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Short report

Seasonal accumulation of anthraquinones in leaves of cultivated *Cassia podocarpa* Guill *et* Perr

KA Abo and AA Adeyemi*

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

Summary

Seasonal variations and spectrophotometric determination of anthraquinones in cultivated *Cassia podocarpa* are presented. The study shows that combined anthraquinones are concentrated in the leaves at peak flowering (2.43%) and lowest in the bark (0.21%). Anthraquinone glycosides reached peak levels during the months of October to March (dry season), the maximum being recorded during January to March. There was significant drop in glycosidic content during the period April to September (rainy season). There was slight increase in concentration of aglycones during the rainy season which may be due to inter-conversion of some glycosides to the aglycones. However, the free aglycone content is much lower than the glycosides. This is desirable for optimum laxative activity and reduced toxicity. This study is significant because it provides useful information on the seasonal distribution of anthraquinones and the best period for harvesting leaves of *C. podocarpa* for drug development. The inclusion of *C. podocarpa* in the African Pharmacopoeia will, no doubt, enhance its commercialization as laxative and for its antimicrobial effect.

Keywords: *Cassia podocarpa, anthraquinones, seasonal variations*

Résumé

Les variations saisonnières et la détermination spectrophotométrique d'anthraquinneoes dans *Cassia podacarpa* cultivé ont été introduites. L'étude montre que la combination d'anthraquinones est concentrée dans les feuilles lors de la floraison maximum (2,43%) et le plus bas dans l'écorce (0,21%). Les glycosides d'antraquinone ont atteint leur niveaux maximum entre les mois d'octobre à mars (en saison sèche), le maximum a été enregistré de janvier à mars. Il y avait une baisse significative dans le contenu glycosidique pendant la periode d'avril à septembre (saison de pluvieuse). Il y avait une légère augumentation dans la concentration de l'aglycone pendant la saison pluvieuse qui pourrait être dû a l'inter-conversion de quelques glycosides aux aglycones. Pourtant, le libre contenu aglycone est bien plus bas que les glycosides. Ceci est souhaitable pour une activité de laxative optimale et une réduction en toxicité. Cette étude est très importante car, elle fournit des informations utiles sur la distribution saissonnière d'anthraquinones et la meilleur période de moissonner les feuilles de *C.podocarpa* pour la fabrication de médicament. L'addition de *C.podocarpa* dans le pharmacopée encouragera sans doute sa commercialisation comme laxatif et pour son effet anti-microbien.

Introduction

Cassia podocarpa Guill and Perr (family Leguminosae-Caesalpinoideae) is one of the 33 *Cassia* species growing in Nigeria [1]. It is known as "Asunwon" in Yoruba and is used in traditional medicine for the treatment of skin diseases, gonorrhoea, sores and as an arbotifacient [2,3].

Previous investigation has shown that *C. podocarpa* contains appreciable quantities of hydroxyanthraquinone derivatives such as sennosides, rhein, physcione and aloe-emodin [4,5] in common with Senna (*Cassia acutifolia*, official natural anthraquinone laxative) which is not found in Nigeria, but commercially available as "Herb Tea". Consequently, *C. podocarpa* has been recommended for chemical and biological standardization for use as a substitute for Senna, as vegetable laxative and for its antimicrobial activity [6–8].

We have noticed variations in hydroxyanthraquinone content of many wild *Cassia* species collected around Ibadan. For this reason, we report for the first time results of seasonal accumulation of anthraquinones and alcohol soluble extractives of *Cassia podocarpa* cultivated under standardized condition since this species is of interest for drug development in Nigeria.

Materials and methods

Plant material. *Cassia podocarpa* Guill and Perr was raised from wild seeds. These were grown 4ft apart on loose soil of about 0.64% total nitrogen and maintained with abundant farmyard manure. The species was authenticated at the Forestry Research Institute of Nigeria, Ibadan (FRIN) where herbarium specimen is kept. Fresh morphological parts were collected in the mornings and for seasonal 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.

Quantitative micro-chemical test. The free and combined anthraquinones in various leaf batches were extracted into chloroform and detected by the Borntrager reaction as previously described [9]. The result is shown in Table 1.

Determination of ethanol soluble extractive value. Ethanol soluble extractive values were determined on 4.00 gm of the air-dried powdered sample as described in the African Pharmacopoeia [10].

Quantitative assay for total anthraquinones. Standard procedure for the determination of total anthraquinone content (aglycones and glycosides) of *Cassia* species was adopted (8,11). 500mg of defatted.powdered sample of *C. podocarpa* was refluxed with 50ml of 20% methanol for 15 min. The extract was filtered and made up to 50ml with 20% methanol. 10ml was taken and further refluxed with 1ml of concentrated hydrochloric acid and 2ml of 20% (w/v) ferric chloride solution for 15 min. The cooled reaction mixture was partitioned into chloroform (2 x 30 ml) and the pooled organic phase made to 100 ml with fresh chloroform and dried over anhydrous sodium sulphate. Aliquots

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

(10ml) of the diluted extract were evaporated to dryness, cooled and reconstituted to 10ml with IM potassium hydroxide solution. The absorbance of the solution was immediately measured at 500nm 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 dilutions of 1,8-dihydroxyanthraquinone (Sandoz, London).

