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Microscopic evaluation and seasonal variations of anthraquinone glycosides of cultivated *Cassia fistula* Linn.

K A Abo, A A Adeyemi*, and A O Sobowale+

Department of Pharmacognosy, Faculty of Pharmacy, College of Medicine, University of Ibadan, Ibadan, Nigeria.
**Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo College of Health Sciences, Ogun State University, Sagamu, Nigeria. + Department of Biological Sciences, Ogun State University, Ago-Iwoye, Nigeria.*

Summary

In this report, we present the results of the microscopy, seasonal variations and spectrophotometric estimation of hydroxy-anthraquinone glycosides of culitivated *Cassia fistula* Linn. The total glycoside contents of the morphological parts of this species at different stages of growth are also presented. The study shows that anthraquinone glycosides are concentrated in the leaves (1.75%) and flowers (1.58%) at peak flowering. Notable seasonal variations were observed in the cultivated species. Hydroxyanthraquinones reached peak levels druing the months of September (1.08%) and October (2.20%). There was a significant drop ($P < 0.05$) in glycoside content during most part of the rainy season. It has been established that anthraquinone glycosides, rather than the aglycones, are the active forms and hence an estimation of the total glycoside content of a sample is a reliable indication of biological activity. The advantages of using the natural anthraquinone laxative when the glycoside content is highest are discussed. The study has provided useful information on the best period for harvesting the morphological parts of *C. fistula* for drug development.

Keywords: *Cassia fistula, microscopy, anthraquinone glycosides, seasonal variations.*

Résumé

Dans ce rapport, nous presentons les resultats microscopiques les variations saisoniéres et l'estimation spectrophotometrique dé glycosides d'hydroxy-anthraquinone dans la culture de Cassia fistula Linn. La totalite de glycosides de la partie morphologique de ces especes a' des stages differents de croissance est aussi representées. Cette etude montre que les glycosides d'anthraquinone sont concentrées dans les feuilles (1,75%) et les fleurs (1,58%) au plus haut point de floraison. Les variations saisoniéres avaient ete observees dans les especes cultivees. Les hydroxyanthraquinones atteing-naient le n'veau le plus eleve pendant les mois de Septembre (1, 08%) et Octobre (2, 20%). Il y avait une diminution significative ($P <$ 0, 05) en glycoside pendant la majeur partie de la saicison pluvieuse. Il a ete etabli que les glycosides d'anthraqui'none au lieu des aglycones zont les formes actives et parconsequent une estimation du raux de glycoside dans un speciment est une indication significative de l'activite biologique. Les avantages d'utulisation de laxatif d'anthraquinone naturel lorsque la contenance de glycoside est trés elévée sont discutés. Cette etude a permi de connaitre la meillaire periode pour ceuillir les parties morphologiques de *C. fistula* pour le development des medicaments.

Correspondence: Dr. K A Abo, Department of Pharmacognosy Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria.

Introduction

Cassia fistula Linn. (Leguminosae-Caesal pinioideae) is one of the 33 *Cassia* species growing in Nigeria [1]. It is used in traditional medicine for the treatment of venereal and skin diseases and as a purgative [2,3]. This species was introduced into Nigeria from India where it is widely used as a tonic, astringent, and for the treatment of rheumatism, snake bite and fever [4,5].

C. fistula growing in India has been reported to contain sennosides, rhein [6,7], 7-methylphyscion [8] and rhamnetin-3-0-gentiobioside [9] which are established hydroxyanthraquinones derivatives. The seeds of this species have been reported to exhibit antidiabetic properties [8]. Furthermore, the hexane fraction of the fruits has been shown to exhibit reasonable antibacterial activity against *Klebsiella* species [10].

Recent studies in our laboratory show that the Nigerian variety contain appreciable quantities of anthraquinone derivatives and it exhibits potent dose related laxative activity in rats. We have also previously reported the antimicrobial potential of leaves and pods of *C. fistula* (growing in Ibadan) on *A. flavus, A. niger, C. albicans, S. aureus, E. coli, F. oxyporum, Ps. aeruginosa and P. mirabilis* [11].

