

DETERMINATION OF SCOPOLETIN IN *PHYSALIS ALKEKENGY* AND *SOLANUM DULCAMARA* BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

CLAUDIA BUTNARU ^{1*}, ELENA BUTNARU ¹, LAURIAN VLASE ²,
MIHAI IOAN LAZĂR ¹

¹University of Medicine and Pharmacy „Gr. T. Popa”, Faculty of
Pharmacy, 16 University Street, 700115, Iași, Romania

²University of Medicine and Pharmacy „Iuliu Hațieganu”, Faculty of
Pharmacy, 13 Emil Isac, 400023, Cluj-Napoca, Romania

*corresponding author: butclaudia@yahoo.com

Abstract

The paper presents the results of the qualitative and quantitative determination of scopoletin (SLT) in *Physalis alkekengi* and *Solanum dulcamara* (*Solanaceae*) by high-performance liquid chromatography tandem mass spectrometry (HPLC/MS). The plant samples were harvested around Iași on July 2007, July 2008, August-September 2009. Scopoletin was extracted from dried leaves by two methods: with methanol 70% by reflux and with ethanol 70%. Hydrolyzed samples showed significant levels of scopoletin: for *Physalis alkekengi* the values were between 83.32 and 267.90 mg/ 100 g product and for *Solanum dulcamara*: 123.13 and 438.93 mg/ 100 g product. The mean values are lower for *Physalis alkekengi* compared with those for *Solanum dulcamara*.

Rezumat

Lucrarea prezintă rezultatele analizei calitative și cantitative a scopoletinei din *Physalis alkekengi* și *Solanum dulcamara*, familia *Solanaceae*. Determinările s-au efectuat printr-o metodă modernă de cromatografie de înaltă performanță cuplată cu spectrometrie de masă. Probele au fost recoltate din regiuni din jurul municipiului Iași în trei perioade (iulie 2007, iulie 2008, august-septembrie 2009). Scopoletina s-a izolat din frunzele uscate prin două metode: refluxare cu metanol 70% și extracție alcoolică, cu alcool etilic 70%. În probele hidrolizate s-au găsit concentrații semnificative de scopoletină. Pentru *Physalis alkekengi* valorile au variat între 83,32 și 267,90 mg/ 100 g produs, iar pentru *Solanum dulcamara* între 123,13 și 438,93 mg/ 100 g produs. Valorile medii de scopoletină pentru *Physalis alkekengi* sunt mai mici comparativ cu cele pentru *Solanum dulcamara*.

Keywords: scopoletin, *Physalis alkekengi*, *Solanum dulcamara*, HPLC/MS

Introduction

Physalis alkekengi L. (*Solanaceae*) is a perennial herb, spontaneous, present across all the country. All plant parts, except the fruits, are poisonous. In folk medicine, fruits are used for diuretic action and for soothing the pain of kidneys and bladder. In tropical countries the fruit is

used for asthma (Amazon), for its anti-inflammatory action (Colombia) and for the treatment of malaria and hepatitis (Peru). *Solanum dulcamara* L. (*Solanaceae*) is a woody perennial plant with thickened and branched rhizomes. It grows on the banks of streams, rivers, and cuts through forest fences. It is common Romania. All plants parts, especially fruits and seeds are toxic.

In an extensive theme of research it was performed the phytochemical determination on these two species of the *Solanaceae* family. This paper presents the results of the qualitative and quantitative determination of scopoletin (SLT) by high-performance liquid chromatography tandem mass spectrometry (HPLC/MS).

Scopoletin, 7-hydroxy-6-methoxy-coumarin, is known to possess biological activities such as abirritant and anti-tumor, it can quench intrinsic fluorescence of bovine serum albumin and the fluorescence intensity of itself is enhanced. So, SLT is used as a fluorescence probe for the quantitative determination of proteins. SLT acts as a monoamine oxidase inhibitor (MAOI) in mice and may also act in humans, but this has not yet been demonstrated. SLT also inhibits plant growth *in vivo*. SLT showed very good growth inhibitory properties. Recently, it was reported that SLT showed inhibition of nitric oxide synthesis in murine macrophages. The production of nitric oxide by macrophages mediates killing or growth inhibition of tumor cells, bacteria fungi and parasites. Therefore it will be valuable to develop a potent and economic source of SLT [1, 2, 4, 5, 7].

HPLC/MS technique has been widely accepted as the most sensitive and specific method in the identification and quantitative analysis of phyto-constituents and drugs. There is no data in the scientific literature about the SLT content in *Physalis alkekengi* and *Solanum dulcamara* [3, 6].

