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High-Performance Liquid Chromatographic Method for the Quantitative Determination of Hypoxoside in African Potato (*Hypoxis hemerocallidea*) and in Commercial Products Containing the Plant Material and/or Its Extracts

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Hypoxoside is a norlignan diglucoside present in the corms of African potato (*Hypoxis hemerocallidea*). The latter is used as a popular African traditional medicine for it's nutritional and immune-boosting properties. A reverse phase high-performance liquid chromatography method was developed and validated for the determination of hypoxoside using a mobile phase consisting of acetonitrile:water (20:80, v/v). The method was linear throughout the range of 10−100 μg/mL and provided a high degree of accuracy (100 ± 4%). The recovery of the method was found to be 100 ± 5%, and the precision of the study, % relative standard deviation intraday and interday (over three separate days), was better than 6.15 and 5.64%, respectively. The limits of detection and quantification were calculated to be 0.75 and 3.5 μg/mL, respectively. This method was applied to the analysis and quality control of African potato corms as well as 12 commercially available products. The daily intake of hypoxoside through traditionally prepared African potato decoction was also evaluated.

KEYWORDS: ***Hypoxis hemerocallidea*; hypoxoside; RP-HPLC; validation; quality control**

INTRODUCTION

African potato (AP), *Hypoxis hemerocallidea*, also known as *Hypoxis rooperi* of the family Hypoxidaceae, apart from it's perceived nutritional value, is of great medical interest (*1*) and is purported as possibly being the best-known medicinal plant by many South Africans (*2*). Extracts of the corms have been ingested by man for a diversity of ailments (*3*) including the treatment of urinary diseases (*4*), prostate hypertrophy, and internal cancer (*5*). Furthermore, it has recently gained increased prominence as an alternative medicine for nutritional use in the daily diet of HIV/AIDS patients as a result of strong recommendations by the South African Minister of Health (*6*). Hypoxoside is the trivial name for (*E*)-1,5-bis-(4′-B-D-glucopyranosyloxy-3′-hydroxyphenyl)pent-1-en-4-yne (CAS registry no. 83643-94-1), which is a norlignan diglucoside present in the corms of hypoxis plants (*4*) (**Figure 1**). This compound has an uncommon aglycone structure consisting of a diphenyl-1-en-4-yne-pentane skeleton. The glycoside possesses low toxicity, and AP is used as a food (*7*), whereas it's aglycone, rooperol, is purported to possess antiphologistic (*8*), bacterostatic, and bactericidal properties (*9*). The vast pharmacological and clinical reports (*10*−*12*) have led to the registration of several patents (*13*−*15*) and the commercialization of the extract of AP in Germany under the trade name Harzol, for the treatment of prostrate adenoma (*16*). There are numerous other commercially available formulations containing AP with various therapeutic claims. Traditionally, AP is cut into cubes or shredded and boiled for 20 min before the decoction is orally consumed. It has been claimed that sterols and sterolins present in AP are responsible for its medicinal properties, but this is yet to be scientifically proven. Whereas sterols and sterolins are ubiquitous in nature, the relatively abundant presence of the compound, hypoxoside, is a unique feature of plants in this genus (*4*). Thus, hypoxoside is an appropriate analytical marker for use in the standardization and quality control (QC) of AP and related products.

Several methods have been reported for the extraction, isolation, and determination of hypoxoside (*4*, *17*, *18*) from plants belonging to the genus, Hypoxis. However, very little information has appeared in the literature to describe methods for the quantitative analysis of hypoxoside.

In 1990, Vinesi et al. (*5*) reported a high-performance liquid chromatography (HPLC) method for the separation of the glycoside fraction from plant material of *Hypoxis obtusa.* Validation data to support the use of this method for quantitative analysis of hypoxoside were, however, not provided.