In previous studies, we have noticed significant variations in the hydroxyanthraquinone content of various *Cassia* species collected around Nigeria [11-14]. There are also close morphological similarities between *C. fistula* and some indigenous *Cassia* species.

For these reasons, we report for the first time results of seasonal accummulation of hydroxyanthraquinones in leaves of cultivated *C. fistula*. The total anthraquinone content of the morphological parts of this species at difference stages of growth is presented. This communication also describes some characteristic microscopic features of the leaf because of its close similarity with some indigenous *Cassia* species which may not be as medicinally useful as *C. fistula* Linn.

Materials and methods

Plant material: Morphological parts of *Cassia fistula* Linn. were collected between February 1997 and April 1998 from a cultivated species in Ibadan and authenticated at the Forestry Research Institute of Nigeria, Ibadan, where a herbarium specimen (Voucher No. FHI 102266) is kept. Fresh organs were collected in the mornings and for seasonal variation studies, leaves were harvested weekly over a period of one

year. Samples were dried at 45°C, powdered and stored in sealed amber glass containers until ready for analysis. Five replicate determinations were performed on each batch.

Identification test: The presence of hydroxyanthraquinone derivatives in various batches was ascertained following extraction into chloroform and detection by the Borntrager reaction as previously described [15].

Microscopy: Surface (epidermal) preparations and transverse section of leaf were prepared for microscopic examination as described in the African Pharmacopoeia [16]. The powdered sample and specimens were cleared over a bunsen flame or bleached by soaking in chloral hydrate solution B.P. for 2-4 days. These were subsequently stained with phloroglucinol/ conc. HCL, mounted in glycerol and examined under the Olympus binocular research microscope. The result are shown in Figures 1-3.

Determination of water soluble extractive value: Water soluble extractive values were determined on 4.0gm of the air-dried powdered sample as described in the African pharmacopoeia (16).

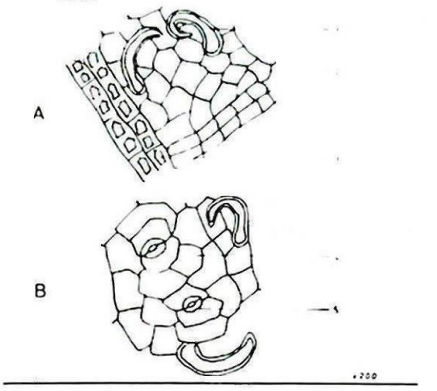
Spectrophotometricestimationof hydroxyanthraquinone content: Standard procedure for the determination of hydroxyanthraquinone derivatives of *Cassia* species was adopted [11,17,18]. 500mg of defatted powdered sample of *C. fistula* was refluxed with 50ml of 20% methanol for 15 min. The extract was filtered and made up to 50ml with 20% methanol. About 10ml was taken and further refluxed with 1ml of concentrated hydrochloric acid and 2ml of 20% (w/v) ferric chloride solution for 15min. The cooled reaction mixture was partitioned into chloroform (2 x 30ml), made to 100ml with fresh chloroform and dried over anhydrous sodium sulphate. 10ml of the diluted extracted was evaporated to dryness, cooled and reconstituted to 10ml with 1M potassium hydroxide solution. The absorbance of the solution was immediately measured at 500mm in a 1cm cell using a Gallenkamp SPR-500 UV spectrophotometer. The assay was verified by comparison with data obtained from a linear calibration curve derived from dilution of 1,8-dihyroxyanthraquinone (Sandoz, London).

Results and discussion

Cassia fistula Linn. Is a moderately sized spreading deciduous ornamental tree. It possess compound leaves with four to five pairs of leaflets arranged in opposite direction. The leaves are paripinnate, glossy and coriaceous. The flowers are yellow and cone-shaped and it gives the tree a very beautiful look during flowering season. The smooth black cylindrical pods are sometimes 30 inches long with numerous one-seeded cells,

The epidermal characters of *C. fistula* is shown in figure 1. The upper and lower epidermal cells are polygonal with straight anticlinal walls showing numerous unicellular, conical and appressed covering trichomes. Paracytic stomata were found only on the lower epidermis. Calcium oxalate prisms were evident around the vascular bundles.

