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Phytochemical and Microscopic Analysis of Tubers of *Ipomoea mauritiana* Jacq. (Convolvulaceae)

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ABSTRACT

Tubers of *Ipomoea mauritiana* that are used as Vidari in Ayurveda are important ingredients in popular Ayurvedic nutraceutical products such as *Chyavanprash*. The objective of the current study was to develop distinct phytochemical and microscopy protocols and standards that can be used in quality control of the crude drug. Chromatographic analyses of methanol extracts of the tuber gave typical fingerprints, where Scopoletin, a standard reference marker, resolved at R_f 0.56 under TLC and at Rt 21.5 min under HPLC conditions. Three bright blue fluorescent bands at R_f 0.70, 0.56 and 0.45 under the TLC conditions were characteristic of the raw drug. Scopoletin was quantified to be 0.029–0.034 % using the HPTLC and HPLC methods. Powder microscopic features of authentic tubers of *Ipomoea mauritiana* were characterized by the presence of cluster crystals, simple and compound starch grains with a range of 10–50 µm and orangish-brown cell fragments of laticiferous elements. Longitudinal sections showed the laticifers (latex cells) of articulated anastomosing type and the transverse section of the tubers were mostly characterized by the specific successive cortical cambial activity.

Keywords: Ayurveda, HPTLC, HPLC, *Ipomoea mauritiana*, Microscopy, Scopoletin.

INTRODUCTION

Ipomoea mauritiana Jacq. (Convolvulaceae) is a glabrous twining shrub with large tuberous root and leaves that are palmately (5–9) lobed, to 8cm long. It is occasionally found in semi-evergreen forests, mostly in open areas (1–2). The tubers of the species are considered as restorative of high value in traditional medicine (3). Many of the Ayurvedic industries use *Ipomoea mauritiana* as Vidari instead of the permitted raw drug *Pueraria tuberosa* (Roxb. ex Willd.) DC. The Ayurvedic Pharmacopoeia of India correlates *Ipomoea mauritiana* as Kshiravidari (4). Tubers of *Vidari* are used in more than 45 formulations of Ayurveda, and in many instances also used as single drug. Vidari is also an important component of the popular ayurvedic formulation *Chyavanaprasha* (5). It is useful as aphrodisiac, cardiotonic, demulcent, diuretic, refrigerant, galactogogue and tonic (6). It is used in consumption, emaciation, enteric fever and spermatorrhoea (7). Taraxerol, Taraxerol acetate, β-Sitosterol, Scopoletin and 7-O-β-D-Glycopyranosyl scopoletin (scopolin) have been isolated from the methanol extract of root tubers (8). Even though scopoletin has been reported in methanolic extract of *Ipomoea mauritiana* tubers it has not been quantified. We have used scopoletin as an important bioactive marker and quantified it in tubers of *Ipomoea mauritiana*. Scopoletin belongs to the class of coumarin (6-methoxy-7-hydroxy coumarin) type of compounds and is reported in at least 27 families (9) including Convolvulaceae members like *Convolvulus pluricaulis*, *Evolvulus alsinoides, Clitoria ternatea* and *Canscora*

decussata (10). Scopoletin is a fluorescent compound with λ_{max} 230, 254, 260, 298, 346 nm (11). Scopoletin is reported to possess anti-inflammatory, immunomodulatory and antioxidant activity (12–14). The present investigation reports pharmacognostic, phytochemical profiles and quantification of Scopoletin content in the tubers of *Ipomoea mauritiana.*

MATERIALS AND METHODS

Plant material

Mature tubers of authentic *Ipomoea mauritiana* were collected from Maharashtra and Kerala. The tubers were authenticated by qualified plant taxonomists from Foundation for Revitalisation of Local Health Traditions (FRLHT), Bangalore, India. Voucher specimens were deposited at FRLH Herbarium, Bangalore [Herbarium collection No: 107576] and Raw Drug Accession numbers L/07/07/050, L/07/10/024, L/07/11/010, L/08/05/001, L/08/06/001. The tubers were cleaned, chopped into 1cm cube and dehydrated at 45 ± 2 °C in a dehydrator. The dried pieces were coarsely powdered with the help of domestic electrical mixie and the powder was passed through sieve No.10 for phytochemical extraction. For powder microscopy the powder was passed through Sieve No. 22 and not more than 10% through Sieve No. 44.