Results and discussion

The cultivated species, after 24 months of growth, was an erect, glaborous shrub about 11ft high. The leaflets are simple, petiolated and membraneous. The flowers are yellow on a dense raceme.

The pink colour observed in the Borntrager test confirmed presence of hydroxyanthraquinone derivatives (aglycones and glycosides) in the leaf batches of *C. podocarpa* (Table 1). Table 2 shows the total combined anthraquinone content of various morphological parts of the cultivated species. It shows that combined anthraquinones are concentrated in the leaves at peak flowering (2.43%) and lowest in the bark (0.21%).

Table 1: Quantitative reactions of leaves of *Cassia podocarpa* to Borntrager test

Months of harvest	Colour reactions of harvested leaves to the identification test*	
	Aglycones	Glycosides
January	+	+++
February	++	+++
March	+	+++
April	+	++
May	++	+
June	+++	+
July	+++	+
August	++	+
September	++	+
October	+	+++
November	+	++
December	+	++

* *+= Faint Pink; ++ = Rose Pink; +++ = Cherry red.*

Table 2: Total anthraquinone content of organs of cultivated *Cassia podocarpa*

Plant organ	Period of harvest	Total anthraquinone content (% w/w)*
Leaf	Peak flowering	2.43 ± 0.34
Flower	Peak flowering	1.17 ± 0.41
Pod	Maturity (before fruit dehiscence)	0.53 ± 0.19
Bark	Peak fruiting	0.21 ± 0.16

Value are mean ± S.D., n = 5
**Calculated as 1, 8 - dihydroxyanthraquinone*

Table 3 shows monthly variation of ethanol soluble extractive values and seasonal distribution of anthraquinones in the leaves. The figure for each month is the mean (± S.D.) of the appropriate weekly determination. Anthraquinones glycosides reached peak levels during the months of October to March (dry season), the maximum being recorded during January to March. The was significant drop in glycosidic content during the months of April to September (rainy season). A similar trend was recorded for the ethanol soluble extractives. These findings are consistent with earlier reports which suggested that rains may considerably lower the accumulation of some biologically active secondary metabolites in medicinal plant tissues [12–15].

Table 3: Seasonal accumulation of total leaf anthraquinones in cultivated *Cassia podocarpa*

Month of harvest	Av. Rainfall (mm/day)	EtOH sol Ext. Value+	Total anthraquinone content*	
			% Aglycones	%Glycosides*
January	0.0	20.0 ± 0.12	0.09 ± 0.71	2.29 ± 0.31
February	0.8	13.9 ± 0.16	0.21 ± 0.32	2.07 ± 0.17
March	1.1	20.3 ± 0.26	0.08 ± 0.19	2.05 ± 0.08
April	1.3	7.4 ± 0.23	0.13 ± 0.53	1.31 ± 0.19
May	3.2	10.9 ± 0.31	0.29 ± 0.12	0.47 ± 0.00
June	11.6	10.3 ± 0.16	0.33 ± 0.46	1.03 ± 0.42
July	3.4	10.1 ± 0.16	0.31 ± 0.71	0.95 ± 0.36
August	3.8	12.1 ± 0.14	0.28 ± 0.00	0.74 ± 0.12
September	5.4	13.6 ± 0.45	0.21 ± 0.17	0.89 ± 0.00
October	5.3	16.1 ± 0.28	0.07 ± 0.27	1.83 ± 0.38
November	1.8	17.2 ± 0.16	0.04 ± 0.18	1.79 ± 0.41
December	0.0	13.2 ± 0.29	0.06 ± 0.48	1.50 ± 0.00

Values are mean ± S.D.: n = 5; +Ethanol soluble extractive value
**%w/w; calculated as 1, 8-dihydroxyanthroquinone.*

On the contrary, the aglycone content was lowest in the dry season. Perhaps the relative increase in concentration of aglycones during the rainy season is due to inter-conversion of some glycosides in the leaves to the corresponding aglycones. However, it is note-worthy that the free aglycone content is much lower than the glycosides (Table 3). This is desirable for optimum laxative activity and reduced toxicity [16]. Aglycones are less active than the glycosides. It has been shown in a previous report that the glycosides, rather than the free aglycones, are the active forms and hence an estimation of the total glycosides of a sample is a reliable indication of biological activity [16,17]. Furthermore, it is generally accepted that the aglycones eventually enter the general blood circulation through the liver because of their lipophilic nature. For this reason, aglycones may exhibit anthraquinone toxicity (notably hepatotoxicity).

Anthraquinones glycosides, on the contrary, pass through the stomach and intestine unabsorbed because of the hydrophilic nature of the sugar moiety. The glycosides exert laxative activity direct on the colon by irritation and retention of water and electrolytes [18]. The advantages of using natural anthraquinones laxatives when the glycoside content is highest include faster onset of action in situ, reduced toxicity and the overall shorter stay in the body.

This study is significant because it provides useful information on the seasonal accumulation of anthraquinones and the best period for harvesting the leaves of *C. podocarpa* for drug development. The inclusion *C. podocarpa* in the African Pharmacopoeia will no doubt, enhance its commercialization as laxative and for its antimicrobial effect.

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