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Quantification of Triterpene Saponins of *Hedera Helix* Spray-Dried Extract by HPLC

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Abstract: Traditionally, ivy leaf (*Hedera helix)* has been used for a large number of ailments. In the 19th century, water-based extract of young leaves was used for respiratory diseases. The aim of the current study was to quantify the triterpene saponin named hederacoside C of ivy leaf spray-dried extract of *Hedera helix* using high-performance liquid chromatography (HPLC) method coupled with UV-visible detector. The solvent system consisted of solvent A (phosphoric Acid 85%: acetonitrile: water with the ratio of 2: 140: 860) and solvent B (phosphoric acid 85%: acetonitrile with the ratio of 2:998). A gradient elution was used for separation at 205 nm at a flow rate of 1.5 mL/min. The separation was performed using a C18 column at a temperature of 40°C. The method was validated as per ICH guidelines for precision, accuracy, recovery, ruggedness, and robustness. The method was found to be precise, accurate, robust, and reproducible according to the guidelines of United States Pharmacopeia 2014 and European Pharmacopeia 8.0. The content of triterpene saponins was found to be 17.6%. Despite many studies reported on the method development and quantification of compounds of *Hedera helix*, there is insufficient work reported on the spray-dried extracts of this plant. This study quantifies the hederacoside C from the spray-dried extract of the plant by developing an accurate, cheap, robust, and precise method. The proposed method can be of significant usage in the pharmaceutical industry.

Keywords: *Hedera helix*, spray drying, triterpene saponins, hederacoside C, high-performance liquid chromatography.

高效液相色谱法定量测定常春藤喷雾干燥提取物的三萜皂苷

摘要：传统上，常春藤叶（常春藤螺旋）已被用于治疗大量疾病。在 19 世纪，幼叶的水基提取物被用于治疗呼吸道疾病。本研究的目的是使用高效液相色谱(高效液相色谱)方法和紫外可见检测器对常春藤叶喷雾干燥提取物常春藤苷 C 中的三萜皂苷进行定量。溶剂体系由溶剂一个（磷酸 85%：乙腈：水 2：140：860）和溶剂乙（磷酸 85%：乙腈 2：998）组成。梯度洗脱用于在 205 纳米处以 1.5 毫升/分钟的流速进行分离。使用 C18 柱在 40°C 的温度下进行分离。该方法已根据非物质文化遗产指南对精密度、准确度、回收率、耐用性和稳健性

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进行了验证。根据美国药典 2014 和欧洲药典 8.0 的指南，该方法被证明是精确、准确、稳健和可重复的。三萜皂苷含量为 17.6%。尽管许多研究报告了常春藤化合物的方法开发和定量，但关于这种植物的喷雾干燥提取物的工作报道不足。本研究通过开发一种准确、廉价、稳健和精确的方法，从植物的喷雾干燥提取物中量化常春藤苷 C。所提出的方法可以在制药工业中具有重要用途。

关键词：常春藤，喷雾干燥，三萜皂甙，常春藤苷 C，高效液相色谱。

1. Introduction

Hedera helix belongs to genus Hedera and the family Araliaceae (ginseng), found present in Europe [1]. It has almost 70 genera and 700 plant species [2]. The common names of the species include Ivy, common ivy, and English ivy [3]. Different morphologies of the species and genome are from different areas includes continental France, southern Italy and typical Europe. It gives better results at the salinity level of 2.5dS/m [4, 5]. Traditionally it has been used for many diseases such as pain, diarrhea, rheumatoid arthritis, lung infection, and whooping cough. Its decoction was reported for anti-lice, anti-scabies, and as a sun screen. Its sap was used to treat aches of the head and ear [6]. In the 19th century, water-based extract of its young leaves was used for respiratory diseases due to its expectorant and bronchorelaxant properties [7].

