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Chemical and biological analyses of Nigerian *Cassia* species for laxative activity*

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Abstract: The leaves of 10 *Cassia* species (Leguminosae), cultivated in Nigeria, were assayed spectrophotometrically for combined anthraquinone content and also pharmacologically for their laxative properties in male albino rats using official senna leaves (*Cassia acutifolia* Del.) as the reference standard. Leaves of *C. podocarpa* Guill. and Perr. and of senna had identical laxative potency. The results of both the chemical and the biological experiments suggested that *C. alata* L. and *C. podocarpa* are the most likely candidates for drug development in Nigeria. The use of a laxative index is proposed for the comparative study of *Cassia* (or any plant species) and its possible application to the quality control of these drugs is discussed.

Keywords: Cassia *species; Leguminosae; laxative index; anthraquinone content.*

Introduction

The genus *Cassia* (Leguminosae) is represented in Nigeria by 33 species most of which possess a reputation in folklore for laxative properties. Some, such as *C. acutifolia* Del. have been reported to contain anthraquinone derivatives [1–3], the principal laxative constituents of many plants used as purgatives. On the other hand, with the exception of senna (not found in Nigerian flora), the laxative potency of most members of the genus has not been investigated. The present communication represents part of the authors' continuing work in examining the laxative activity of the Nigerian *Cassia* species for subsequent drug development.

Experimental

Plant materials

Young leaves of *Cassia alata* L., *C. biflora* L., *C. fistula* L., *C. hirsuta* L., *C. occidentalis* L., *C. podocarpa* Guill. and Perr., *C. rotundifolia* Pers., *C. siamea* Lam., *C. sophera* L. and *C. tora* L. (Leguminosae) were collected from Ile-Ife (southern Nigeria) between February and March (dry season) and in August (rainy season), identified and authenticated in the Forest Research Institute of Nigeria, Ibadan, where the herbarium

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specimens (Nos 102227–102235) had been deposited. The leaves were dried at 50°C, powdered and stored in sealed amber containers until ready for use.

Cassia acutifolia Del. (senna) leaves (reference standard) were purchased as "Herb Tea" (Alpine UK Ltd).

For the animal experiments, an infusion of leaves of each *Cassia* species, equivalent to 100 mg ml^{-1} on a moisture-free basis, was prepared with boiling distilled water. An aliquot was taken each time for administration of the desired dose.

Chemical analysis

Extraction and qualitative assay. Free anthraquinones in the leaves of *Cassia* species were extracted with $CHCl_3$, detected by Bonträger's test and TLC using published methods [3, 4]; the combined anthraquinone derivatives were examined by previously described methods [5] prior to the tests. For TLC, silica gel G plates were used; the solvent for development was benzene–ethyl acetate–acetic acid (7:2:1, v/v/v). A 5% (m/v) alcoholic solution of KOH was used as the spray reagent; the sprayed plates were heated at 100°C. Authentic samples including rhein, chrysophanol and aloe-emodin were kindly supplied by Dr P.P. Rai.

Quantitative assay. 100 mg of the defatted, powdered leaves of each *Cassia* species were refluxed for 20 min with 20 ml of 10% (m/v) $FeCl_3$; 2 ml of 10 M HCl was added and the mixture was refluxed for a further 20 min. The cooled mixture was extracted with diethyl ether and the ethereal extract was evaporated to dryness. The residue was treated with 10 ml of 5% (m/v) NaOH containing 2% (v/v) of NH_3 and after 20 min the absorbance of the solution at 520 nm was measured using a Varian 634 UV spectrophotometer. The corresponding concentration of the combined anthraquinones, as 1,8-dihydroxyanthraquinone (Sandoz, London), was then calculated from a linear calibration graph [6].

Pharmacological analysis

Laxative assay. White male albino rats were kept in individual cages for one week during which a "dummy assay" was performed and any rat producing wet faeces at this stage was rejected. Established procedures [7, 8] were carried out on each rat with the infusion of each species of *Cassia* leaf. The faeces were examined for wetness hourly for 12 h.

Five rats per group (in quadruplicate) were treated per dose of each *Cassia* leaf infusion (including senna). The results were first expressed as the mean per cent of total faeces that were wet per kg rat and then with reference to senna.

General screening. Each infusion of the various *Cassia* species (including *C. acutifolia* standard) was administered at a dose of 500 mg kg^{-1} [5] to each of 10 male rats and the mean percentage of total faeces that were wet was calculated following the procedure described above. When compared with *C. acutifolia*, the various *Cassia* species could be arranged in order of their "senna-equivalents" (potencies relative to that of senna) as an index of laxative property.

Results and Discussion

The presence of anthraquinone derivatives in the Nigerian samples of *C. alata*, *C.*

Table 1
Anthraquinone content of *Cassia* species cultivated in Nigeria

Cassia species	Brontäger's test		% *Combined anthraquinones	
	dry season	rainy season	dry season	rainy season
C. alata	++	++	1.24 ± 0.01	1.32 ± 0.01
C. biflora	−	−	0.00	0.00
C. fistula	n	±	n	0.04 ± 0.00
C. hirsuta	n	±	n	0.02 ± 0.00
C. occidentalis	±	n	0.04 ± 0.00	n
C. podocarpa	++	++	2.80 ± 0.03	2.56 ± 0.02
C. rotundifolia	±	±	0.04 ± 0.00	0.04 ± 0.00
C. siamea	±	±	0.01 ± 0.00	0.01 ± 0.00
C. sophera	−	−	0.00	0.00
C. tora	n	+	n	0.54 ± 0.01
C. acutifolia (reference)	++		1.16 ± 0.01	