Materials and methods

Samples of the plants were harvested around Iasi as follows:

Physalis alkekengi: July 2007 - Botanical Garden, July 2008 - Botanical Garden, August-September 2009 - Aroneanu, Bârnova, Breazu, Bucium

Solanum dulcamara: July 2007 - Botanical Garden, July 2008 - Botanical Garden, August-September 2009 – University of Medicine and Pharmacy area Iasi.

Room temperature dried leaves were taken into work, extraction being carried out by two methods for the determination of SLT: extraction

with methanol 70% (v/ v) by reflux (samples were noted „RN” and were subjected to hydrolysis as described above; hydrolyzed samples were noted with „RH”) and extraction with ethanol 70% (v/ v) (samples were noted „TN”; hydrolyzed samples were noted with „TH”).

Samples were analyzed by A HPLC/MS method as such (unhydrolyzed) and after hydrolysis. For the hydrolyzed samples we worked in the following way: one part of extract was treated with a part of 2N HCl and the mixture was kept in a water bath at 80°C for about 40 minutes. After hydrolysis, samples were filled with distilled water to a volume of 2 parts and were injected into the chromatographic system. The samples as such (unhydrolyzed) were diluted 1: 1 with distilled water before the HPLC analysis.

In order to perform the qualitative determination it was used a standard of scopoletin (Roth), figure 1.

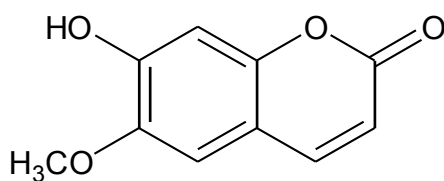


Figure 1
Chemical structure of scopoletin

Equipment

The HPLC/MS system consisted in a HP 1100 Series binary pump, autosampler HP 1100 Series, thermostat HP 1100 Series, mass spectrometer Agilent Ion Trap 1100 SL.

HPLC conditions:

- Column : Zorbax SB-C18 100 mm x 3.0 mm i.d., 3.5 μ m (Agilent, USA)
- The mobile phase: mixture formic acid 0.1% (V/V) and methanol 68/32 (V/V), isocratic elution; flow rate: 1 mL/min, temperature: 40°C
- Detection: MS – isolation and ion fragmentation with m/z 193, adequate for the protonated scopoletin, then ion monitoring with m/z 133 from MS/MS spectrum of the analyte.

MS conditions: ion source: APCI (atmospheric pressure chemical ionisation); ionization mode: positive; nebuliser: nitrogen, pressure: 70 psi; drying gas: nitrogen, flow rate: 7 L/min, vaporiser: 425 °C, drying temperature: 325 °C; capillary potential: -3500 V; mass analysis: m/z 193> 133.

Results and discussion

HPLC/MS analysis of SLT standard:

The full-scan spectrum of a SLT solution is presented in figure 2. According to the molecular mass of SLT ($M=192.1$) and in accordance with the positive ionization mode, the expected ion would be the ion with m/z 193.1, adequate for the protonated scopoletin.

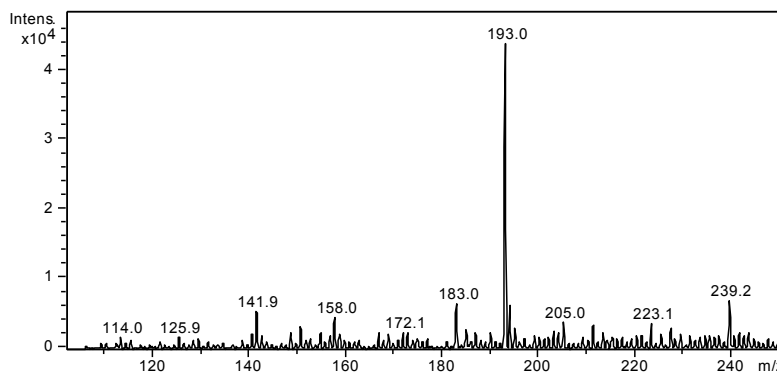


Figure 2
Full-scan spectrum of scopoletin

A further fragmentation of the pseudo-molecular ion with m/z 193 was performed, obtaining the corresponding MS/MS (fragmentation) spectra (figure 3).