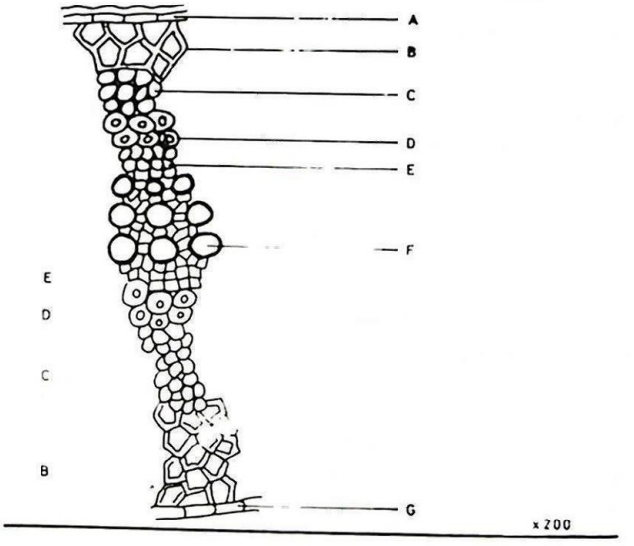
Fig 1 Epidermal characters of Cassia fistula leaf



A = Upper surface view of leaf, B = Lower surface view of leaf; 1 = Unicellular trichome, 2 = Epidermal cells (Upper), 3 = Cal ox-prisms, 4 = Epidermal cells (Lower 5 = Paracytic stoma

The transverse section of the leaf through the mid-rib is presented in Figure 2. It shows a smooth cuticle below which is a single layer of epidermal cells. Below the epidermis were layers of thick-walled angular collenchyma cells followed by numerous layers of smaller parenchyma cells. The vascular bundles were surrounded by rows of thick-walled pericyclic fibers. The lignifiied xylem vessels occur in straight rows .

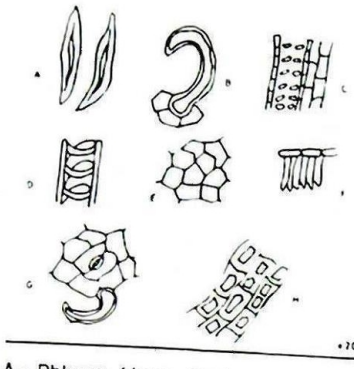
Fig. 2: Transverse section through the mid-rib of the leaf of Cassia fistula



A = Upper epidermis; B = Collenchyma; C = Parenchyma; D = Pericyclic fibres; E = Phloem; F = Lignified xylem vessel, G = Lower epidermis

The powdered leaf sample was characterized by fragments of straight-walled epidermal cells sometimes showing unicellular appressed trichomes as seen on the leaf surface preparations; xylem cells of the spiral and reticulate types; fragments of palisade mesophyll; lignfied phloem fibers and prisms of calcium oxalate sheath occurring around fibers.

Fig 3. Microscopic features of powdered Cassia fistula leaves



A = Phloem fibres, B = Appressed trichome, C = Phloem parenchyma with reticulate xylem; D = Spiral xylem, E = Upper epidermis, F=Fragments of palisade mesophyll, G=Lower epidermis, H = Cal ox (prisms) sheath

The pink to cherry red colour observed in the Bontrager test confirmed presence of hydroxyanthraquinone derivatives in various leaf samples and other morphological parts of the cultivated species.