In 1992, Betto et al. (*18*) described the determination of norlignan glycosides by HPLC using a gradient method, which was claimed to be useful for the separation of the relevant glycosides and for monitoring production in tissue cultures to establish the presence of norlignan glycosides. In 1993 and 1994, Kruger et al. (*19*, *20*) described the use of guanidine hydrochloride and ammonium sulfate for the sorption enrichment of xenobiotics in biological fluids by HPLC, which was subse-

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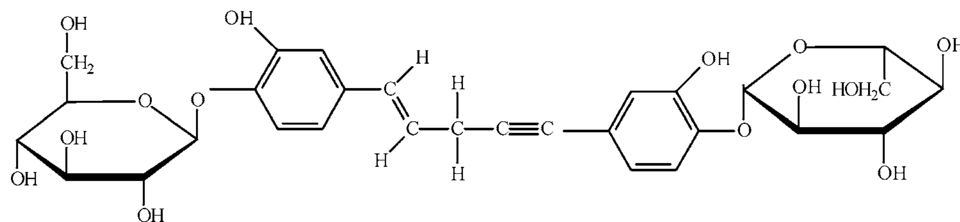

Figure 1. Structure of hypoxoside.

quently applied to study the chromatographic behavior of hypoxoside and rooperol analogues. The method was also applied to analyze human urine and plasma samples for hypoxoside biotransformation products following administration of oral hypoxoside. The same method was used to analyze the methanolic extracts of hypoxoside and rooperol analogues in *H. rooperi* and *Hypoxis latifolia*. The applied method, however, was not validated, and no quantitative data were obtained.

We present here a simple, rapid, accurate, precise, and reproducible quantitative HPLC method, which is suitable for the assay and QC of plant material, extracts, and commercial formulations containing AP.

MATERIALS AND METHODS

Reagents. Acetonitrile and methanol (HPLC grade) were purchased from Romil Ltd., (Cambridge, United Kingdom); butanol, ethyl acetate, and toluene were purchased from BDL Chemicals Ltd. (Poole, United Kingdom); and sulfamerazine (99.1%) was thankfully received as a gift from Orchid Chemicals (Chennai, India). Water was purified in a Milli-Q System (Millipore, Bedford, MA), and Millex HV Hydrophilic (PVDF) filters were purchased from the same source. Silica gel 100−200 mesh (75−250 μm) was purchased from Sigma (St. Louis, MO). Twelve different formulations were purchased from a local pharmacy in Grahamstown, South Africa.

On each of 3 days, stock solutions of hypoxoside (100 mg/L) and the internal standard (IS) sulfamerazine (100 mg/L) were prepared in methanol. Working solutions comprising a set of five calibrators in the concentration range of 10−100 mg/L hypoxoside containing 10 mg/L IS standard were prepared daily by appropriate dilution with methanol. Additional samples were freshly prepared on each day of analysis (30 and 60 μg/mL) for use as QC standards.

Instrumentation. Separate HPLC systems were used for the isolation and quantitative analyses of hypoxoside, respectively.

Isolation of Hypoxoside. The isolation of hypoxoside was carried out using a SpectraSYSTEM P2000 pump equipped with an AS 1000 autosampler and a UV 1000 variable-wavelength UV detector (all supplied by Thermo Separation Products, Riviera Beach, FL). Separation was achieved on a Luna C_{18} (2) (250 mm × 10 mm i.d., 5 μm) semipreparative column (Phenomenex, Torrance, CA). A mobile phase consisting of acetonitrile:water in isocratic mode (20:80, v/v) was used at a flow rate of 5 mL/min. Samples (100 μL) were injected into the column, which was maintained at a temperature of 23 ± 2 °C, and the eluate was monitored at a wavelength of 260 nm.

Analysis of Hypoxoside. The analysis was carried out on an Alliance 2690 HPLC system (Waters Corporation, Milford, MA) equipped with a 2996 photodiode array (PDA) detector, a degasser, a column heater, and an autosampler. A Luna C_{18} (2) (5 μm, 150 mm × 4.6 mm i.d.) column (Phenomenex) was used at 23 ± 2 °C. Separation was achieved using the same mobile phase described for the isolation of hypoxoside but at a flow rate of 1 mL/min using a detection wavelength of 260 nm.