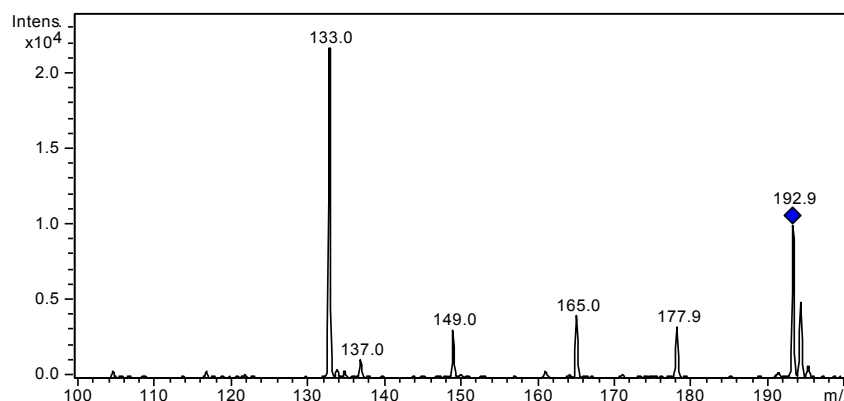


Figure 3
MS/MS spectrum of scopoletin

Note that by fragmentation the protonated scopoletin gave five main fragments with m/z 133, 137, 149, 165, 178 respectively. The ion with

m/z 133 was chosen for quantification, being the most intense. The calibration curve of SLT was performed in the concentration range from 16.3 to 261.6 ng/mL.

LC/MS analysis of SLT from *Physalis alkekengi* and *Solanum dulcamara*

The results are expressed in mg SLT/ 100 g product and are presented in tables I (for *Physalis alkekengi*) and II (for *Solanum dulcamara*).

Table I
Concentration of scopoletin in *Physalis alkekengi*

Sample	Location	Harvesting period	mg SLT / 100 g product
RN1	Aroneanu	August 2009	0.00
RH1			150.40
TH1			107.94
RN2	Bârnova	September 2009	0.00
RH2			199.03
TH2			148.56
RN3	Breazu	September 2009	0.00
RH3			135.10
TH3			95.44
RN4	Bucium	September 2009	0.00
RH4			83.32
TH4			86.53
RN5	Botanical Garden	July 2008	0.00
RH5			161.30
TH5			255.14
RN6	Botanical Garden	July 2007	0.00
RH6			267.90
TH6			179.62
Maximum			267.90
Minimum			155.86
Mean			83.32

where: RN = samples extracted with methanol 70% (v/v); RH= hydrolyzed samples extracted with methanol 70% (v/v); TH=hydrolyzed samples extracted with ethanol 70% (v/v)

Scopoletin not found in the unhydrolyzed samples "RN", likely it is entirely esterified with organic acids. Samples "RH" and "TH" in the two plants show significant levels of SLT. Maximum, minimum and mean values are lower for *Physalis alkekengi* comparative with those for *Solanum dulcamara*. The best extraction was performed by reflux with methanol 70% both for *Physalis alkekengi* and for *Solanum dulcamara* (figures 5 and 6).

The values representing concentrations of SLT are very different; we cannot perform a study depending on the harvesting period.

Table II
Concentration of scopoletin in *Solanum dulcamara*

Sample	Location	Harvesting period	mg SLT / 100 g product
RN7	Botanical Garden	July 2007	0.00
RH7			186.89
TH7			123.13
RN8	Botanical Garden	July 2008	0.0
RH8			220.70
TH8			132.53
RN9	UMF area Iasi	August 2009	153.73
RH9			419.79
TH9			428.80
RN10	UMF area Iasi	September 2009	188.79
RH10			438.93
TH10			415.68
		Maximum	438.93
		Mininum	295.81
		Mean	123.13

where: RN = samples extracted with methanol 70% (v/v); RH= hydrolyzed samples extracted with methanol 70% (v/v); TH =hydrolyzed samples extracted with ethanol 70% (v/v)

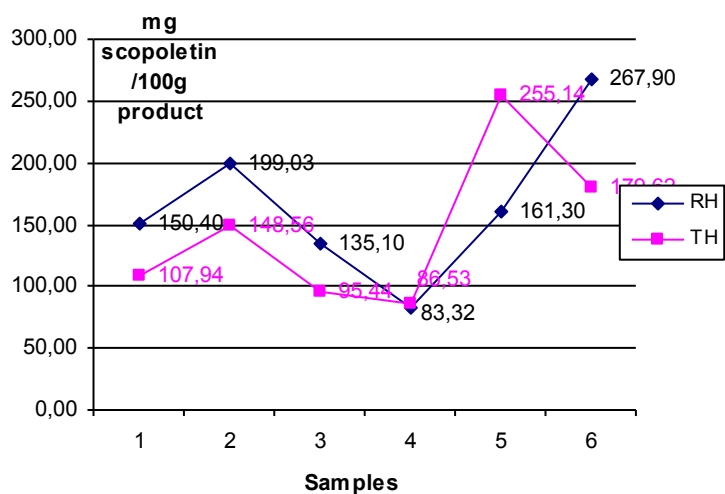


Figure 5
Analysis of the concentrations of scopoletin in *Physalis alkekengi*, depending on the method of extraction

