION

Optimization of dyeing process: Wool was selected for dyeing[13-15] because it is a protein fiber in which both acidic and basic groups are present. Hence, its dye affinity is greater than that of cotton fiber.

Out of three methods of mordanting namely pre-mordanting (A), simultaneous (B) and post mordanting (C), method (A) was considered to be the best with all mordants mentioned in Table-2.

TABLE-2
PERCENTAGE ABSORPTION FOR DIFFERENT
MORDANTS WITH *Cassia fistula* AND *Cassia siamea*

Cassia fistula; Wavelength: 380 nm			
Mordants	Concentration of mordants	Colour	Percentage absorption
Alum	2.0	Pale brown	46.50
Manganese acetate	2.0	Brown	49.09
Ferrous sulphate	1.5	Yellowish brown	42.22
Potassium dichromate	1.0	Dark brown	54.50
Strontium nitrate	1.0	Reddish brown	60.01
Nickel sulphate	1.0	Yellowish brown	48.09
Copper sulphate	1.0	Golden brown	50.05
Barium chloride	2.0	Pale brown	42.09
Chromium nitrate	1.0	Brown	45.08
Cassia siamea Wavelength: 380 nm			
Mordants	Concentration of mordants	Colour	Percentage absorption
Alum	2.0	Reddish Yellow	42.00
Manganese acetate	2.0	Yellowish Green	37.08
Ferrous sulphate	1.5	Brown	54.00
Potassium dichromate	1.0	Orange	51.00
Strontium nitrate	1.0	Yellowish orange	45.96
Nickel sulphate	1.0	Green	38.00
Copper sulphate	1.0	Dark Brown	45.87
Barium chloride	2.0	Brown	42.00

It is evident that percentage of absorption of dye increases with the increase in dye concentration and it reaches maximum when 6 g (Table-1) of dye material was used for both the plants. Similarly 1.5 h of extraction time gave maximum optimum

optical density (Table-3) for both the plants while there was not so significance increase in the optical density even after boiling for 2 h. Out of many mordants, alum, potassium dichromate, nickel sulphate, ferrous sulphate and copper sulphate gave most appealing shades on wool.

TABLE-3
OPTICAL DENSITY AT 380 nm AT DIFFERENT PERIODS OF BOILING OF *Cassia fistula* AND *Cassia siamea* BARK

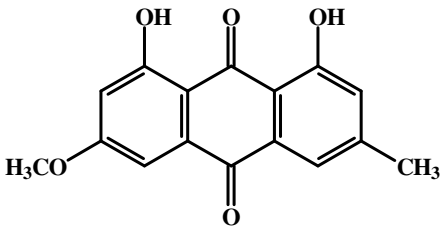
Cassia fistula		
Wavelength (nm)	Time of boiling (h)	Optical density
380	0.5	0.150
380	1.0	0.160
380	1.5	0.185
380	2.0	0.186
Cassia siamea		
Wavelength (nm)	Time of boiling (h)	Optical density
380	0.5	0.100
380	1.0	0.120
380	1.5	0.150
380	2.0	0.125

Out of large number of samples generated during present study, some samples were selected for light and wash fastness.

All the samples dyed, mordanted, using pre-mordanting and dyeing method exhibited fair good to good fastness, while few samples showed poor light and wash fastness (Table-4).

Characterization of anthraquinones from *Cassia fistula* and *Cassia siamea*

***Cassia fistula*:** The brown pigment (m.p)- 270 ºC, $C_{16}H_{12}O_5$, UV (EtOH): 410 nm; IR (KBr, ν_{max}, cm^{-1}) 2940 (phenolics OH), 1660 (non-chelated C=O) and 1635 (chelated C=O); ^1H NMR spectrum of compound **1** showed signals for one C-methyl at δ 2.42 (3 H, S, C-3), one methoxyl group at δ 3.91 (3H, S), two phenolic peri-hydroxyl groups at δ 12.08 and 12.28 (each broad singlet exchangeable with D_2O, 1H, 2×OH, C-8 and C-1) and four aromatic protons at 6.66, 7-34 (1H, S, d, *J* 2 Hz, H-2 and H-4) and 7.05, 7.59 (each 1H, d, *J* 2 Hz, H-7 and H-5) was characterized as 1,8-dihydroxy-6-methoxy-3-methyl anthraquinone (**1**).



1,8-Dihydroxy-6-methoxy-3-methyl anthraquinone (**1**)

TABLE-4
RATING FOR COLOUR FASTNESS OF SAMPLES DYED WITH *Cassia fistula* AND *Cassia siamea* BARK EXTRACTS, TREATED WITH DIFFERENT MORDANTS

Cassia fistula		
Treatment	Rating for colour fastness*	Rating for light fastness**
Samples without mordant	5	4
Manganese acetate	4	5
Ferrous sulphate	3	2
Potassium dichromate	4	5
Strontium nitrate	5	6
Nickel sulphate	5	6
Copper sulphate	3	4
Cassia siamea		
Treatment	Rating for colour fastness*	Rating for light fastness**
Samples without mordant	5	4
Manganese acetate	4	5
Ferrous sulphate	3	2
Potassium dichromate	4	5
Strontium nitrate	5	6
Nickel sulphate	5	6
Copper sulphate	3	4

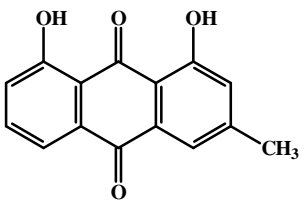
*6 - very good, 5-good, 4- fairly good, 3- fair, 2- poor and v-poor
**6 - very good, 5-good, 4- fairly good, 3- fair, 2- poor and v-poor

***Cassia siamea*:** Two pigments namely, chrysophanol (**2**) and cassiamin (**3**) were isolated by thin layer chromatography of concentrated bark extract.

