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Medicinal Components Recoverable From Sicklepod *(Senna Obtusifolia)* Seed: Analysis of Components by HPLC-MS^n

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Abstract

Sicklepod *(Senna obtusifolia L.)* is synonymous both with *Cassia tora L. and Cassia obtusifolia L.)*. It is usually viewed as a noxious weed in crop fields in southeastern United States. This plant, however, has a long history of use in traditional medicine in oriental countries. It is a prolific seed producer the constituents of which include: polysaccharides, proteins, highly colored low fat content and many phenolic compounds. The phenolic components are usually described as toxic, although recent literature shows many of these to exhibit potent therapeutic properties for human health. Pursuant to this as part of our continuing interest in the plant, we have expanded our study of the seed by extracting a mixture of anthraquinone and naphthopyrone glycosides from *S. obtusifolia* seed obtained from North Carolina. A survey of the composition of the extract was affected using High Performance Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (HPLC-ESI-MS) with a variety of collision induced dissociation (CID) experiments. The major constituents of the mixture produced HPLC-ESI-MS^n data consistent with: chrysophanic acid tetraglycoside; rubrufusarin and toralactone di-, tri- and tetraglycosides; torachrysone ester, di-, tri-, tetra- and pentaglycosides and cassialactone tetraglycoside. The naphthopyrone glycosides and related phenolic compounds in the seed are value-added medicinal co-products to the galactomannan polysaccharides of *S. obtusifolia*. FT-IR spectra of the mixture corroborate the chromatographic information obtained of the mixture of anthraquinone glycosides.

Keywords: *Senna obtusifolia*; Naphthopyrone glycosides; Anthraquinone glycosides; HPLC-MS; FT-IR spectra; Medicinals

Introduction

Senna obtusifolia (L., H.S. Irwing & Barneby) seeds were examined some forty years ago in a survey for new water-soluble gums in southeastern United States [1]. Vershney et al. [2] reported analysis of the carbohydrate profile of a crop of *S. obtusifolia* seeds in southern India. But it was the contamination of the 1989 soybean harvest in North Carolina by *S. obtusifolia* seed that revived interest in this prolific seed producing weed [3]. Subsequent and continuing interest in the plant aims at utilization of the seed components such as the polysaccharides and proteins [4-6]. But the galactomannans and proteins co-exist with phenolic compounds normally thought of as toxicants in the seed. However, several isolates from the seed have been shown to have medicinal value beyond laxatives. In this study, we have expanded our focus on the polysaccharides to include the non-food components of the seed. Thus we describe an aqueous method for recovery of the phenolic co-products of *S. obtusifolia* seed gums that could be useful to medicinal chemists or pharmaceutical manufacturers. The major components of the extracted solute are described by a combination of High Performance Liquid Chromatography (HPLC) retention times, Mass Spectra (MS), and mass spectral fragments produced by collision induced dissociation (CID) experiments. Structural information obtained from HPLC-MS-CID analysis was compared to published reports of fully characterized compounds previously isolated from *S. obtusifolia* or a closely related member of the *Cassia* genus. Identification of constituents were made when the HPLC-MS-(CID) data were found to be consistent with that of a previously identified isolate.

Materials and Methods

Materials and reagents

S. obtusifolia seeds were obtained from the Wilder Farm, Raleigh, via NC State University, Department of Agriculture. Ethanol was purchased from Fisher Scientific (Chicago, IL). Petroleum ether was purchased from Sigma-Aldrich Co. (St. Louis, MO); Amberlite XAD-4 was obtained from SUPELCO, Bellefonte, PA. Anthraquinone standards-physcion, rhein, emodin, aloe-emodin, 1, 8-dihydroxyanthraquinone, chrysophanol- were obtained from ACROS Organics, Fisher Scientific (Chicago IL); and sennaside B and dextran molecular weight standards from 1-150 kD were obtained from Sigma-Aldrich (St. Louis, MO).

