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## TLC-Colourimetric Estimation of Free and Combined Forms of Chrysophanol, Emodin and Physcione in some *Cassia* species

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**Estimation of emodin, chrysophanol and physcione in free and combined form in some *Cassia* species namely *Cassia auriculata*, *Cassia fistula*, *Cassia javanica*, *Cassia roxburghii*, *Cassia siamea*, in the plant parts like bark, flower, leaf, pericarp, seed and stem (wood) has been carried out. The TLC-colorimetric method was employed for the estimation of the same. The total amount of free and combined forms of emodin, chrysophanol and physcione were found in maximum amount in *C. siamea* (1.01%) followed by *C. javanica* (0.80%), *C. fistula* (0.68%), *C. auriculata* (0.53%) and *C. roxburghii* (0.37%). The standards, chrysophanol, emodin and physcione were developed from Indian Rhubarb (*Rheum emodi*) by TLC-spectroscopic techniques.**

The genus *Cassia* belongs to family Leguminosae and subfamily Caesalpinaceae<sup>1</sup>, out of which 23 species are found in India<sup>2</sup>. All the species of *Cassia* contain anthraquinone glycosides as major chemical constituent and traces of tannins and flavonoids<sup>3</sup>. They are identified by Borntrager's test<sup>4</sup>. They are useful in the treatment of constipation and they also have antispasmodic and antiinflammatory action<sup>5</sup>.

Chrysophanol is 1,8-dihydroxy-3-methyl anthraquinone. It occurs in free and combined states in cascara, senna, and rhubarb. Emodin is 1,3,8-trihydroxy-6-methyl anthraquinone. It occurs in free and combined states in rhubarb, cascara rumex. Physcione is 1,8-dihydroxy-3-methoxy-6-methyl anthraquinone. Other anthraquinones present in free and combined states are aloe emodin and

rhein<sup>6</sup>.

Till date the qualitative estimation of anthraquinone glycosides on 23 *Cassia* species has been carried out but no systematic work has been undertaken to find out the exact amount of anthraquinone derivatives in each plant part of *Cassia* species. Therefore, the present work is carried out to see the quantitative distribution of emodin, chrysophanol and physcione within these five *Cassia* species.

The plant parts like bark, flower, leaf, pericarp, seed and stem-wood of *C. auriculata*, *C. fistula*, *C. javanica*, *C. roxburghii* and *C. siamea* have been collected when each and every part of the species got matured. The plant parts were then air dried, powdered and used for estimation.

A TLC-colourimetric method has been employed to estimate the same. The free anthraquinone derivatives produced pink red or violet colour with aqueous ammonia

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or caustic soda solution. There are also other methods for estimation of anthraquinones like flourimetry<sup>7-8</sup> and high performance liquid chromatography<sup>9</sup>.

The research materials were collected from the botanical garden present in the campus of Shri Bhagwan College of Pharmacy, N-6, Cidco, Aurangabad and authenticated in the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, as *C. auriculata*, *C. fistula*, *C. javanica*, *C. roxburghii* and *C. siamea*. They were then separated as bark, flower, leaf, pericarp, seed and stem (wood), were then dried, powdered and used for the estimation. Samples were prepared for the estimation of anthraquinones as outlined below<sup>10</sup>.

For estimation of free anthraquinones 1 g of accurately weighed, powdered either of plant part was taken. It was extracted by refluxing with 50 ml of chloroform for 4 h and was then filtered. The filtrate was then concentrated to about 1 ml and used for determination of free emodin, chrysophanol and physcione. This extract was designated as E-1. The marc left after filtration was used in the next step, for determination of combined-O-form anthraquinones. The marc was heated with 50 ml of dilute sulphuric acid for 4 h and then filtered. The filtrate was extracted two times with 50 ml of chloroform and the combined chloroform extracts concentrated to about 1 ml. The concentrate (designated E-2) was used for determination of free emodin, chrysophanol and physcione, which were present in combined-O-form. The marc left after this step was used for determination of combined-C-form of anthraquinones. The marc was heated with 50 ml of dilute sulphuric acid and 5 g of ferric chloride for 4 h and then filtered. The filtrate was extracted two times with 50 ml of chloroform and the combined chloroform extracts concentrated to about 1 ml. The concentrate (designated E-3) was used for determination of free emodin, chrysophanol and physcione, which were present in combined-C-form.

TLC plates (20 cmx20 cm) were prepared using silica gel-G (35 g of silica gel-G was mixed with 80 ml of water). A mixture of petroleum ether (60 to 80°), ethyl acetate and formic acid (75:25:01) was used as mobile phase<sup>11</sup>.

The 1 ml of chloroform concentrates (E-1, E-2 and E-3) of either of the plant part of either of the species were subjected to thin layer chromatography. Alongwith the row of spots, one spot of each authentic sample (emodin, chrysophanol and physcione) was also spotted with the help of capillary. The solvent was allowed to run up to 15-16 cm from the application of spots. The plates were removed

from the chamber and air dried. The all three compounds, emodin, chrysophanol and physcione were coloured and had shown Rf values as emodin, (0.6) chrysophanol (0.4) and physcione (0.7). All corresponding bands were carefully scraped out with the help of sharp blades and collected individually in separate centrifuge tube.