Microscopy

Microscopic analysis was carried out according to the standard protocols mentioned in Quality Control Methods for Medicinal Plant Materials (1998) (15). The anatomical features such as cambial activity were observed on Transverse Sections (T.S.) and laticiferous system through Longitudinal Sections (L.S.). The powder was subjected to various mounts to observe for authenticating features such as starch grains, cluster crystals, latex cells (laticifers), vessel element types and cambial activity of the tubers. To study anatomy and histology of the tubers free-hand sections of fresh materials were taken and observed under a Compound Trinocular Microscope (Labomed, Bangalore). Photographs were captured using Sony Digital Camera (DSC-W5).

Reagents and chemicals

Standard Scopoletin (90.5 % pure by HPLC) was gifted by Dr.P.S.Rao, Warangal University, Andhra Pradesh. All solvents were supplied by Qualigens (Mumbai, India) of HPLC grade. Chromatography was performed on 20 cm × 10 cm pre-coated silica gel 60 F254 TLC plates from Merck (Darmstadt, Germany)

Extraction procedure

Five grams of coarse powder of dried tubers of *Ipomoea mauritiana* was successively extracted using Petroleum ether, hexane, ethyl acetate, chloroform, methanol and water by Soxhlet apparatus. The extracts were dried under reduced pressure. The dried extract was reconstituted to 10mL in the respective solvents. These extracts were used for qualitative phytochemical analysis. 2.5 – 5g of coarse powder of *Ipomoea mauritiana* tubers were refluxed in methanol until colourless at 50 °C in water bath. The extract was dried under reduced pressure. The dried residue was re-dissolved in 10 ml methanol and used for TLC and HPLC analysis.

Stock solution of Scopoletin

Stock solution of standard scopoletin was prepared by dissolving 2 mg of standard Scopoletin in 10 ml of methanol in a standard volumetric flask to obtain a concentration of 0.2 mg / mL. This was stored in a refrigerator at a temperature of ± 4°C and brought to room temperature before use.

Qualitative Phytochemical Analysis

Qualitative analysis to determine the presence or absence of phytoconstituents was done on the successive extracts as per the procedure of Mukherjee (2002) (16).

HPTLC Analysis

Analysis was performed on 20 cm x 10 cm TLC silica gel 60 F_{254} plates. The different standards and *Ipomoea mauritiana* sample solutions were applied on the plate by means of Linomat 5 (CAMAG, Switzerland) automated spray-on band applicator equipped with a 100 µL Hamilton syringe and operated with the following settings: band length 8 mm, dosage speed 150 nL^{-1}, distance between bands 15.5 mm, distance from the plate edge 15 mm, and distance from the bottom of the plate was 10 mm. Development of the plates was carried out allowing 10 min for solvent saturation of the twin-trough chamber (CAMAG, Switzerland) at ambient temperature. A solvent system consisting of ethyl acetate: methanol: water: ammonia (13:5:1.8:0.2) was used as mobile phase. Total volume of solvent mixture was 20 mL and the migration distance was 80 mm. After development, the mobile phase was evaporated from the plate by drying in a fume hood for 10 min. The compounds of interest were viewed under visible light, at 254 nm, 366 nm and after derivitization with Anisaldehyde — Sulphuric acid reagent consisting of anisaldehyde — glacial acetic acid-methanol-Con. H_2So_4 (0.5:10:85:5, v/v/v/v). The plate was then dried

with a hair dryer and placed for 10 min in an oven at 110°C. The images were recorded using Reprostar 3 (CAMAG, Switzerland). R_f values of the markers and the compounds of interest were calculated automatically by winCATS software (version 1.2.3) of CAMAG, Switzerland. Densitometric scanning was performed using TLC scanner 3 on absorbance mode at 346 nm, 20mm/sec scanning speed. The source of radiation was Hg lamp.

Scopoletin content in *Ipomoea mauritiana* was calculated using scopoletin as standard. Calibration plot for standard scopoletin was obtained as integrated peak areas of different amounts (20–100 ng) of scopoletin at 346 nm.