Fourier transform infrared (FT-IR) spectrometry

FT-IR spectra were measured on an Arid Zone FT-IR spectrometer (ABB MB-Series, Houston, TX) equipped with a DTGS detector. Liquid derivatives were pressed between two NaCl discs (25 mm x 5 mm) to give thin transparent oil films for analysis by FTIR spectrometry. Absorbance spectra were acquired at 4 cm^{-1} resolution and signal-averaged over 32 scans. Interferograms were Fourier transformed using cosine apodization for optimum linear response. Spectra were baseline corrected, scaled for mass differences and normalized to the methylene peak at 2927 cm^{-1}.

Extraction of phenolics and phenolic glycosides

Whole *S. obtusifolia* seeds were dry milled as previously described

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[5]. The seeds were passed through a roller mill for cracking and an impact mill to separate the cotyledons (meats) from the cracked seeds. The remaining meat hull / endosperm mixture was chilled in dry ice and further milled using a Retsch mill and sieved through a 100 mesh screen to yield a clean endosperm fraction. The ground seed meal was defatted with petroleum ether in a Soxhlet extractor for 20 hours after which the meal was dried under vacuum at 20°C. The dried meal was extracted with deionized water and centrifuged until the supernatant was clear as previously described [6]. To precipitate the water-soluble proteins from the extract, the supernatant was heated to 92-93°C for 40 minutes, vacuum filtered through a pad of celite in a medium porosity glass funnel and allowed to cool to room temperature. Next, portions of the filtrate were applied to a preconditioned column of Amberlite XAD-4 (styrene-divinylbenzene nonionic macroreticular resin with particle size 20-60 mesh wet and 40 Å mean pore size) and then washed with deionized water to remove the polysaccharides, free sugars and oligosaccharides. The anthraquinones and other phenolic constituents retained on the column were then eluted with ethanol followed by 0.1 M ammonium hydroxide in ethanol until the pink color generated on the resin was discharged. The combined ethanol eluant was concentrated to a red solid under reduced pressure by rotary evaporation at 40°C.

Analysis of the anthraquinone/phenolic fractions

A portion of the red solid was dissolved in water (10 mg/ml) and analyzed on a high-performance gel permeation chromatographic system consisting of a Spectraphysics Spectrasystem P4000 pump and a Thermosepartions Spectrasystem AS300 autosampler (both Thermoscientific, Waltham, MA), a Waters R401 refractive index detector (Waters Corporation, Milford, CT), an HP Chem Station data acquisition system (Agilent Technologies, Santa Clara, CA) and a Synchropak GPC 100 column (250 mm x 4.6 mm. available from Eprogen, Inc., Darien, IL). Applied sample was eluted with deionized water at 0.5 ml/minute. Retention times of the analyte peaks were compared to Dextran standards from 1 to150 kD. By comparison, the molecular weight distribution of the solution was estimated to be: 55 % greater than 20 kD, 36% less than 1 kD and 8% approximately 3 kD. These results suggested that either polysaccharide had not been fully separated from the anthraquinone/phenol components by the column or that the anthraquinones are glycosylated.

A portion of the dried red solid was analyzed on a Fourier Transform Infrared (FT-IR) Spectrometer (ABB Inc., Houston, TX equipped with a DTGS detector). The sample was prepared by pulverizing 1.0 mg of the dried extract with spectrometric grade dry KBr (300 mg) in a stainless steel vial with two stainless steel balls. The powdered sample was then placed in an IR die and compressed in a Carver press at 24000 lb/in^2 to give a transparent disc. Absorbance spectra were acquired at 4 cm^{-1} resolution. The IR spectrum contained peaks at ν_{KBr} cm^{-1}: 3399 b(-OH) 2932 (-CH_2- stretch), 1718 (-CO_2- stretch), 1625 (-C=C- puckering), 1404 (-CH_2- deform), 1270 (-CO_2- stretch), 1060 (-CH_2O- stretch) which confirmed the presence of a substantial amount of carbohydrate in the red solid. Anthraquinone aglycone spectrum shows bands at 3422 (-OH), 3006 (=CH- arom.), 2956 (-CH_3 asym. stretch), 2921 (-CH_2- asym. stretch), 2856 (-CH_2- sym stretch), 1718 (-CO_2 stretch), 1655, 1631 (-C=C- breathing mode, arom.), 1463 (-CH_2- deform.), 1363 (-CH_3 deform), 1159 (-CHO- stretch), 1089 (-CHO- stretch), 821 (-CH- arom) cm^{-1}.

