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Seasonal variation in the content of sennosides and rhein in leaves and pods of *Cassia fistula*

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The seasonal variations of sennoside and rhein content (both glycosidic and non-glycosidic) in leaves and pods of *Cassia fistula* grown in Calcutta, India was studied. In the leaves, both these anthraquinone glycosides remain high in the mature and old leaves in the months from January to April when the contents of mature pods are low. In the developing green pods the content is high compared to the older ones, while the young leaves have lower glycosidal content compared to their mature stage. In general, leaves are a richer source of these glycosides.

BOTH the leaves and the pods of *C. fistula* L. are traditionally used as mild laxative[1,2]. They contain sennoside A, B, rhein and its glycoside[2,3]. Sennosides have laxative activity[4]. Rhein is used in arthritis[5]. Seasonal variations in the content of sennosides in the leaves and the pods of the two *C. fistula* population of Mexico have been reported by Cano Asseleih *et al*[6]. In the present work, leaves and pods of Indian *C. fistula* were analysed to study the seasonal variations of sennosides and rhein (free and glycosidic form) content.

MATERIALS AND METHODS

The leaves and pods were collected in the first week of every month from Calcutta during January to December, 1993. Voucher specimens (Nos. CfL 1-12 and CfP 1-12) were deposited in the Department of Botany, University of Calcutta. Dried leaves and pods (without seeds) were extracted following the method of Habib and Sebakhy[7] for quantitative determination of sennoside and glycosidic rhein. Rhein present in non-glycosidic form was determined by HPLC from the ether extract before hydrolysis.

HPLC analysis : The HPLC system consisted of a varian 4400 integrator, varian 9050 variable wavelength uv-vis detector, varian 9010 solvent delivery system, Bondesil CN 5 µm 4.6 mm (I.D.) X25 cm column. Mobile phase was methanol and water 9:1. The flow rate was 0.5 ml/min. The eluant was monitored at 260 nm. The chart recorder was set at 0.5 cm/min. speed.

Different concentrations of rhein solutions (6 µg/ml to 14.15 µg/ml), prepared from the stock solution prepared dissolving 1 mg of sample in 4 ml of acetonitrile, were injected. Concentration of rhein in leaves and pods present in free form were calculated from the regression equation Y=106.8 X -2.85 (correlation coefficient-0.99) prepared from the peak height (y) and concentration (x) of the authentic anthraquinone.

RESULTS AND DISCUSSION

The leaves showed seasonal variation of sennoside and rhein content (Table 1). The percent sennoside content varied from 0.05 ± 0.003 to 1.23 ± 0.01, the percent glycosidic rhein content varied from 0 to 0.905 ± 0.005 (not detectable in the month of June) and percent non-glycosidic rhein content varied from 0 to 0.236 ± 0.001 (not detectable in the months of February, March and June). High sennoside content was detected in the months of

Table 1 : Seasonal variation in percentage of sennoside and rhein content

Months	LEAF			POD		
	Sennoside	Rhein glycoside	Rhein	Sennoside	Rhein glycoside	Rhein
January	1.16±0.01	0.90±0.005	0.14±0	0.25±0	0.04±0.01	0.003±0
February	0.61±0.02	0.43±0	-	0.21±0.007	0.02±0.005	0.04±0
March	1.23±0.01	0.88±0	-	0.20±0.005	-	-
April	1.10±0.03	0.71±0.005	0.24±0	0.15±0	0.004±0	0.05±0
May	0.14±0	0.09±0.02	0.16±0.01	0.10±0.005	0.02±0	-
June	0.33±0.01	-	-	0.09±0.01	0.03±0	-
July	0.70±0.01	0.40±0.01	0.06±0			
August	0.48±0.007	0.37±0.005	0.12±0.004			
September	0.06±0	0.07±0	0.05±0	0.30±0.01	0.15±0.005	-
October	0.39±0	0.11±0	0.17±0	0.37±0.01	0.17±0.01	-
November	0.45±0.02	0.15±0.08	0.08±0	0.34±0.01	0.15±0	-
December	0.70±0.008	0.37±0	0.03±0	0.33±0	0.14±0	-

Results expressed as mean (percentage dry weight) ±standard deviation; n=2

January, March and April (content was found to be decreased slightly in February) when the leaves are fully mature and gradually fall from the tree. In the young and full sized leaves that are found in the month of May, sennoside content was low. This anthraquinone glycoside then increased in quantity (except being very low in the month of September).

The present observations in the Indian *C. Fistula* plant differ from those reported by Cano Asseleih *et al.*[6] in the Mexican population. The peak sennoside content in leaves of Mexican plants was observed in June when new leaves appeared, whereas, in the Indian plant, the sennoside content was high in the mature leaves at the time of leaf-fall and low in the new leaves.

The glycosidic rhein content was highest in the month of January and showed similar pattern of seasonal variation to that of sennoside. Rhein was present in free form in the leaves at a very low concentration, which showed no remarkable seasonal variation.

The pods appeared in the plant at the end of July and showed seasonal variation of sennoside and rhein content (Table 1). Percent sennoside content varied from 0.08±0.01 to 0.37±0.008. Percent glycosidic rhein content varied from 0 to 0.17±0.008. Highest glycosidic rhein content was observed in full sized green and unripe pods in the month of October when the sennoside content was also highest. The content of both these anthraquinone glycosides then gradually decreased with ripening of the pods. The non-glycosidic rhein did not show any major variation in content.

Our observations with the Indian *C. fistula* plant differ from the observations made by Cano Asseleih *et al.*[6] with the Mexican plant where sennoside content is reported to be low in green pods and high in pale brown pods at the midstage of fruit maturation. However, they also have reported decrease in sennoside content with maturation of fruit. But our observations show similarity with the observation of Fairbairn and Shrestha[8] in *Cassia senna* L. where high content of glycosides (calculated as sennosides) was observed in pale pods in contrast to dark pods.

The amount and composition of classes of compounds are governed by geographical distribution of plants and its habitat (an ecological component). Geographical and ecological variation may be the result of the plasticity of individual genotypes (modifications) or of a genetic heterogeneity of plant taxa[9]. Different cytotypes have been recognised in *C. fistula* collected from different localities in India (Dutta and De-unpublished). Differences in the pattern of seasonal variations in sennoside content in *C. fistula* grown in India and Mexico might be due to one of the above mentioned factors.

The leaves and pods showed opposite pattern of variation in sennosides and rhein content. In the developing green pods the content was high when the leaves had lower glycosidal content. This may be due to translocation of the glycosides from the leaves to the developing pods as has been presumed in *C. fistula* by Cano Asseleih *et al.*[6]. Decrease in content in the mature pods might be due to conversion of these glycosides to some other anthraquinone or non-anthraquinone compounds.

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