HPLC Analysis

HPLC analyses were done using Shimadzu (Tokyo, Japan) Analytical system with LC-10ATVP pump, a rheodyne injector, SPD-10AVP UV-Visible detector and Class VP 6.12 SP5 integration software. The stationary phase was Shim-pack C_{18} column from Shimadzu (250 × 4.6 mm, 5µm). Standard Scopoletin (25–100 ng) and raw drug (0.23g/mL) were injected using 20µL Hamilton syringe under a gradient methanol: water mobile system (0 min — 100% water: 0% methanol to 45 min – 0% water: 100% methanol) at a flow rate of 1 mL/min. Calibration plot of scopoletin was obtained by injecting different amounts (25–100 ng) of standard scopoletin at 254 nm.

RESULTS AND DISCUSSION

Macro-Morphology:

The tubers were cylindrical to sub-cylindrical or ellipsoid; oblong or globose in outline, with a tapering base. The size was 16–24 cm in length × 8-10 cm in breadth. It had a few thick filial roots laterally. It was dark brownish with milky latex. Inner portion was pale white with starchy mass.

Histology and Anatomy:

The tubers had an irregular wavy outline. A many layered periderm with phellem or cork formed the outermost tissue. There was an inner zone of tangentially elongated phellogen (cork cambium) and phelloderm (secondary cortex). These phelloderm cells had either cluster crystals of calcium oxalate (one cluster per cell) or possessed numerous starch grains of both simple and compound type. There were concentric rings of successively produced cortical vascular cambia; the number of rings ranged with the girth of the tuber. Each cambial ring was made of four to five layers of radially disposed cells, contributing bidirectional cells. Most cambial derivatives on either side of the cambial zone differentiated into parenchyma cells while some on the inside differentiated into perforate tracheary elements in narrow radial patches broadly interspersed with parenchyma cells. Some derivatives on the outside that lie in radial alignment with xylem differentiated into sieve elements and companion cells, again broadly interspersed with parenchyma cells. Thus, unlike in most plants with secondary growth, the cambium in this case produced 'collateral vascular-bundle-like' disposed secondary xylem and phloem elements very characteristically; neither secondary xylem nor secondary phloem had sclereids or fibres. One very characteristic feature of the tuber was the differentiation of laticifers from some of the outer derivatives of the vascular cambium. The laticifers were of the articulated anastomosing type (*Figure: 1*) where several very short latex cells were joined together, with terminal cross-walls intact, to form a highly branched laticiferous vessel system. The contents of these cells were orangish brown in colour.

Powder analysis:

Powder was of light brown shade to dark brown, coarse to moderately coarse. The vessel elements were short with predominantly slit-like lateral wall pitting, although circular to elliptical pitting was also commonly seen; vessel elements were with terminal to sub-terminal, simple, perforation plates (*Figure: 2* & *3*). Parenchyma cells were numerous with plenty of starch grains or with druse-type cluster crystals. Starch grains were spherical to sub-spherical, some with distinct hilum, minute to large size with a range from 10 µm to 50 µm; a few compound starch grains were also noticed. Fragments of orangish-brown cells were seen, which were identified to be portions of laticiferous elements. A few fragments of corky periderm were also noticed. Neither fibres nor sclereids could be seen.

Most characteristics of the tuber that Iyer and Kolammal (1993) (17) described were confirmed by the present study. However, there were no sclereids observed by us unlike what was described by them. Again the lateral wall pitting as belonging to the scalariform and reticulate types mentioned by them was not found since the pitting belonged to psuedoscalariform, slit-like and circular to elliptical crowded types as described by Carlquist (1988) (18). There were no fibres or resin ducts as mentioned by The Ayurvedic Pharmacopoeia of India (2006) (4). However, there was an extensive articulated, anastomosing laticiferous system as described in Rao and Krishnamurthy (1984) (19). Usually such laticiferous systems harbour secondary metabolites such as alkaloids, phytosterols etc.