Additional portions of the red solid were dissolved in acetonitrile / water and analyzed by HPLC-ESI-MS-CID spectroscopy. The HPLC system consisted of an autosampler and quaternary gradient pump (Thermo-SpectraPhysics Spectrasystem AS3000, P4000, ThermoFisher, Waltham, MA), and Inertsil 5 ODS 3 column (150 mm x 3 mm id) (Metachem Technologies Inc., Torrence, CA). Samples were eluted at 0.3 ml/min with a gradient program from 0.25% acetic acid in water (A) to 0.25% acetic acid in acetonitrile (B) as follows: 0-5 min. 100% A; 5-45 minutes linear gradient from 100% A to 100% B. The HPLC eluant was analysed on a ThermoFinnegan LCQ-Decca mass spectrometer (ThermoFisher, Waltham, MA) equipped with an electrospray ionization source operated in negative ion mode at collision energy settings of 45 to 55 with automated data dependent collision induced dissociation scanning of the first and second most intense ions detected during MS monitoring of the chromatographic separation.

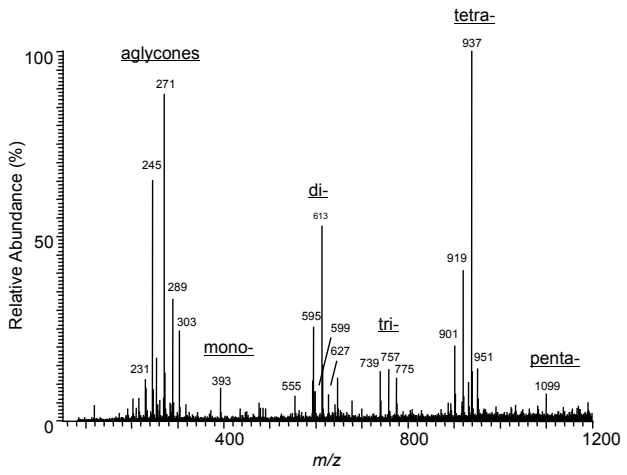


Figure 1: Mass Spectrum of the glycosides fraction eluted between 22.6 and 26.5 minutes from a C-18 HPLC column. Glycosides of 1 to 5 units are seen

Identification of components

Components were identified by HPLC retention times and mass spectrometric analysis. When possible, these were compared to standards. When standards were not available, structural information was gleaned from detailed study of the product ions generated by collision induced dissociation experiments. To confirm identification, the structural information was compared to literature references of fully characterized compounds isolated from *Cassia* species.

Results and Discussion

In this study, naphthopyrone, torachrysone and anthraquinone glycosides in a water extract of defatted *Senna obtusifolia* seed meal were separated from proteins by heat treatment of the aqueous extract followed by filtration of the precipitated proteins. Polysaccharides were removed by application of the de-proteinated extract to a hydrophobic polyaromatic resin column which retained the phenolic glycosides but excluded the polysaccharides. The retained material was recovered from the column by application of an initial ethanol wash, followed by dilute ammoniacal ethanol and the solute was sequentially analyzed by FTIR and HPLC-ESI-MS-CID.

