



A Study of Extraction and Characterization of Anthocyanin Pigments from *Indigofera* (L.) species

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ABSTRACT

Anthocyanins are water soluble polyphenol natural pigments. They are responsible for the wide range of colors in the petals of flowering plants and they may vary the color from salmon-pink, through scarlet, magenta and violet to deep blue (Haslam, 1995). Chemically anthocyanin are B-glycosides of anthocyanidins. The anthocyanins are sub divided into the sugar-free anthocyanidin aglycones and the anthocyanin glycosides. The anthocyanins have as well been used in chemotaxonomy, where they have provided a positive contribution in the reclassification of certain plants. The objectives of the study were to extraction and characterization of anthocyanin pigments from three species of *Indigofera* (Linn.) viz., From spectral value and compatibility of Rf value, eight spots were identified as anthocyanins from all three species of *Indigofera*. Delphinidin 3, 5 diglucoside and Cyanidin 3-(2G-glucosylrhamnosylglucoside) were found in all three species; Cyanidin 3-rhamnosylglucoside was found in *Indigofera enneaphylla* and *Indigofera linifolia*. Cyanidin 3-rhamnosylglucoside-5-glucoside and Pelargonidin 3-glucoside was detected in *Indigofera endecaphylla*, Pelargonidin 3,5-diglucoside in *Indigofera enneaphylla* and that of Peonidin 3,5-diglucoside & Cyanidin 3-glucoside in *Indigofera linifolia*.

KEY WORDS

Anthocyanin, *Indigofera*, Polyphenol,
Glycosides, Chemotaxonomy, Paired affinity.

Anthocyanins are responsible for the wide range of colors in the petals of flowering plants and they may vary the color from salmon- pink, through scarlet, magenta and violet to deep blue (Haslam, 1995). They are present in flower petals to attract pollinators and in fruits and seeds as attractants for seed dispersers (Willson and Whelen, 1990).

Chemically anthocyanin are B-glycosides of anthocyanidins. The anthocyanins have as well been used in chemotaxonomy, where they have provided a positive contribution in the reclassification of certain plants.

As anthocyanins are almost universal in flowering plants they are chemical compounds that are useful for systematic purposes both at the family and genus level. Systematic studies have been made using the distribution of anthocyanins present in the floral petals of many major plant groups including Poaceae (Harborne and Clifford, 1967); Umbelliferae (Harborne, 1976); Onagraceae (Harborne *et al.*, 1976); Commelinaceae (Harborne and Stirton, 1980); the Araceae (Harborne *et al.*, 1981); Lamiales (Harborne, 1992a); Leguminosae (Fabaceae) (Harborne *et al.*, 1970), and the Compositae (Asteraceae) (Harborne, 1996), and at genus level in Collomia (Harborne *et al.*, 1982) and Patersonia (Harborne *et al.*, 1989b).

For the present study three species of *Indigofera* (Linn.) were selected viz., *Indigofera endecaphylla* Jacq., *Indigofera enneaphylla* Linn. and *Indigofera linifolia* (L.f.) Retz.

Materials and Methods

Fresh petals were collected from all the population of three species of *Indigofera*. After air-drying anthocyanins were extracted from these petals at room temperature following procedures of Harborne, 1967. The chromatograms were developed into two solvent systems one by one, first in BAW (n-butanol-glacial acetic acid - water; 4:1:5) and then in 1% HCl (conc. HCl -water; 3:97).

Colour of spots were observed under ammonia fumes and NH₃+UV. The relative distance (Rf) of each spot was calculated. Anthocyanins and related compounds were identified by comparing the obtained Rf values and colours.

Observations

Table 01: Rf and spectral properties of Anthocyanins

Spot No.	Colour in NH ₃ Vapour	Rf (x 100) in solvents		Species			Spectral values max ()nm MeOH+HCl	Chemical Identity
		BA W	1% HCl	I	II	III		
1	Red	12	10	+	+	+	545	NI
2	Blue green	14	08	+	+	+	543	Delphinidin 3,5 diglucoside
3	Purple	25	38	+	-	-	539	Cyanidin 3-rhamnosylglucoside 5-glucoside
4	Light purple	26	60	+	+	+	537	Cyanidin 3-(2G glucosylrhamnosyl glucoside
5	Purple	30	17	-	-	+	534	Peonidin 3,5 diglucoside

6	Orange red	32	24	-	+	-	536	Pelargonidin 3,5 diglucoside
7	Magenta	35	18	-	+	+	535	Cyanidin 3-rhamnosylglucoside
8	Purple	38	08	-	-	+	530	Cyanidin 3-glucoside
9	Light red	45	14	+	-	-	520	Pelargonidin 3-glucoside
10	Yellow	61	-	-	-	+	527	NI
11	Light yellow	64	-	+	+	-	525	NI
12	Lemon yellow	95	-	+	+	-	515	NI

NI = Not identified.