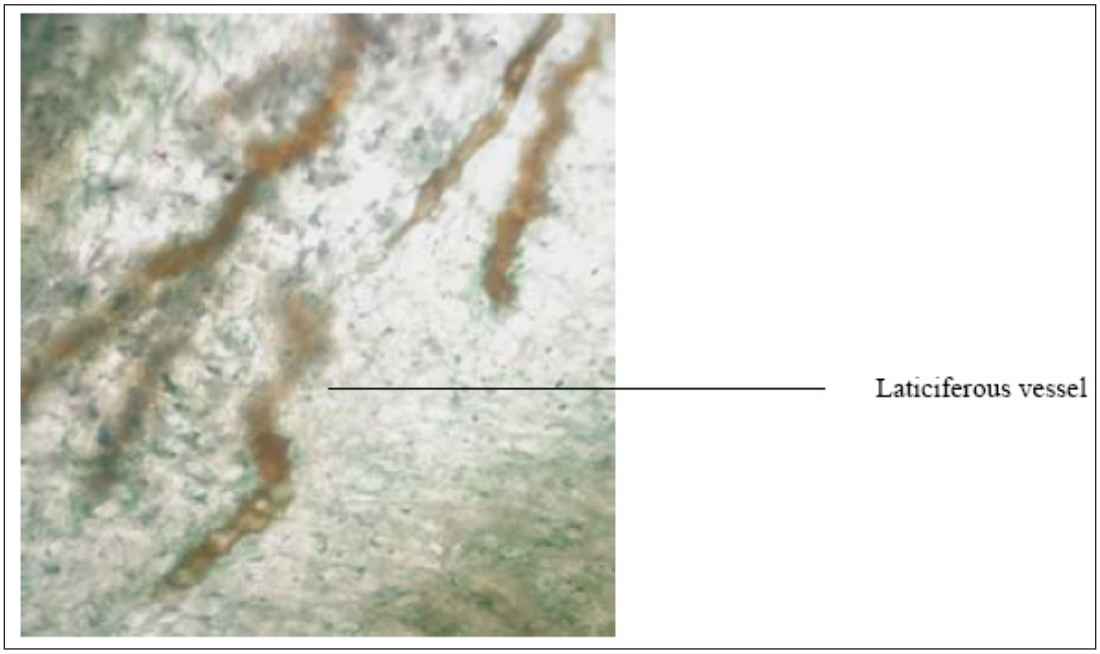

Figure 1: Articulated Anastomosing Laticiferous system (x100)

Figure 2: Vessel element with slit-like and psuedoscalariform lateral wall pitting (x400)

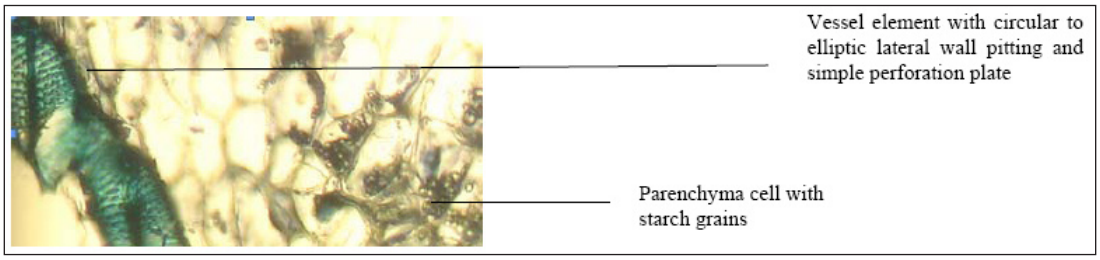

Figure 3: A portion of L.S. of tuber showing vessel elements with circular to elliptical lateral wall pitting and terminal simple perforation plate. Parenchyma cells with starchgrains are also seen (x100)

Table 1: Qualitative Phytochemical Analysis of tubers of Ipomoea mauritiana

Chemical Constituent	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Water extract
Alkaloid-	-	-	+	+	
Carbohydrates	+	+	+	+	+
Glycosides	-	-	-	+	+
Saponins	-	-	-	+	+
Phytosterols	-	-	+	+	+
Fats and oil	+	-	-	-	-
Resins	+	+	-	-	-
Phenolic acids and tannins	-	-	-	-	-
Flavonoids	-	-	+	+	-
Proteins	-	-	-	+	+
Gums & mucilage	-	-	-	+	+