Samples. The fresh corms of AP were collected, and samples were assigned accession numbers and vouchers and stored in the herbarium (Faculty of Pharmacy, Rhodes University). The corms were carefully washed with water and dried with tissue paper. They were cut into approximately 0.5 cm^3 pieces and segregated into four lots of known weight (ca. 250 g). Until a constant mass was achieved, replicates of 250 g of fresh AP samples (cubes) were dried according to the following four procedures: (i) evenly spread and allowed to dry by exposure to sunlight for 8 h daily for 3 weeks (SUN); (ii) dried under the shade (the same loss as above was reached in a month) (SHD); (iii) immediately frozen in liquid nitrogen and lyophilized for 12 h (FZD); and (iv) microwaved (750 W) for 4 min (high) to assist dehydration (MWD). In all instances, a constant mass was reached, i.e., 40% of initial mass.

Twelve different commercially available formulations (A−L) were chosen as follows: Four (A−D) were hard gelatin capsules, three (J−L) were in liquid form, and the remaining five products (E−I) were formulated as solid dosage forms (tablets). Four different batches of product D were purchased to study the interbatch variability. The average weights of all of the solid formulations were calculated.

Extraction and Isolation of Hypoxoside. Fresh corms (~250 g) were placed into a blender and slurried with 50 mL of methanol. The slurry was extracted with 500 mL of methanol for 30 min using a magnetic stirrer and strained through a muslin cloth. Because aqueous solutions of hypoxoside are photolabile at 1.2 × 10^6 lux hours (Nair, unpublished results) the above extraction was repeated three times under subdued light to prevent photodegradation of hypoxoside. The methanol extract was subsequently filtered (Whatman filter paper no. 44) to remove fibers. The filtered extract was dried in a rotary evaporator under vacuum at 45 °C, and the evaporated extract (~35 g) was then dissolved in 250 mL of water by shaking for 10 min. The water-soluble extract was again filtered through Whatman filter paper (no. 44) and transferred to a 500 mL separating funnel. It was first partitioned with water-saturated ethyl acetate (250 mL × 3) to remove nonpolar impurities. The aqueous portion was further partitioned with water-saturated *n*-butanol (250 mL × 3), and the nonaqueous layer was removed and thoroughly dried under vacuum to obtain a yield of approximately 30 g (butanolic extract). This procedure facilitated the removal of polar and other mucilaginous impurities. A glass column (500 mm × 50 mm i.d.) was packed with silica gel (60−120 mesh, 75−150 μm) and was eluted with toluene:butanol:water:methanol (6:4:2:1) at a flow rate of 0.5 mL/min. Two grams of butanolic extract was loaded onto the column, and the first 200 mL was discarded. The column was further eluted with toluene:butanol:water:methanol (4:4:2:1) at a flow rate of 0.5 mL/min. The first 50 mL was discarded, and the remaining 200 mL was collected and dried in a rotary evaporator at 45 °C under vacuum.

The isolation of hypoxoside from the above enriched extract was effected by injecting 100 μL samples of an aqueous solution (5 mg/mL) into the semipreparative HPLC previously described. Hypoxoside fractions were collected, combined, freeze-dried, and stored in a deep freeze (−20 ± 2 °C) until required for use.

Purity. Confirmation of the purity of hypoxoside was established using ^{1}H, ^{13}C NMR, liquid chromatography−mass spectroscopy (LC-MS), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and HPLC prior to use as a reference standard for quantitative analysis.

Extraction Procedures. Ten hard gelatin capsules of each of products A−D were emptied and separately well-mixed, and aliquots of the powder mix (~50 mg) were accurately weighed into a 10 mL volumetric flask. The flasks were made up to volume with methanol and sonicated (Branson, Parrott Drive, Shelton, CN) for 20 min. After the mixtures were allowed to cool for 5 min, 1 mL of each filtered solution was transferred to a 10 mL volumetric flask, 1.0 mL of the IS solution (100 mg/L) was added, and their volumes were made up with methanol. The solutions were filtered using durapore (PVDF) membranes (0.45 μm) prior to injecting 10 μL into the HPLC system.

Ten tablets of each of products E−I were crushed in a mortar, powdered, and well-mixed using a pestle. Fifty milligram aliquots of each product were accurately weighed into a 10 mL volumetric flask, and methanol was added and treated as described above for capsules.

The liquid formulations (products J−L) were well-shaken, 1 mL of each preparation was transferred into a 20 mL volumetric flask, and the volume was made up with methanol after the addition of the IS, sulfamerazine.