An average of the mass spectral data acquired from 22.6 to 26.5 min showed masses corresponding to phenolic glycosides, with tetraglycosides being the most abundant regardless of the individual base aglycones (Figure 1). In CID experiments, these compounds were distinguished by the characteristic loss of the intact tetraglycosyl unit, *m/z* 648, from the phenolic tetraglycoside. Di- and triglycosides displayed corresponding losses of *m/z* 324 and *m/z* 486, respectively.

The masses corresponding to glycosides in Figure 1 were: *m/z* 1099, torachrysone pentaglycopyranosyl ester; *m/z* 951, cassialactone tetraglycopyranoside; *m/z* 937, torachrysone tetraglycopyranosyl ester; *m/z* 919, rubrofusarin/toralactone tetraglycopyranosides; *m/z* 901, chrysophanol tetraglycopyranoside; *m/z* 775 torachrysone triglycopyranosyl ester; *m/z* 757, rubrofusarin/toralactone triglycopyranosides; *m/z* 739, chrysophanol triglycoside; *m/z* 627, cassialactone diglycoside; *m/z* 613, torachrysone diglycopyranosyl ester; *m/z* 599, hydroxymusizin diglycoside ester; *m/z* 595 rubrofusarin\ toralactone diglycosides; *m/z* 577, chrysophanol diglycoside; *m/z 555*, hydroxymusizin diglycoside; *m/z* 393 hydroxymusizin monoglycoside. The aglycone fragments were: *m/z* 303, cassialactone; *m/z* 289, torachrysone ester; *m/z* 271, rubrofusarin/toralactone; *m/z* 253, chrysophanol; *m/z* 245, torachrysone; and *m/z* 231, hydroxymusizin. The bulk of the mass spectrometrically detectable material in the water extract consisted of rubrofusarin and toralactone glycosides (*m/z* 919, 595), torachysone glycoside esters (*m/z* 937; 613) and their derivatives; cassialactone glycosides (*m/z* 951) and anthraquinone glycosides (*m/z* 901, 739). Extracted ion chromatograms of the predominant glycoside series are shown in Figure 2. Figure 2A top panel displays a partially resolved eic of the geometric isomers Rubrofusarin and Toralactone tetraglycopyranosides. Rubrofusarin tetraglycoside eluted slightly faster than its geometric isomer Toralactone tetraglycoside and so is observed as the abutting peak [7]. In the extracted ion chromatograms of torachrysone glycopyranosyl esters (Figure 2B), the third chromatogram (eic 775) contains two peaks. The major peak at 25.16 is Torachrysone triglycopyranosyl ester (Table 2) whereas the smaller peak at 23.92 is an intact fragment of *m/z* 1099 (seen in the top chromatogram at 23.92) that seemed to have resisted loss of the diglycoside to form the triglycopyranosyl ion (Table 2).

Cassiasides

In MS^2 experiments, the most intense fragment across the entire HPLC separation was *m/z* 271 which was indicative of rubrofusarin, a naphtho-γ-pyrone, and its positional isomer, toralactone, a naphtho-α-pyrone, (Figure 3). These isomers were present primarily as their tetraglycosides, namely: *cassia*side B2 (rubrofusarin tetraglucoside) and cassiaside C2 (toralactone tetraglycoside) (Figure 3). These components separated on HPLC and were identified by their retention times, CID losses and previous reports of their characterization from cassia species [8-11]. Di and tri-glycosides of rubrofusarin and toralactone were also present in significant quantities. The structures are shown in Figure 3 and the HPLC retention times and mass spectral data are presented in Table 1.

Medical significance of cassiasides

Several studies have reported a variety of medicinal bioactivities of cassiasides. Recently, rubrofusarin and toralactone diglycosides have been recommended as potential therapeutic agents for diabetic complications and related diseases [12]. The diglycosides of both isomers were reported to inhibit the production of advanced glycation end products (AGEs) which are causative agents in diabetes complications. In addition, the naphtho-α-pyrone tetraglycoside isomer, toralactone tetraglucoside or cassiaside C2, has been reported to inhibit histamine release far more than the potent anti-inflammatory drug, indomethacin [9]. The interest in cassiaside C2 as an anti-allergic agent was such that a complex procedure to synthesize it has been reported [13].