+ : Detectable, - : Not detectable

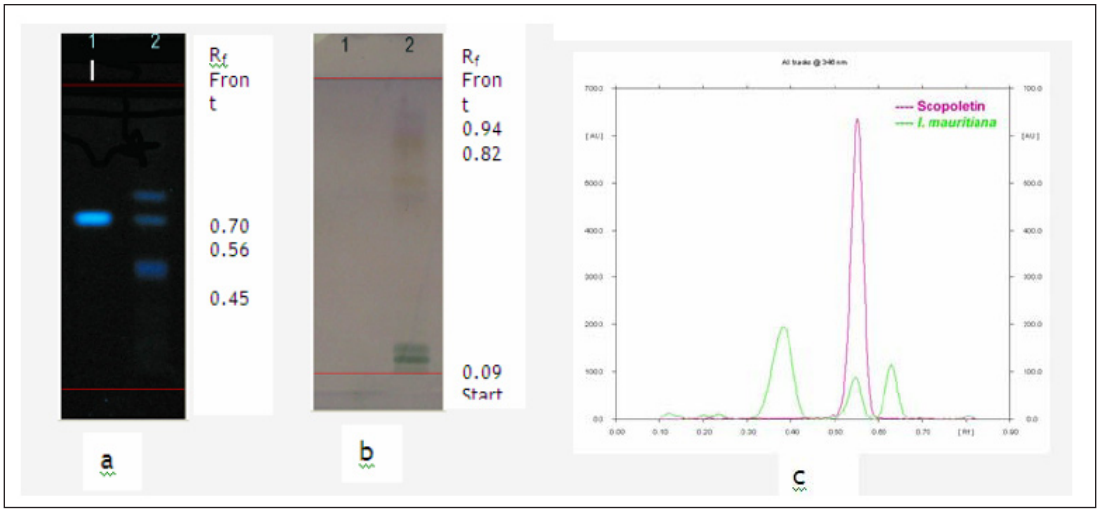

Figure 4 (a) Chromatogram of standard scopoletin (100ng/spot) (Track 1) and methanol extract of Ipomoea mauritiana at 366 nm (Track 2)
(b) Chromatogram of standard scopoletin (100ng/spot) (Track 1) and methanol extract
(c) Densitometry scanning of Scopoletin at 346 nm

Qualitative Phytochemical Analysis

The present study carried out on the plant samples revealed the presence of medicinally active constituents in tubers of *Ipomoea mauritiana* as summarized in *Table 1*. Alkaloids, Glycosides, Phytosterol, Flavonoids, Proteins and Gums and Mucilage were present in Methanol and Water extracts. Carbohydrate was present in all the extracts, Fats and oils, resins were present in Petroleum Ether and Chloroform extracts.

HPTLC

Different compositions of mobile phase for HPTLC analysis were tried in order to obtain high resolution and reproducible peaks. Ethyl acetate: methanol: water: ammonia (13:5:1.8:0.2) was found to be the most suitable mobile phase. One of the major components in the TLC fingerprint of methanolic extract of *Ipomoea mauritiana* was scopoletin with an R_f value of 0.56. Scopoletin showed blue fluorescence under UV 366 nm (*Figure: 4a* & *c*). However, densitometric scanning at 346 nm was found optimal for the band, providing maximum sensitivity. An excellent linear relationship (R^2= 0.998) was obtained within the range of 20–100 ng /spot for scopoletin at 346 nm. Scopoletin content in the tuber was found to be in the range of 0.029 ± 0.0011 % in samples of *Ipomoea mauritiana* as quantified by the HPTLC method. Two other blue fluorescent bands at R_f 0.45 and 0.70 were also observed in any typical fingerpint of *I. mauritiana* (*Figure: 4a* & *c*). After spraying with Anisaldehyde Sulphuric Acid, four bands were observed at R_f 0.92, 0.84, 0.09 and 0.05 respectively (*Figure : 4b*)

HPLC

HPLC chromatogram of standard scopoletin and *Ipomoea mauritiana* extract were standardized. Scopoletin showed a retention time of 21.5 min (*Figure: 5*) linear relationship