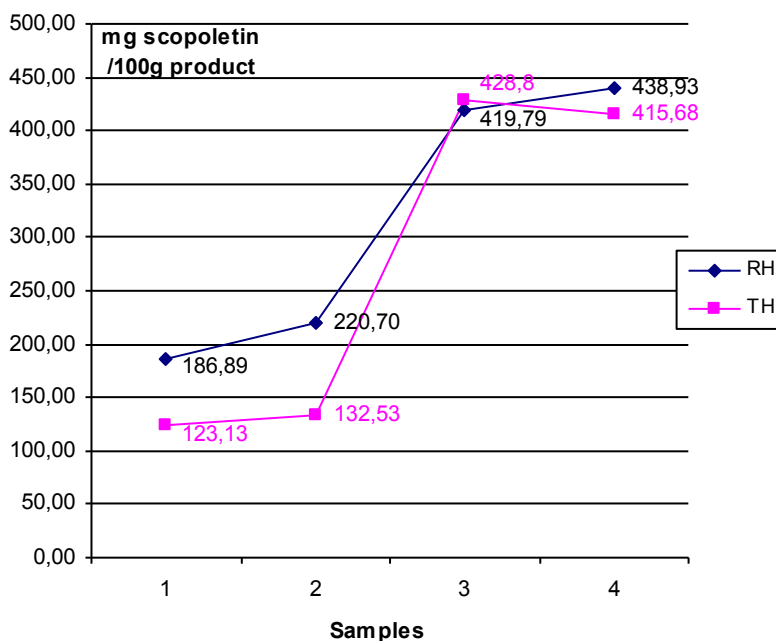


Figure 6
Analysis of concentrations of scopoletin in *Solanum dulcamara*,
depending on the method of extraction

Conclusions

We identified and assayed scopoletin in two species of the *Solanaceae* family: *Physalis alkekengi* and *Solanum dulcamara*. Scopoletin was extracted from the dried leaves by two extraction methods: with methanol 70% (v/v) by reflux and extraction with ethanol 70% (v/v). Extracts were analyzed by HPLC/MS. Scopoletin was not found in the non-hydrolyzed samples, it is likely to be entirely esterified with organic acids. The hydrolyzed samples of the two plants show significant levels of scopoletin: for *Physalis alkekengi* values were between 83.32 and 267.90 mg/ 100 g product and for *Solanum dulcamara*: 123.13 and 438.93 mg/ 100 g product. The best extraction was performed by reflux with methanol 70%. Values of scopoletin concentration are lower for *Physalis alkekengi* compared with those for *Solanum dulcamara*.

References

1. D'Arcy W.G., Solanaceae: biology and systematic, Columbia University Press, New York, 1986
2. Dornberger K., The potential antineoplastic acting constituents of *Physalis alkekengi* L. Var. *franchetii* Mast.. *Pharmazie*, 1986, 41(4), 265-268
3. Moffat A.C., Osselton M.D., Widdop B., Clarke's analysis of drugs and poisons, Pharmaceutical Press, New York, 2004
4. Panda S., Kar. A., Evaluation of the antithyroid, antioxidative and antihyperglycemic activity of scopoletin from *Aegle marmelos* leaves in hyperthyroid rats. *Phytother Res.*, 2006, 20(12), 1103-1105
5. Raible D.G., Mohanty J.G., Jaffe J.S., Stella H.J., Sprenkle B.E., Glaum M.C., Schulman E.S., Hydrogen peroxide release from human eosinophils on fibronectin: scopoletin enhances eosinophil activation. *Free Radic. Biol. Med.*, 2000, 28(11), 1652-1660
6. Vlase L., Mindruta I., Muntean D., Iacob D., Leucuta S.E., High throughput quantification of quinidine in human plasma by LC/MS/MS for therapeutic drug monitoring, *Farmacia*, 2010, 58(2), 184-189
7. Xia Y., Dai Y., Wang Q., Liang H., Determination of scopoletin in rat plasma by high performance liquid chromatographic method with UV detection and its application to a pharmacokinetic study. *J Chromatogr B Analyt Technol Biomed Life Sci*, 2007, 857(2), 332-336

Manuscript received: January 20th 2010