The sun dried (SUN), shade-dried (SHD), freeze-dried (FZD), and microwave-dried (MWD) cubes of the AP corms were powdered using a pulverizer (Retsch KG, 5657 Haan, West Germany) fitted with a 40 mesh sieve. In addition, a further two AP raw material samples were commercially procured; one of those was labeled to contain powder of dried AP to be filled in capsules and the other was labeled as containing *Hypoxis* extract (10:1). Separate samples of each powder were well-mixed, and 100 mg of each was accurately weighed into a 20 mL volumetric flask; approximately 15 mL of methanol was added, sonicated for 20 min, and allowed to cool for 5 min before making up to volume. Thereafter, 1.0 mL of this solution was added to a 10 mL volumetric flask, 1.0 mL of the IS added, and the volume was made up to 10 mL with methanol. The solution was vortexed for 1 min before filtering through a PVDF (0.45 μm) membrane prior to analysis by HPLC.

An aqueous decoction of AP was prepared according to the usual method used by traditional healers (Sangomas) in the Eastern Cape region of South Africa. Authenticated, fresh AP was peeled and then shredded, and approximately 20 g samples were accurately weighed out into round-bottomed flasks, and 250 mL of water was added. The mixture was boiled for 20 min and strained through a clean muslin cloth. It was then filtered and made up to an approximate volume of 250 mL (weight of AP equivalent to 80 mg fresh AP/mL). One milliliter of this aqueous extract was serially diluted to provide a concentration equivalent to approximately 8 mg AP/mL, the IS solution was added, and the mixture was then filtered using PVDF (0.45 μm) filters prior to injecting 10 μL for analysis by HPLC.

Stability. The stability of methanolic solutions of standards and samples was assessed by storing for 10 days at room temperature (∼23 °C). The stability of standard solutions of hypoxoside was also assessed by storing in the refrigerator at 4 °C.

RESULTS AND DISCUSSION

Isolation and Purity of Hypoxoside. The freeze-dried sample is pure white in color but changes to pale yellow when exposed to sunlight. SEM showed hypoxoside to be uniformly fluffy and not crystalline. The DSC yielded a melting point of 154.6 °C. The infrared (IR) spectrum in the fingerprint region confirmed the identity from the presence of the functional groups of hypoxoside. The ^{13}C NMR and ^{1}H NMR analysis yielded data that corresponded to the values previously reported for hypoxoside (*4*). The isolated compound was readily soluble in methanol and yielded the same UV absorption spectrum as previously reported (*20*). Chromatographic peak purity of hypoxoside using PDA detection data confirmed the absence of any UV-absorbing impurities. It also confirmed hypoxoside peak homogeneity. Analysis of hypoxoside by LC-MS using electrospray ionization provided a fragmentation pattern showing an *m*/*e* peak of 607.18 and other prominent *m*/*e* peaks at 443.56 and 281.02. This indicates the loss of one and two glucose moieties, respectively, to form the aglycone, rooperol.

Method Validation.

Linearity. Calibration curves were constructed by plotting the peak area ratio of hypoxoside/IS vs concentration of hypoxoside on each of three separate days and were linear with determination coefficients higher than 0.99 in all cases (**Table 1**).

Limits of Detection (LOD) and Quantification (LOQ). The LOD (signal/noise > 3) and LOQ (signal/noise > 10) were determined by analyzing serial dilutions of known concentrations of hypoxoside standard solutions. With PDA detection, the spectra of both the hypoxoside and the IS showed good absorbance at 260 nm, which was used as the detection wavelength throughout the analyses. The LOD and LOQ were 0.75 and 3.5 μg/mL, respectively. During the recovery study using product D, which was spiked with various concentrations of hypoxoside, the LOD and LOQ were found to be of the same order as above since the signal/noise ratios were similar.

Table 1. Linearity[a]

	$y = mx + c$ linear model	determination coefficient (r^2)	concentration range (μg/mL) ($n = 3$)
day 1	$y = 0.0515x - 0.1346$	0.9998	5, 10, 20, 40, 80, 100
day 2	$y = 0.0572x - 0.1608$	0.9998	
day 3	$y = 0.0366x - 0.0449$	1	

[a] y = peak area ratio; x = concentration; n = number of injections. Each concentration (5, 10, 20, 40, 80, and 100 μg/mL) was injected three times. The LOD and LOQ of hypoxoside were 0.75 and 3.5 μg/mL, respectively, and the relative standard deviation for all data points was less than 3%.