Table 1: Total hydroxyanthraquinone content of morphological parts of *Cassia fistula* at different stages of growth

Period of harvest	Total hydroxyanthraquinone content* (% w/w)			
	Leaf	Flower	Bark	Pod
Before flowering	0.85±0.68	NA	0.22±0.52	NA
Peak flowering	1.75±0.72	1.58±0.34	0.15±0.06	NA
Peak fruiting	0.46±0.24	NA	0.19± 0.62	0.78 ± 0.09
Fall of leaves	Tr	1.07 ±0.42	0.23±0.06	0.45±0.16

*Values are mean ± S.D.; n = 5; NA = Not available; *Calculated as 1,8-dihydroxyanthraquinone; Tr = Traces.*

Table 1 shows the total anthraquinone content of the morphological parts of *C. fistula* at different stages of growth. It shows that anthraquinone glycosides are concentrated in the leaves (1.75%) and flowers (1.58%) at peak flowering. At peak fruiting, there was a noticeable drop of anthraquinones in the leaf (0.46%). At the fall of leaves, anthraquinones predominate in the flower. The bark recorded lowest content throughout the period of study.

Table 2 shows monthly variations of water soluble extractive values and seasonal distribution of hydroxyanthraquinone glycosides in the leaf. The figure of each month is the mean (± S.D.) of the appropriate weekly determinations. Anthraquinones reached peak levels during the dry season (September-February) in Ibadan, the maximum content being recorded in September (1.08%) and October (2.20%). There was significant drop ($P < 0.05$) in glycoside content at the onset of the rainy season and the level remained low during most part of the season (Table2). This finding is consistent with earlier reports that rains may considerably lower accummulation of some bioactive secondary plant metabolites [19-21].

Table 2: Seasonal distribtuion of total leaf hydroxyanthraquinones of cultivated *Cassia fistula Linn.*

Mth of Harvest	Av. Rainfall (mm/day)	Water-soluble Extractive value	ID Test*	Total Anthraquinone Glycoside Content (% w/w)
January	0.0	25.0 ± 0.21	+++	0.50 ± 0.08
February	0.8	20.6 ± 0.00	++	0.33 ± 0.11
March	1.1	35.8 ± 0.32	+	0.17 ± 0.37
April	1.3	30.2 ± 0.05	+	0.21 ± 0.01
May	3.1	30.4 ± 0.00	+	0.29 ± 0.04
June	11.6	32.8 ± 0.17	+	0.13 ± 0.00
July	3.4	24.5 ± 0.00	+	0.10 ± 0.09
August	3.8	28.2 ± 1.46	++	0.46 ± 0.15
September	5.4	15.0 ± 0.28	+++	1.08 ± 0.14
October	5.3	20.1 ± 1.08	+++	2.20 ± 0.03
November	1.8	15.3 ± 0.33	+++	0.71 ± 0.16
December	0.0	15.5 ± 0.25	++	0.42 ± 0.07

*Values are mean ± (S.D.; n=5 + = Faint pink, ++ = Rose pink, +++ = Chery red; * Calculated as 1,8-dihydroxyanthraquinone.*

It has been established that hydroxyanthraquinone glycosides, rather than the aglycones, are the active forms and hence an estimation of the total hydroxyanthraquinone glycoside content of a sample is a reliable indication of biological activity [22]. According to Fairbairn and Moss [23], the activity of the whole leaf and pods of *Cassia acutifolia* Delile (Senna) is higher than could be predicted from the percentage of pure sennosides and that the therapeutic use of the whole Senna products in standardised in form is the most physiological and rational stimulant of the disordered bowel. It has also been shown that the crude extracts of Senna are more potent laxatives than the pure sennosides and it was concluded that the crude extracts contain some unknown compounds more potent or synergistic with the active constituents [24]. These pieces of evidence rightly support our recommendation for the use of simple aqueous crude extracts (as practiced by herbalists) of appropriate morphological parts of *C. fistula* as safe laxatives.

The advantages of using the natural anthraquinone laxatives when the glycoside content is highest include faster onset of action *in situ*, reduced toxicity [25] and the overall shorter stay in the body [24]. The glycosides exert laxative activity direct on the colon by irritation and retention of water and electrolytes [24,26].

This study has provided useful information on seasonal accummulation of anthraquinone glycosides and the best period for harvesting the morphological parts of *C. fistula* for drug development in Nigeria.

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