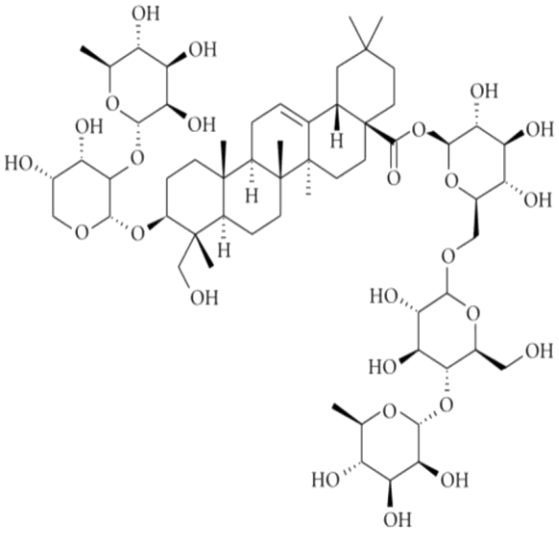

Fig. 1 Chemical structure of hederacoside C

The German Commission E approved it as an effective drug for long-term inflammatory diseases of the lung, sputum producing coughs owing to bronchorelaxant and expectorant activities [8, 9]. However, its leaves and sap contain saponins and polyacetylenes that produce irritation and allergic skin reaction [10]. Ivy leaf reported to contain triterpene saponins, which include derivatives of hedergenin as bidesmosides, hederasaponin C (hederacoside C), and hederasaponin B, D,E,F,G,H, I monodesmosides: α-hederin [11, 12].

A method using high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was developed for the determination of hederacoside C in rat plasma [11]. HPLC-DAD method was used for simultaneous determination of saponins and flavonoids in Ivy leaf extract [12]. Hederacoside C European Pharmacopeia identified hederacoside C by thin layer chromatography [13]. HPLC-PDA profiling of *Hedera helix* leaves collected from different regions was performed by Dhawan et al. [14]. Blumenthal et al. explained Hederacoside C in methanolic extract of ivy leaf by LC-MS [15]. Another study quantified six phenolic compounds in ivy leaf extract using HPLC [16]. One more study determined hederacoside C in Ivy-thyme cough syrup [17, 18]. Spray-dried extracts (SDE) manufactured by spray drying technology are unique and have multiple advantages in the pharmaceutical industry [19]. This is the latest and utmost technology in pharmaceutical, food, and other industries and is currently obtaining the interest of investors and manufacturers [20]. Spray drying produces good quality products with minute impact of water action and mass reduction resultant with easy portability and storage [21]. No study on the analysis of hederacoside C from spray-dried extract of ivy leaf has yet been presented. So, the current study was established for the quantification of hederacoside C from spray-dried extract of Ivy leaves using HPLC-UV method.

2. Methodology

2.1. Formulation of Spray-Dried Extract of *Hedera Helix*

Crushed leaves of the plant were mixed in a solvent mixture composed of purified water and ethanol (1:2.33) for extraction. The crushed leaves and solvent mixture were taken in a ratio of 1:5, and the mixture was heated for 120 ± 5 min while maintaining the temperature up to 77 ± 5°C and pressure up to 430 ± 30 mmHg. The resulted thick mixture was decanted. After decantation, the extract was collected in a receiving container. The next stage was filtration through the

sieve of 350 mesh size into a holding container. The fourth step was evaporation that began when temperature approximated to 90°C and was up to 100°C in evaporation columns throughout the evaporation process. The thick liquid extract was carried toward the feeding container of spray dryer and spray drying process was carried out at 183 ± 2°C. After completion of drying, the extract was blended in a blender at a speed of 20 ± 2 rpm. At the end spray-dried extract in the powder form was collected in an air-tight and double-seal polybag. Place this sealed bag into another polybag with 40 gm silica gel. The powder was water-soluble and yellowish brown to brown in color and partly soluble in water.