Accuracy and Precision. Accuracy and precision studies were performed and assessed within and between runs using two sets of QC samples, which were separately prepared on each of the 3 days of analysis as previously described. The accuracy of the method was found to be between 97.3 and 103.00%, and the relative standard deviation (RSD) for interday precision was less than 3% (**Table 2**).

Recovery. The recovery of hypoxoside was evaluated to assess the extraction efficiency of the method. Products D and E were chosen for the intraday and interday recovery studies. The first batch of product D (D1) was chosen for the recovery studies and contained 16.35 mg hypoxoside per unit, whereas product E was chosen for use as a "blank matrix" since it did not have any detectable amount of hypoxoside. These formulations were individually and separately spiked with low, medium, and high concentrations of hypoxoside, i.e., 15, 30, and 45 μg/mL, respectively. This was done on three different days, and the analyses were performed in triplicate. Recovery values of product D1 were between 94.39 and 104.49%, and product E recoveries were between 94.18 and 98.53%. The intraday precision for product D1, expressed as % RSD, was better than 6.15% throughout the 3 days, and the interday precision ($n = 3$) was better than % RSD of 5.64. Similarly, good intra- and interday precisions were seen for product E, which was better than % RSD of 3.03 and 2.34%, respectively (**Table 3**). Although the spiked samples used for the recovery studies showed slightly higher % RSD values than data obtained from the QC samples, the data are generally in close agreement, indicating that the method is accurate and precise.

Stability. Methanolic solutions containing hypoxoside were found to be stable on storage at room temperature (∼23 °C) as well as the samples stored in a refrigerator at 4 °C for 10 days.

Application to the Analysis of Samples.

Commercial Formulations. **Figure 2** shows the HPLC-PDA chromatogram of a standard solution (20 mg/L). PDA analysis showed the relevant peaks at 10.23 (hypoxoside) and 4.98 min (IS) to be homogeneous. Extraction efficiency using methanol was investigated by sonicating about 50 mg of product C over a period of 1 h, and samples were withdrawn and analyzed at 10 min intervals. It was found that ultrasonication for 20 min was the minimum time required for optimum extraction of hypoxoside from the capsules. The assay values and the individual label claims of each of the analyzed commercial

Table 2. Accuracy and Precision

day 1				day 2				day 3				
μg/mL				μg/mL				μg/mL				
actual weight	calculated weight	% accuracy	% RSD	actual weight	calculated weight	% accuracy	% RSD	actual weight	calculated weight	% accuracy	% RSD	interday precision % RSD ($n = 3$)
29.68	29.42	99.13	0.24	30.06	30.11	100.19	0.73	30.02	30.18	100.53	0.86	0.73
59.36	57.75	97.30	0.34	60.12	61.92	103.00	0.56	60.04	59.55	99.18	0.34	2.91

Table 3. Recovery

recovery SD ($n = 3$)												
day 1				day 2				day 3				
spiking level (mg/500 mg dosage form)	observed content (μg/mL)	% recovery	% RSD	spiking level (mg/500 mg dosage form)	observed content (μg/mL)	% recovery	% RSD	spiking level (mg/500 mg dosage form)	observed content (μg/mL)	% recovery	% RSD	interday recovery % RSD ($n = 3$)
sample product D												
14.41[a]			0.98	14.29[a]			0.44	14.66[a]			1.86	1.30
29.47 (+15.06)	30.94	104.49	2.00	29.42 (+15.13)	30.60	104.01	6.15	29.77 (+15.11)	28.10	94.39	4.53	5.64
44.53 (+30.12)	44.68	100.33	2.35	44.55 (+30.26)	44.13	99.05	2.72	44.88 (+30.22)	43.86	97.71	0.71	1.32
59.59 (+45.18)	59.14	99.24	3.57	59.68 (+45.39)	58.15	97.43	3.51	59.99 (+45.33)	58.03	96.73	2.29	1.33
sample product E												
a				a				a				
15.06 (+15.06)	14.84	98.53	2.00	15.13 (+15.13)	14.68	97.02	2.34	15.11 (+15.11)	14.79	97.88	0.32	0.77
30.12 (+30.12)	28.56	94.82	0.94	30.26 (+30.26)	28.63	94.61	1.87	30.22 (+30.22)	28.70	94.97	3.03	0.19
45.07 (+45.18)	44.08	97.79	2.25	45.39 (+45.39)	42.75	94.18	2.95	45.33 (+45.33)	44.60	98.38	2.66	2.34