I = *Indigofera endecaphylla*

II = *Indigofera enneaphylla*

III = *Indigofera linifolia*

Table 02: Paired affinity value (PA) of Anthocyanins

	<i>Indigofera endecaphylla</i>	<i>Indigofera enneaphylla</i>
<i>Indigofera endecaphylla</i>	-	-
<i>Indigofera enneaphylla</i>	71.42 %	-
<i>Indigofera linifolia</i>	42.85 %	57.14 %

Table 03: Group affinity, number of unique spots and isolation value of Anthocyanin compounds.

Species	GA	No. of unique spots	Isolation value (%)
<i>Indigofera endecaphylla</i>	214.27	2	16.66
<i>Indigofera enneaphylla</i>	228.56	1	8.33
<i>Indigofera linifolia</i>	199.99	3	25.0

Results and Discussion

Altogether 21 spots were obtained in all three species, out of which *Indigofera endecaphylla*, *Indigofera enneaphylla* and *Indigofera linifolia* each contained 7 spots. Among three species of *Indigofera* 12 type of spots were observed (Table).

Indigofera endecaphylla was characterized by two unique spots (spot no. 3 & 9). *Indigofera enneaphylla* had only one unique spot i.e spot no.6 and *Indigofera linifolia* was characterized by three spots (spot no. 5, 8 & 10). From spectral value and compatibility of Rf value, eight spots were identified as anthocyanins from all three species of *Indigofera*. Delphinidin 3, 5 diglucoside and Cyanidin 3-(2G-glucosylrhamnosylglucoside) were found in all three species ; Cyanidin 3-rhamnosylglucoside was found in *Indigofera enneaphylla* and *Indigofera linifolia*. Cyanidin 3-rhamnosylglucoside-5-glucoside and Pelargonidin 3-glucoside was detected in *Indigofera endecaphylla*, Pelargonidin 3,5-diglucoside in *Indigofera enneaphylla* and that of Peonidin 3,5-diglucoside & Cyanidin 3-glucoside in *Indigofera linifolia*.

On the basis of Anthocyanins study the paired affinity (PA) was highest between *Indigofera endecaphylla* and *Indigofera enneaphylla* (71.42%) ; intermediate between *Indigofera enneaphylla* and *Indigofera linifolia* (57.14%) and lowest between *Indigofera endecaphylla* and *Indigofera linifolia* (42.85%).

Group affinity (GA) values also showed the highest for *Indigofera enneaphylla* (228.56), intermediate for *Indigofera endecaphylla* (214.27) and lowest for *Indigofera linifolia* (199.99). The isolation value (IV) was found to be highest in *Indigofera linifolia* (25%), intermediate in *Indigofera endecaphylla* (16.66%) and lowest in *Indigofera enneaphylla* (8.33%).

From the study of anthocyanins it was clear that Cyanidin 3-rhamnosylglucoside-5-glucoside and Pelargonidin 3-glucoside was found only in *Indigofera endecaphylla*; Pelargonidin 3, 5-diglucoside in *Indigofera enneaphylla* and Peonidin 3,5-diglucoside & Cyanidin 3-glucoside was restricted to *Indigofera linifolia*. Similar types of anthocyanin were extracted from the flowers of *Mucuna sempervirens* (leguminosae) by Ishikura and Shibata in 1973. In which he detected glycosides of cyanidin, peonidin, delphinidin, petunidin and malvinidin.

CONCLUSION

The frequency of the glycosides of Cyanidin was maximum in all the chromatograms of studied species of *Indigofera*, whereas the glycosides of Delphinidin and Cyanidin were present as main pigment in all three species of *Indigofera*. The paired affinity was highest between *Indigofera endecaphylla* and *Indigofera enneaphylla*. In this way *Indigofera linifolia* showed its affinity both towards *Indigofera endecaphylla* and *Indigofera enneaphylla*. Group affinity value showed the high affinity of *Indigofera enneaphylla* towards both *Indigofera endecaphylla* and *Indigofera linifolia*. The patterns of unique spots were different in all three species proving the distinctness of the species.

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