The rhizomes of Indian Rhubarb (*R. emodi* Family Polygonaceae) commonly known as Revandchini were collected from Shabbar Dawasaz, Pan Dariba market, Aurangabad and authenticated in the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Then pure emodin, chrysophanol and physcione were isolated from *R. emodi* in our laboratory and homogeneity of the compounds was confirmed by spectral studies (UV, IR, <sup>1</sup>HNMR and Mass). The stock solutions of chrysophanol, emodin and physcione separately were prepared by dissolving 10 mg each of the anthraquinone in aqueous potassium hydroxide solution in three separate volumetric flasks. From the stock solutions 0.2, 0.4, 0.6, 0.8 and 1.0 ml were transferred to clean dry tube. Then 9.8, 9.6, 9.4, 9.2 and 9.0 ml of distilled water were added to give working dilutions of 20, 40, 60, 80 and 100 µg per 10 ml. The working dilutions were set aside for 15 minutes and their optical density were measured on colorimeter at 480 nm against 1N aqueous potassium hydroxide solution as a blank. The standard curves were prepared by plotting optical density against concentration in µg per 10 ml. The calibration curves for chrysophanol; emodin and physcione were found to obey Beer's law. The compounds, emodin, chrysophanol and physcione, embedded in silica gel G, were extracted with chloroform. The extracts were collected in different petri dishes and evaporated to dryness. Then 10 ml of 1 N aqueous potassium hydroxide solution was added and thoroughly stirred for 10 minutes. The optical densities of all the compounds were measured on colourimeter at 480 nm against 1N aqueous potassium hydroxide solution as a blank. Then the concentrations of all the compounds were calculated. (Table 1)

It was found that the total amount of free and combined forms of emodin, chrysophanol and physcione were found in maximum amount in *C. siamea* (1.01%) followed by *C. javanica* (0.80%), *C. fistula* (0.68%), *C. auriculata* (0.53%) and *C. roxburghii* (0.37%). (Table 2)

The quantitative distribution of anthraquinone glycosides in five local *Cassia* species shows that the plant parts like bark, flower, leaf, seed, pericarp and stem (wood)

of *C. auriculata*, *C. fistula*, *C. javanica*, *C. roxburghii*, *C. siamea* contain chrysophanol, emodin and physcione in considerable amount.

It has been observed that *C. auriculata* contains total free chrysophanol (0.10%), free emodin (0.14%), free physcione (0.23%) and O-glycosidic physcione (0.06%). *C. fistula* contains total free chrysophanol (0.28%), free physcione (0.26%) and O-glycosidic physcione (0.15%). *C. javanica* contains total free chrysophanol (0.36%), O-glycosidic chrysophanol (0.04%), free emodin (0.05%), O-glycosidic emodin (0.08%), free physcione (0.14%) and O-glycosidic physcione (0.14%). *C. roxburghii* contains total free chrysophanol (0.10%), free emodin (0.09%), O-glycosidic emodin (0.07%), free physcione (0.10%) and O-glycosidic physcione (0.02%). While *C. siamea* contains total free chrysophanol (0.71%), free emodin (0.05%), free physcione (0.23%). (Table 2)

It is found that not a single plant part from either of the *Cassia* species contains chrysophanol, emodin and physcione in their C-glycosidic form. The finding of the higher percentage of chrysophanol, emodin and physcione in *C. siamea* (1.01%) reflects that *C. siamea* is very important as far as the chemotaxonomical point of view is concerned. It is suggested that along with chrysophanol, emodin and physcione quantitative estimation of aloe emodin and rhein should be carried out in all the plant parts including root of every *Cassia* species. This will help a phytochemist, exactly, to draw the chemotaxonomical significance of anthraquinone glycosides in local *Cassia* species.

It is also suggested that further along with these five *Cassia* species, quantitative estimation of aloe emodin, chrysophanol, emodin, physcione and rhein including roots of *C. angustifolia*, should again be carried out because *C.*

TABLE 1: QUANTITATIVE DISTRIBUTION OF ANTHRAQUINONE GLYCOSIDES IN FIVE CASSIA SPECIES PRESENT IN AURANGABAD REGION

Plant Part	Form	<i>C. auriculata</i>			<i>C. fistula</i>			<i>C. javanica</i>			<i>C. roxburghii</i>			<i>C. siamea</i>		
		Cr	E	P	Cr	E	P	Cr	E	P	Cr	E	P	Cr	E	P
Bark	F			68				35		28		20		310		126
	C									32						
Flower	F	35	45		108		54					35	62		38	
	C						51					22				
Leaf	F		63	64	121		75	121		54			96		44	
	C						51			103						
Fruit* (Pericarp)	F			61			79	114			52	45	61	84		
	C										45					
Seed	F		36				51	52	51			20		80	51	47
	C						47		84	46	20					
Stem (Wood)	F	62		32	46			40		54				73		
	C			62				36								
Total	F	97	144	225	275		259	362	51	136	98	85	96	705	51	225
	C			62			149	36	84	135		65	22			
Grand Total			528			683		804		366		1011				

The abbreviations indicate Cr-Chrysophanol, E-Emodin, P- Physcione, F-Free and C-Combined.

The numbers indicate concentration of the compounds Chrysophanol, Emodin and Physcione in µg/10 ml.

TABLE 2: PERCENTAGE OF FREE AND COMBINED FORMS OF CHRYSOPHANOL, EMODIN AND PHYSCIONE IN SOME CASSIA SPECIES.

Species	Form	Chrysophanol (%)	Emodin (%)	Physcione (%)	F and C Total (%)
<i>C. auriculata</i>	F	0.097	0.144	0.225	0.528
	C			0.062	
<i>C. fistula</i>	F	0.275		0.259	0.683
	C			0.149	
<i>C. javanica</i>	F	0.362	0.051	0.136	0.804
	C	0.036	0.084	0.135	
<i>C. roxburghii</i>	F	0.098	0.085	0.096	0.366
	C		0.065	0.022	
<i>C. siamea</i>	F	0.705	0.051	0.225	1.011
	C				

The abbreviations indicate F-Free and C-Combined.

*angustifolia* is used widely in several herbal preparations. This type of study will help a phytochemist evaluate the comparative distribution of these phytoconstituents.

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