In another study, the diglycoside of the naphtho-γ-pyrone isomer, rubrofusarin diglucoside, was reported to exhibit significant hepato-

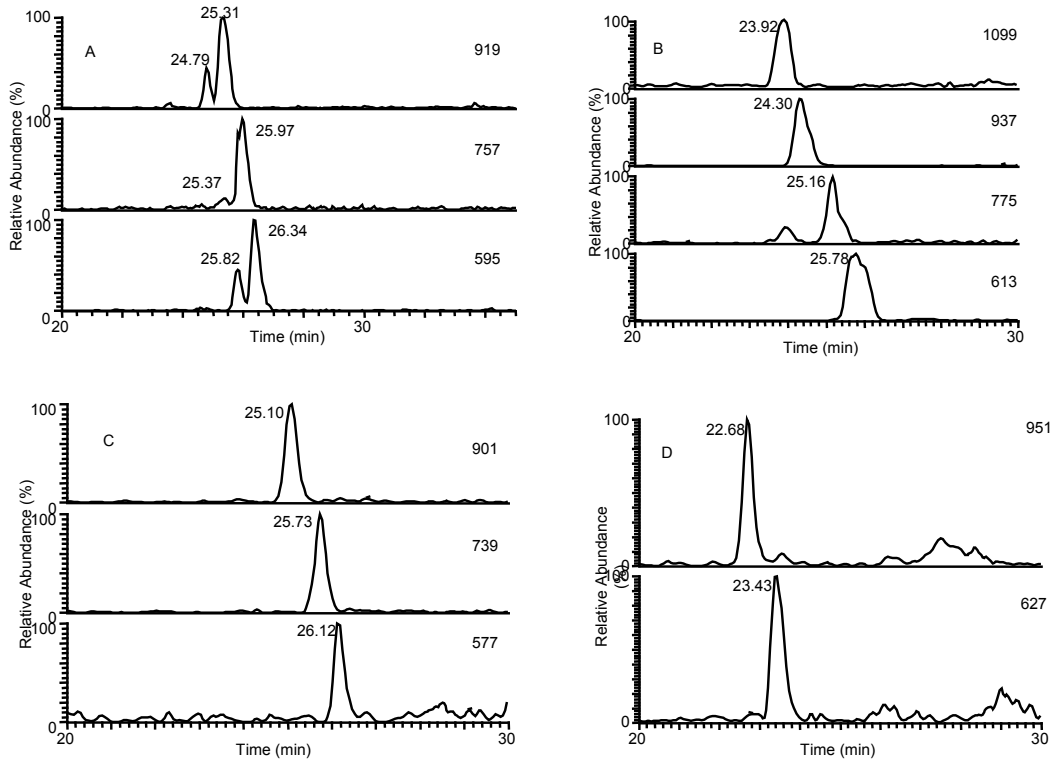
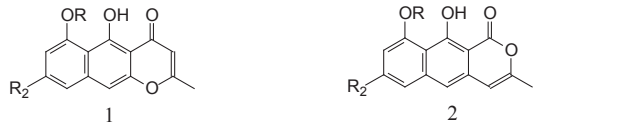


Figure 2: Extracted ion chromatograms of: A) Rubrofusarin and Toralactone tetraglycopyranosides; B) Torachrysone pentaglycopyranosylesters; C) Chrysophanol glycopyranosides and D) Cassialactone glycopyranosides



R= H, R_2=OCH_3 rubrofusarin
R= tetraglycopyranosyl, R_2= OCH_3
R= triglycopyranosyl, R_2= OCH_3
R= diglycopyranosyl, R_2=OCH_3

R=H, R_2=OCH_3 toralactone
R= tetraglycopyranosyl, R_2=OCH_3
R= triglycopyranosyl, R_2=OCH_3
R= diglycopyranosyl, R_2= OCH_3