Abbreviations: n, not investigated; ±, trace; +, positive; ++, strongly positive; −, absent.
*Calculated on moisture-free basis.

fistula, *C. occidentalis*, *C. podocarpa* and *C. tora* (Table 1) is in agreement with literature reports [1–3]; the absence of anthraquinones in *C. siamea* (contrary to previous reports [9] could be attributed to geographical, seasonal or developmental variation as earlier reported on senna [10]. To the authors' knowledge, the anthraquinone contents of *C. biflora*, *C. hirsuta*, *C. rotundifolia* and *C. sophera* have not been reported previously. The seasonal effect on the anthraquinone yield is not obvious from this investigation. Quantitatively, *C. alata* and *C. podocarpa* gave the highest contents of bound anthraquinones; when further examined by TLC alongside *C. acutifolia* using several solvents with authentic samples, both species gave rhein and aloe-emodin in their acid hydrolysates, thus supporting earlier findings by Rai and Abdullahi [3]. However, in contrast to their results on samples from northern Nigeria of *C. alata* (0.1%) and *C. podocarpa* (1.0%) in the present work, 1.32 and up to 2.80% combined anthraquinones, respectively, were found in samples collected from southern Nigeria. Although the possible effects of geographical variation on the anthraquinone content cannot be ruled out, arbitrary collection of the leaves from the plant could also explain the inconsistency of results since it is well known that old leaves give low yields of anthraquinones.

Figure 1 shows the laxative activity profile in rats for four *Cassia* species including senna. *Cassia tora* possessed the least laxative activity, which lends some support to the choice of the species in folklore as a mild laxative for young children and pregnant women. *Cassia podocarpa* and *C. acutifolia*, which gave identical and higher activity, were further compared at different times (Fig. 2). Their potencies were not significantly different (Student's *t*-test). Laxative activities generally increased with time although no wet faeces were observed after 12 h in any experiment. The other *Cassia* species investigated in this work did not produce a considerable amount of wet faeces when subjected to the preliminary general screening procedure (see Experimental).

Based on this method, a significant laxative activity of any *Cassia* species has been previously defined [5] as the 50% level or more of wet faeces at a 500 mg kg^{-1} dose in 12 h. *Cassia podocarpa*, with 62% wet faeces when compared to *C. acutifolia* (69%) under these conditions, would possess a senna-equivalent of 0.90, *C. alata* 0.70 and *C.*

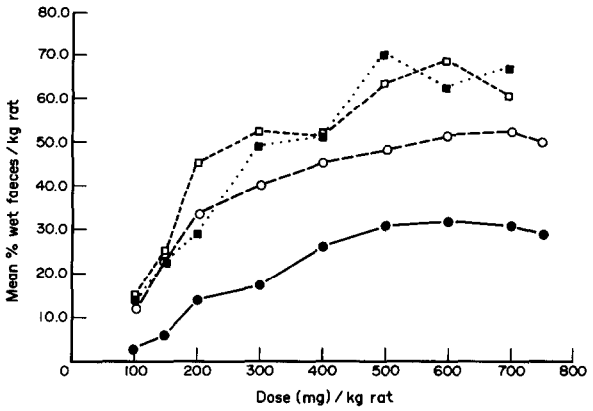

Figure 1
Dose–laxative activity profile for some *Cassia* species 12 h after administration. (Expressed in milligram-equivalent of moisture-free plant material per kg rat.) ■ *C. acutifolia* Del. (reference); □ *C. podocarpa* Guill. and Perr.; ○ *C. alata* L.; ● *C. tora* L.

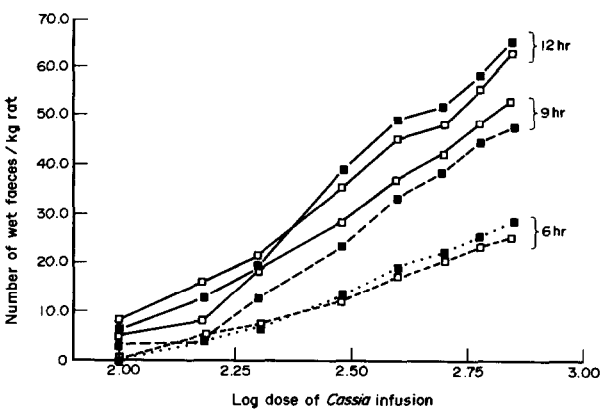

Figure 2
Comparative laxative activity for *C. podocarpa* and *C. acutifolia* at different times. (The dose was based on milligram-equivalent of plant material per kg rat.) ■ *C. acutifolia* Del.; □ *C. podocarpa* Guill. and Perr.

tora 0.45. Hence, an index of 0.70 and above is required to represent a potent laxative species of *Cassia*. This biological index is considered to be a more realistic tool than chemical assays for quality control purposes; the index is also useful as a comparative screening yardstick for a large number of plant species. Chemical assays which determine the total anthraquinone content irrespective of the forms, will not necessarily correspond to purgative activity. This has been demonstrated by the results for *C. podocarpa* which had a far higher content of combined anthraquinones than that of senna and yet possessed identical laxative potency. Results from both the chemical and biological assays in this work suggest that there is promise for development of an acceptable vegetable laxative in Nigeria using *C. podocarpa* and/or *C. alata*.

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