[a] The actual content before spiking the sample 500 mg dosage form. A blank cell means that the amount is below LOD (3.75 mg/500 mg).

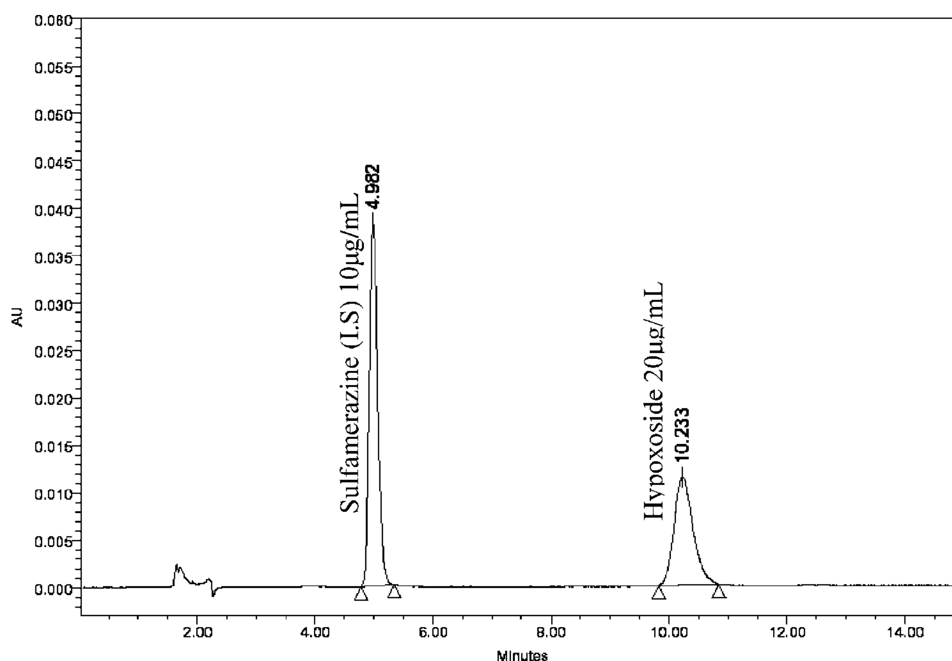

Figure 2. HPLC-PDA chromatogram of standard solution of hypoxoside with the IS.

products are given in **Table 4**. It should be noted that the label claims the presence of AP in one or other form and not hypoxoside per se. Products (C, D1, D2, F, G, H) showed the presence of hypoxoside among which product H (43.32 mg/dosage unit) was found to have the highest content of hypoxoside, whereas the other products (products A, B, D3, D4, E, I, K, and L) did not reveal the presence of hypoxoside despite the label claim indicating a high content of AP. Four batches of product D (D1, D2, D3, and D4) were used in this study, and only batches D1 (16.35 mg/dosage unit) and D2 (21.67 mg/dosage unit) indicated the presence of hypoxoside. Among the products (J−L) in liquid form, only product J contained hypoxoside (344.07 mg/mL). This could be attributed to the fact that this liquid dosage form is labeled as an aqueous alcoholic extraction of AP.