2.2. HPLC Analysis

2.2.1. Instrumentation and Reagents

The HPLC system Dionex Ultimate 3000 is equipped with UV-visible detector and binary pump. The analyses were performed on a C18 column (YMC Hydrosphere L 150 × 4.6 mm i.d., 5 μm) protected by a guard column. Methanol, acetonitrile, phosphoric acid were of HPLC grade and purchased from Sigma-Aldrich. Sartorius syringe filters were used for filtration of the sample and standard before injection into the system. Standard hederacoside C was purchased from Sigma-Aldrich. *Hedera helix* leaves for preparation of extract were procured from Herbion Lahore plant. The plant was botanically identified from the botany and R&D department of Herbion, Karachi Pakistan. For further authentication and identification, plant specimens were deposited in the R&D Department at Herbion for future references.

2.2.2. Sample Preparation

The sample of SDP (spray dried powder) was prepared by dissolving 500 mg (weight) of SDP in diluted methanol (20% water) in 50-mL volumetric flask. The mixture was sonicated for 15 min and diluted to 50 mL with diluted methanol. Finally, solution was filtered through 0.45 micron syringe filter.

2.2.3. Standard Preparation

Standard hederacoside C weighing 10 mg was placed in 10-mL volumetric flask, dissolved in diluted methanol (20% water), sonicated for 2–3 min and filtered.

2.2.4. Analytical Procedure

A volume of 20 μL was injected for both test and standard solution. The mobile phase (composition mentioned in Table 1) was run at a flow rate of 1.5 mL/min, detection was performed at 205 nm, column temperature was maintained at 40°C and gradient elution system (mentioned in Table 2) was used.

Table 1 Mobile phase composition

Mobile Phase	A (V/V/V)	B (V/V)
Phosphoric Acid	2	2
Acetonitrile	140	998
Water	860	-

Table 2 Gradient elution program of mobile phase for the quantification of biomarker in the SDE of Ivy leaf

Time (Min)	Mobile Phase A	Mobile Phase B
0	100	0
30	60	40

2.3. Method Validation

For validation of the analytical procedure, ICH guidelines were followed. For accuracy, recovery, precision, repeatability, and reproducibility, the guideline CPMP/ICH/281/95 was followed. The method has been validated for the detector's linear responses in the range of 50–400 μg/mL (06 injections). The linear regression analysis was done by plotting the average peak area (mAU/min) against concentration (μg/mL), and the correlation coefficient was calculated. Instrument precision was measured by injecting standard solution of one concentration six times and results were presented in RSD form that was less than 2%. Method precision was determined by the evaluation of three different standard concentrations on inter and intra-day. Recovery studies were used to evaluate the accuracy of the method. A known amount of standard was added to a sample of known concentration at 80, 100, and 120% of standard and analyzed in triplicate, and percent recovery was calculated. Ruggedness was determined by changing the analyst and robustness was checked by evaluating the effect of the analytical parameter's deviation on retention time. The limit of quantification (LOQ) and limit of detection (LOD) were determined using regression analysis according to the ICH guidelines, as follows: LOD = 3.3 (SD_y/S), LOQ = 10 (SD_y/S) where SD_y for standard deviation of y-intercept and S for the slope of the calibration curve.

3. Results and Discussion

In the current study, we quantified and standardized the triterpene saponins in ivy leaf SDE by HPLC fingerprinting.

3.1. Estimation of Triterpene Saponins by HPLC

HPLC is the accurate and precise method for quantitative analytical studies. Hederacoside C was quantified using the HPLC method. The chromatographs for hederacoside C standard and extract are shown in Fig. 2 and 3, respectively. The

peak of the hederacoside C reference standard was eluted at 20.1 min, and a peak with a similar retention time was also notified in ivy leaf SDE. The content of biomarker was calculated to be about 17.61% (w/w).