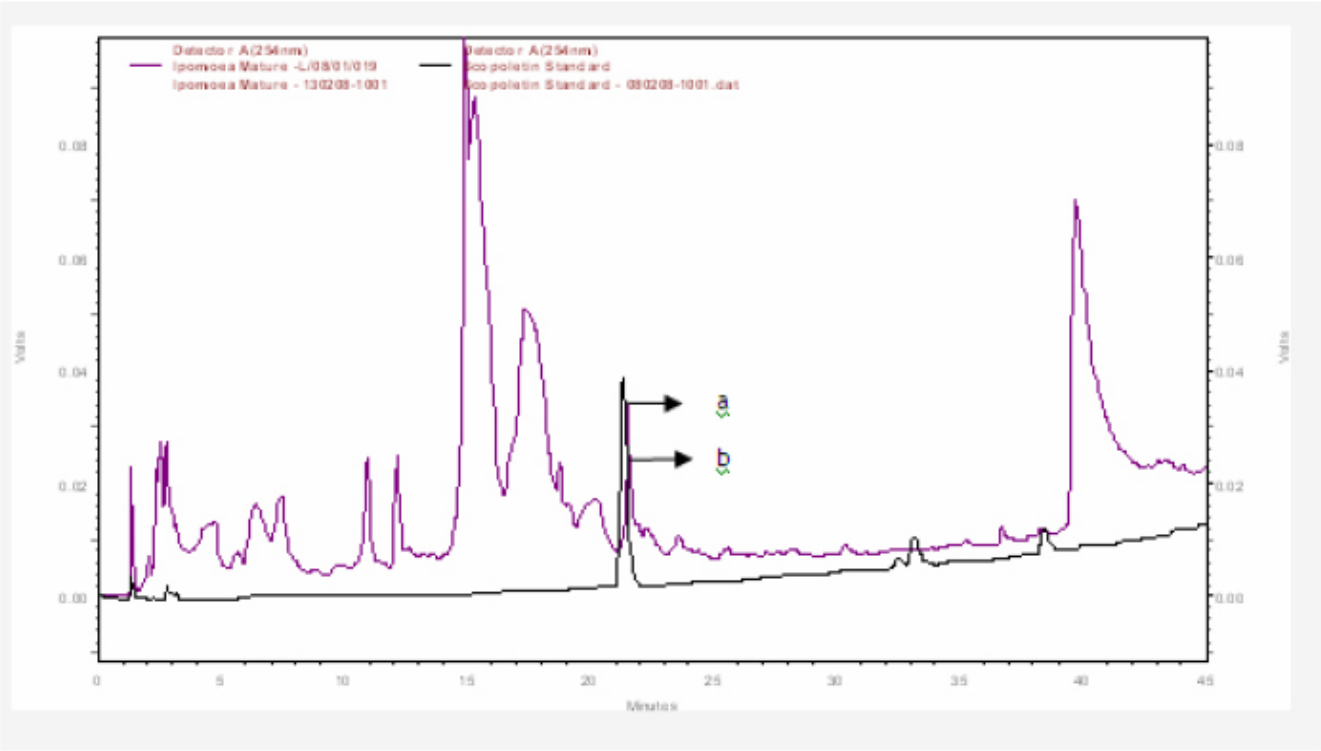

Figure 5: HPLC chromatogram of (a) standard scopoletin and (b) Ipomoea mauritiana Legends

(R^2= 0.9912), obtained within the range of 25–100 ng. Scopoletin content in the tuber was found to be 0.034 + 0.0015 % *as* quantified by the HPLC method.

CONCLUSION

Ipomoea mauritiana is a traditionally valuable raw drug and the tubers are used in several Ayurvedic formulations by the industries. It is important to check the authenticity and quality of the drug to ensure batch-to-batch consistency in performance of the medicine. The curent study has developed protocols and standards using microscopy and phytochemical techniques. The microscopic study revealed the characteristic articulated anastomosing type of laticiferous system and the successive bidirectional cortical cambial activity which would help in quality control of the crude drug Qualitative phytochemical screening of tubers *Ipomoea mauritiana* showed that the tubers were rich in Carbohydrate, Phytosterol, Glycosides, Flavonoids, Saponins along with Alkaloids, Proteins, Gums, mucilage, Fats, oil and, Resins. HPTLC fingerprint of the methanol extract of tubers of *I. mauritiana* showed three characteristic fluorescent compounds at R_f 0.45, 0.56 and 0.70. HPLC fingerprint shows Scopoletin at Rt 21.5 min. Scopoletin content in the tuber was found to be 0.029–0.034 % in HPTLC and HPLC method. The protocols developed are simple, sensitive and precise which can be used for quantification of Scopoletin.

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