Crude AP Corms. Results from the various drying methods are depicted in **Table 5**. The hypoxoside content, expressed in terms of the percentage dried weight (g/100 g, w/w) indicate that lyophilized AP (FZD) contained the highest content of hypoxoside (10.18%, w/w) followed by MWD (5.85%, w/w), SHD (2.79%, w/w), and SUN (1.29%, w/w). The procured commercially available AP raw materials used in formulations

Table 4. Assay of Formulations

sample no.	sample name	average weight of the formulation (g)	labeled content/weight	hypoxoside (mg/dosage unit)	% average dry wt of hypoxoside (g/100 g)	% RSD
1	product A	0.551	232 mg *H. hemerocallidea*			
2	product B	0.638	15 mg *Hypoxis* extract			
3	product C	0.590	AP 70 mg	12.42	2.60	6.18
4	product D1	0.690	*H. rooperi* 250 mg	16.35	2.89	5.64
5	product D2	0.660	*H. rooperi* 250 mg	21.67	4.09	3.22
6	product D3	0.740	*H. rooperi* 250 mg			
7	product D4	0.664	*H. rooperi* 250 mg			
8	product E	0.599	*Hypoxis* extract 12 mg			
9	product F	1.214	*Hypoxis* powder (AP) 275 mg	18.75	1.60	5.17
10	product G	1.117	*Hypoxis* powder 200 mg	14.05	1.26	6.27
11	product H	1.099	*Hypoxis* powder 200 mg	43.32	3.94	5.07
12	product I	1.160	*Hypoxis* (AP) 200 mg			
13	product J	50 mL	*H. rooperi*, ethanol, and water	344.07 (mg/mL)	34.41 (g/100 mL)	2.30
14	product K	500 mL	extract of 500 mg *Hypoxis*/7.5 mL			
15	product L	500 mL	2 teaspoons/extract of 1000 mg *Hypoxis*			

Table 5. Assay of Corms and Preparations

sample no.	sample name	% average dry wt (g/100 g) of hypoxoside	SD	% RSD
1	FZD	10.18	0.52	5.14
2	SHD	2.79	0.12	4.19
3	SUN	1.29	0.03	2.36
4	MWD	5.85	0.29	3.43
5	commercial AP decoction			
6	extemporaneous traditional decoction	6.13	0.39	6.48
7	raw material 1 *Hypoxis* extract (10:1)			
8	raw material 2 (NRP-158)			
9	raw material 3 (NRP-166)	0.61	0.03	5.85
10	raw material 4 (NRP-167)	9.79	0.36	3.71

of AP dosage forms were also analyzed. Among the four raw materials (raw materials 1−4), only 3 and 4 contained quantifiable amounts of hypoxoside.

Traditional Extract of AP. The analysis of the aqueous extract prepared using the preparation procedure suggested by the traditional doctors (Sangomas) was also analyzed for hypoxoside content. From the survey carried out with different traditional doctors in this region, who are in the practice of prescribing AP, we calculated the dose level. An average amount of about 20 g of freshly shredded AP boiled in 250 mL of water was prescribed for daily consumption for patients to boost their immunity against various ailments. In 2006, Steenkamp et al. (*21*) also mentioned a daily dose intake of approximately 37 mg/mL AP decoction three times a day but did not specify the volume administered. The content of hypoxoside was found to be 6.13% (**Table 5**, sample 6) of the mass of fresh AP, which equates to an average daily consumption of about 1.22 g hypoxoside/patient. No hypoxoside was found in the aqueous extract of AP sold by unqualified vendors, which was meant for ready consumption by patients (**Table 5**, sample 5).

Conclusions. The proposed method for the isolation of hypoxoside provided an excellent yield of the compound. This paper describes a simple, accurate, and precise HPLC-UV method to quantitatively determine hypoxoside and which can be readily applied to analyze raw materials as well as formulations containing AP. In addition, sample preparation and assay procedure for the formulations and for AP raw material were rapid and repeatable and can be used for the QC and routine assay of AP and formulations thereof. The results indicated that vast discrepancies in hypoxoside content exist among the various materials and products that were analyzed. These results thus clearly suggest that suitable QC measures need to be implemented to ensure consistent quality of marketed products.

The study of traditional AP preparations also showed large differences in the content of hypoxoside in the commercially available AP preparation as compared to the extemporaneously prepared aqueous decoction. A further notable finding is that the content of hypoxoside in the latter preparation is considerably higher than in any of the commercially available formulations analyzed. This is, indeed, food for thought considering that if hypoxoside is associated with pharmacological activity, the various commercially available products studied would not be expected to provide the necessary treatment for which AP is indicated.

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