Figure 3: Naphtho-γ-pyrone (1) and Naphtho-α-pyrone (2) glycopyranosides

M-1	MS2 Product	MS2 Loss	Retention Time (min)	M-1 Intensity
919	271	648 (4 gly)	24.79, 25.31	530
757	271	486 (3 gly)	25.37, 25.97	165
595	271	324 (2 gly)	25.82, 26.34	412

Table 1: MSn Spectral Fragmentation of Rubrofusarin/Toralactone glycopyranosides

protective activity in an activity guided fractionation of liver protective agents and naphtho-γ-pyrone glucosides were identified as the main anti-hepatotoxic principles in *Cassia tora* seeds. The study thus recommended naphtho-γ-pryone glycosides (rubrofusarin and the monoglycopyranoside or nor-rubrofusarin glycoside) as a new class of anti-hepatotoxic natural products of pharmaceutical interest [14,15].

Torachrysone derivatives

In addition to the glycosides of the *m/z* 271 isomers, a second series of compounds were prominent in the water extract. Under multiple stages of CID and MS selection, these compounds fragmented to a core molecule of *m/z* 245 and were determined to be derivatives of torachrysone (Figure 4 and Table 2). Torachrysone was previously found in rhubarb [16] and in *Cassia tora* seed [8]. Most of the torachrysone existed as torachrysone glycosyl esters. The torachrysone derivatives were identified from a series of glycosides which fragmented to an aglycone of *m/z* 289 in MS^2 experiments. Upon further fragmentation, the *m/z* 289 aglycone consistently lost 44 mass units (CO_2) to yield torachrysone *m/z* 245. Di-, tri- tetra- and pentaglycosides of torachrysone ester were tentatively identified (Table 2, Figure 4). Additionly present in the aqueous extract were cassialactone glycosides and hydroxymusizin glycosides; their structures are shown in Figure 4 with the HPLC retention times and some mass spectral losses shown in Table 3. Although torachrysone derivatives have not yet been studied for medicinal activities, they may have medicinal potential. Torachrysone, along with the naphtho-α-pyrone, toralactone and the anthraquinones aloe-emodin, rhein and emodin extracted from Cassia tora seeds have been reported to demonstrate significant antibacterial activity against methicillin-resistant *Staphylococcus aureus*, which is a serious problem in hospitals [8]. In the latter study, the authors observed a lack of activity of the glycosides of the phenolics but recommended the glycosides not be overlooked because they are known to be hydrolyzed in intestines, releasing the active aglycones *in situ*.

Anthraquinone glycosides and anthraquinones

The major anthraquinone glycosides in the aqueous extract were identified as chrysophanol tetraglycoside (*m/z* 901) and chrysophanol triglycoside (*m/z* 739) (Table 4, Figure 5). In the CID experiments these compounds exhibited characteristic losses in the MS^2 spectra of the intact tetraglycosyl unit *m/z* 648 and the intact triglycosyl unit (*m/z* 489) from the chrysophanol tetraglycoside and triglycoside, respectively, generating the aglycone (*m/z* 253). These compounds separated on HPLC, as expected, with more highly glycosylated compounds eluting earlier in the chromatogram (Table 4, Figure 5). These findings are consistent with those of Wong and co-workers who isolated both chrysophanol tri- and tetraglucosides from seeds identified both as *Cassia tora* and Sicklepod. They fully characterized these isolates including the finding that the tetraglucoside and the corresponding chrysophanol triglucoside exhibit mild protective effect upon liver

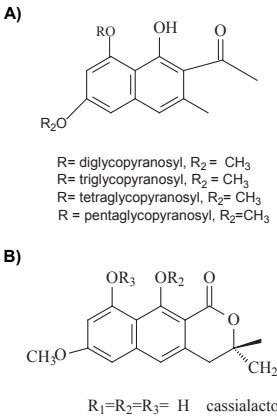
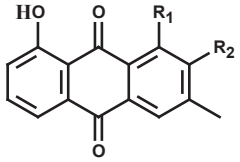