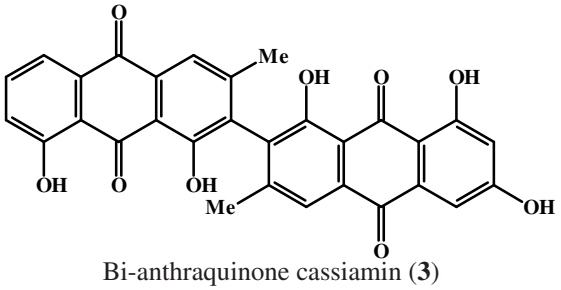
Compound **2**: Crystallized from chloroform:methanol (1:1) as yellow needles, m.p. 195 ºC, $C_{15}H_{10}O_4$ (M^+ 254), R_f value 0.28 (chloroform:benzene:methanol 16:3:1 v/v/v); UV (EtOH) 257, 277, 287, 429 nm; IR (KBr cm^{-1}): 3400, 1670, 1620; NMR ($CDCl_3$) one methyl group at δ 2.42 (3H, s, C-3), two chelated phenolic hydroxyl group at δ 12.10 and 11.99 (2×OH, C-8 and C-1), 5 aromatic protons at δ 7.03 (1H, s, H-2) and 7.10-8.10 (4H, m, H-4, H-5, H-6, H-7). It was characterized as 1,8-dihydroxy-3-methyl anthraquinone (chrysophanol) (**2**).

Compound **3**: Crystallized from tetrahydrofuran as yellow orange prism, m.p. 356-357 ºC (decomp.), R_f value 0.42 (chloroform:benzene:methanol 16:3:1 v/v); UV (EtOH) 228, 259, 288 and 444 nm, resembles those of 1,8-dihydroxy anthraquinone such as chryosophanol UV (EtOH) 257, 277, 287 and 429 nm and emodin UV (EtOH) 222, 261, 289, 429 nm.

The IR carbonyl absorption of the compound at 1670 cm^{-1} indicated the presence of CO group. The IR absorption band in the region of 1620 cm^{-1} was assigned to olefinic/aromatic linkage vibration, singly chelated anthraquinone carbonyl absorb in the region 1637-1631 cm^{-1}, in which compound has no absorption maximum, indicating that the α-hydroxyl of neither anthraquinone moiety were in 1,4 or 1,5-relationship.



1,8-Dihydroxy-3-methyl anthraquinone (chrysophanol) (**2**)



Bi-anthraquinone cassiamin (**3**)

The ^{1}H NMR ($CDCl_3$) spectrum showed signels for two methyl groups at δ 2.40 (s), δ 2.42 (6H,s,C-3,C-3'), seven aromatic protons at 7.93 (1H, s, H-4), 7.66 (3H, m, H-5, H-6, H-7), 7.84 (2H, s, H-4', H-5'), 7.93 (1H,S,H-7'). This was further supported by closed resembles of electronic spectrum of bianthraquinone cassiamin to the spectra of chrysophanol and emodin (**3**).

Conclusion

The colour components isolated from the bark of *Cassia fistula* and *Cassia siamea* mainly anthraquinone moiety. From the above study it can be concluded that a unique bands of colours can be obtained from natural dyes by using chemical mordants. Their light and wash fastness is only fair to good be the majority of cases. These dyes can replace synthetic dyes as their intermediates are usually toxic and carcinogenic to mankinds.

REFERENCES

1. S.B. Kalidhar, *Phytochemistry*, **28**, 2455 (1989).
2. L. Misra and H. Wagner, *Indian J. Chem.*, **40B**, 801 (2006).
3. S. Khatoon and S. Mehrotra, *Natural Medicines*, **54**, 171 (2000).
4. R. Bhuyan and C. N. Saikia, *Indian J. Fiber Textile Res.*, **29**, 470 (2004).
5. L. Ledwani, S. Seghal and K.C. Gupta, *Asian J. Chem.*, **21**, 5179 (2009).
6. S.R. Shukla, S.C. Shinde, A.S. Banye and M.P. Patil, *Indian J. Fiber Textile Res.*, **29**, 350 (2004).
7. L. Ledwani and M. Singh, *Indian J. Chem.*, **44B**, 1970 (2005).
8. L. Ledwani and M. Singh, *J. Indian Chem. Soc.*, **83**, 383 (2006).
9. L. Ledwani and M. Singh, *Indian J. Chem.*, **43B**, 2257 (2004).
10. M.A. Sayeed, M.Z. Islam and M.H. Hossain, *Indian Textile J.*, **100**, 114 (1990).
11. R.B. Chowan, *Clotheline*, **8**, 96 (1995).
12. M.L. Gulrajani, *Indian Textile J.*, **102**, 86 (1992).
13. K. Nishidha and K. Kobayashi, *Am. Dyestuff Reporter*, **8**, 96 (1995).
14. F.N. Olmez, *J. Graduate School of Nat. Appl. Sci.*, **6**, 113 (2002).
15. M.L. Gulrajani, D. Gupta and S. R. Maulik, *Indian J. Fiber Textile Res.*, **24**, 294 (1999).

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