Figure 4: Torachrysone (A) and Cassialactone (B) glycopyranosides



R_1= tetraglucopyranosyl; R_2=H (FM 902)
R_1= triglucopyranosyl; R_2=H (FM 740)
R_1= diglucopyranosyl; R_2=H (FM 578)

Figure 5: Chrysophanol glucopyranosides

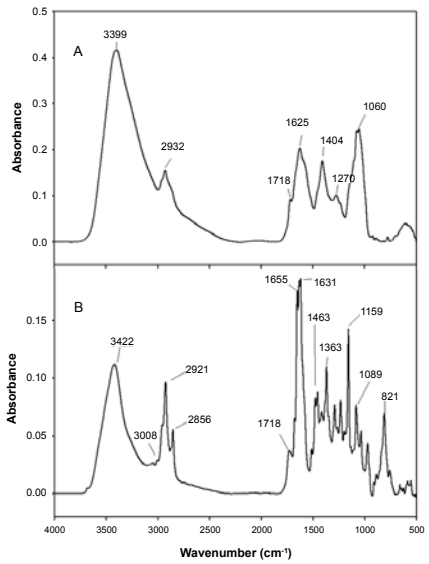


Figure 6: FT-IR spectra of: A) the red solid isolate from the column; B) Non-glycoside mixture of anthraquinones from an earlier batch of seed partitioning with diethyl ether

M-1	MS2 Prodution	MS2 Loss	MS3 Production	MS3 Loss	Retention Time (min)	M-1 Intensity
1099	775	324 (2 gly)	-	-	23.92	124
937	289	648 (4 gly)	245	44	24.34	1330
775	289	486 (3 gly)	245	44	25.16	174
613	289	324 (2 gly)	245	44	25.78	539

Table 2: MSn Spectral fragmentation of Torachrysone glucopyranosides

M-1	MS2 Product	MS2 Loss	MS3 Product(s)	MS3 Loss	MS4 Product	MS4 Loss	Retention Time(min)	M-1 Intensity
951	303	648(4 gly)	259	44	244	15	22.7	374
627	259	368 (2 gly)	217, 244	42, 15			23.4	107
641	317	324 (2 gly)	273	44	229	44	28.6	215
479	317	162 (1 gly)					31.8	79
393	231	162 (1 gly)	187	44			27.2	159
555	231	324 (2 gly)					26.08	93
599	555	44	393	162			23.12	127

951 Cassialactone tetraglycoside

627 Cassialactone diglycoside

393 hydroxymusizin monoglycoside

555 hydroxymusizin diglycoside

599 hydroxymusizin diglycosy carboxylester

Table 3: Other molecular species present in the aqueous extract

M-1	MS2 Prodution	MS2 Loss	Retention Time (min)	M-1 Intensity
901	253	648 (4 gly)	25.10	367
739	253	486 (3 gly)	25.73	246
577	253	324 (2 gly)	26.19	42

Table 4: Chrysophanol glucopyranoside fragmentation Data

cells exposed to the hepatotoxin, carbon tetrachloride [14,15]. Small amounts of the free anthraquinones: emodin, obtusin, chrysophanol, physcion, chryso-obtusin and aurantio-obtusin were identified as well in the aqueous extract. Anthraquinones are widely known to be present in *Cassia* seeds but these are usually extracted with methanol because of their sparse solubility in water.

In a recent study of activity guided fractionation of *Cassia obtusifolia* seeds in a search for inhibititors of blood platelelet aggregation, the monoglycosides of chryso-obtusin, aurantio-obtusin and obtusifolin were identified as exhibiting strong inhibition of platelet aggregation [17]. The authors of that study suggested the glucose functionality may increase the activity of the anthraquinones by increasing the H_2O solubility of each compound. The activities of compounds with higher degrees of glycosylation were not examined since that study was limited to the methanol soluble portion of the seeds.

The IR spectrum of this red isolate Figure 6A, shows absorbances characteristic of both carbohydrate and phenolic components in the extract. The broad band centered at 3399 cm^{-1} is a composite of OH bands of carbohydrates and phenolics and so also is the 1060 cm^{-1} band. The intermediate bands between 1800 and 1400 cm^{-1} are a blend of phenolic frequencies since carbohydrates usually are transparent in this region of the spectrum except for water of crystallization around 1640 and the -CH_2- bending mode at 1460 cm^{-1}. The second derivative of this IR spectrum clearly showed strong puckering absorption modes of the aromatic rings as well as strong vibratory modes indicative of carbohydrate moieties (3500, 1000-1200 cm^{-1}), spectrum not included. The second FT-IR spectrum (Figure 6B) is that of an earlier sample of ground sicklepod seeds extracted with diethyl ether following defatting. The isolate was a dark-red powder, which is the anthraquinone aglycones with spectral features in KBr as shown, ν cm^{-1}: 3422 b (-OH stretch), 3008 (H-C=C-), 2921 (-CH_2- asym. stretch), 2856 (-CH_2- sym stretch), 1718 (-CO_2- ester stretch), 1655, 1631 (carboxyl stretching modes), 1514, 1463 (-CH_2- deform), 1363 (-CH_3 deform), 1235 (carboxyl stretch), 1159, 1089 (-C-O stretch), 821 (arom C-H bend).

Toxins

Cassia occidentalis, seeds are reported to be both toxic [18,19] and medicinal and therefore some care is required in recovery of useful products from Cassia. Ingestion of whole untreated seeds or ground whole seed meals of Cassia species are known to cause illness and death to cattle, horses, pigs and chickens [19]. Aqueous sodium bicarbonate or sodium citrate extractions of the seeds are reported to remove the toxin and leave it bound to particulate matter in the extract [20]. In our laboratory, a separate experiment aimed at the direct, rapid extraction of base soluble proteins from defatted *S. obtusifolia* seed meal with very dilute alkali (0.015 M NaOH) followed by acidification of the extract to precipitate the soluble proteins, resulted in the release of a volatile material which produced numbing of the nose and mouth of the analysts. So as a cautionary note, therefore, we would recommend care in carrying out the quick procedure on sicklepod seed meal. This phenomenon is, however, not experienced when one sequentially isolates the defatted meal proteins first with saline solution for the water-soluble albumin/globulins fraction, followed by aqueous alcohol treatment of the residual pellet for prolamines and finally with dilute alkaline extraction for the glutelin components [5,6,21].

Several techniques for decreasing the toxic principle in Cassia have been described in the literature. Treatment of the seed with heat and humidity is reported to eliminate the toxic principle in Coffee senna, i.e., autoclaving [22]. Roasting of Coffee Senna *(Cassia occidentalis)* seeds reportedly made them safe for use as a coffee substitute during World War II [22]. Storage of the seed at ambient temperatures in tropical climates for a year has also been reported as a means to reduce toxicity in Cassia preparations before use as a folk medicine [3].

Conclusions

Previous studies have shown that *S. obtusifolia* seed is a potential domestic source of unique gums or galactomannans and proteins that are usable as hydrocolloids in pet foods when the toxic non-food components are removed or at low concentration. The present work emphasizes extraction and utilization of the non-food co-products of this seed which in southern Asia have been shown to be bioactive against hepatotoxins, diabetic complications, allergies, *Staph. aureus* and as a potent anticoagulant. A mixture of these co-products have been easily recovered from *S. obtusifolia* seed and monitored by HPLC-ESI-MS^n analysis using the methods described here. The approach describes a simple "green" technique for extracting the mixture and identifying most of the important medicinal compounds extant in the seed. The overall goal is a continued effort to expand utilization of the seed through these value-added co-products and therefore improve the potential of this regional weed to become a domestic alternative economic new crop.

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