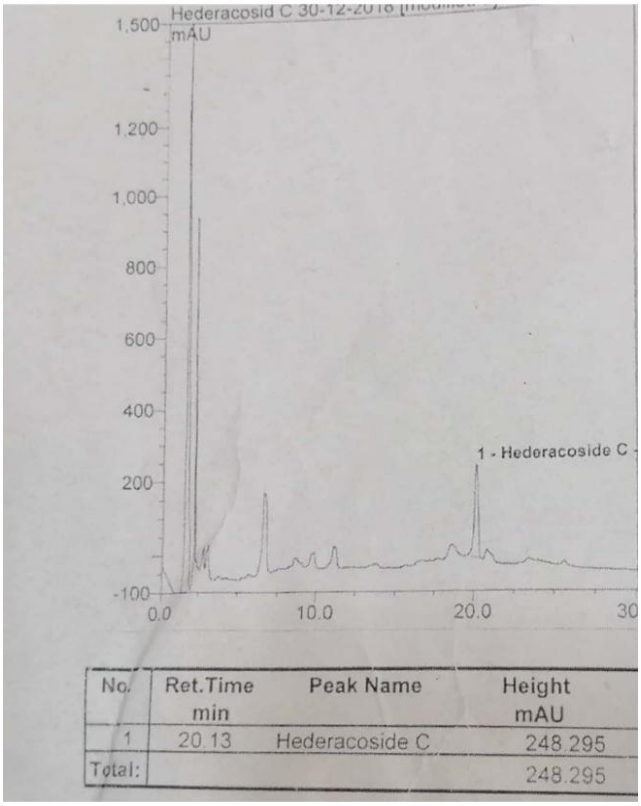

Fig. 2 Chromatogram showing the peak of hederacoside C

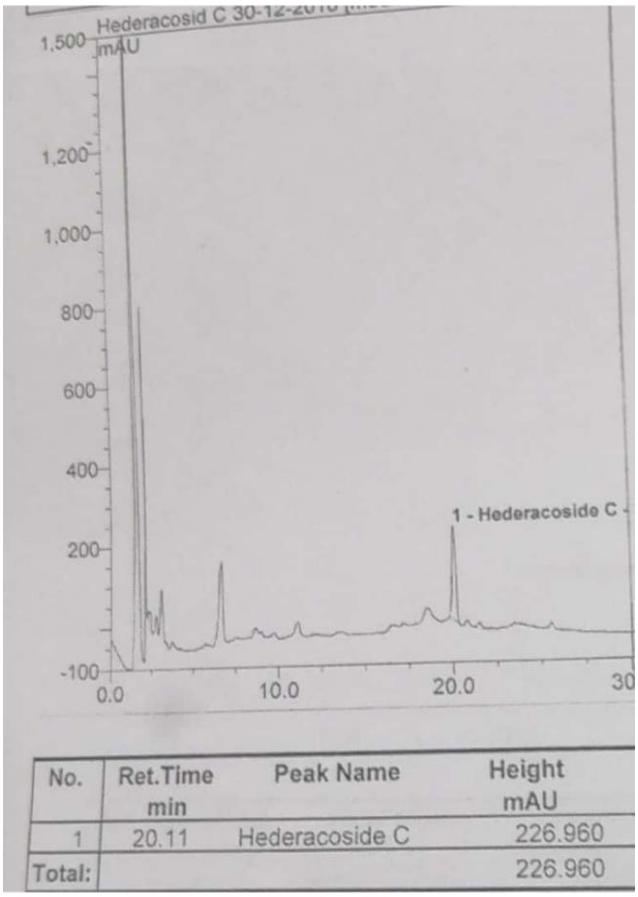

Fig. 3 Chromatogram showing the peak of hederacoside C in Ivy leaf SDE

3.2. Method Validation Studies Linear Response

The method has been validated for detector linear response. The response was found to be linear in the range of 50-400 µg/mL (06 injections). The linear regression analysis was performed by plotting the average peak area (mAU/min) against concentration (µg/mL), and the correlation coefficient was found to be $r^2 > 0.9999$ indicating a good linear correlation, as mentioned in Table 3.

Table 3 Data of regression analysis for hederacoside C quantification by HPLC method

Parameters	**Values**
Concentration (ug/ml)	50–400
Detection wavelength (nm)	205
No. of injections	6
Slope of equation	0.037
Y- intercept	0.015 ± 4.91
r^2	0.9999
Regression equation	Y = 0.037x + 0.015
SD of y-intercept calculated by LINEST function in excel	0.044161
LOD	3.93 ug/mL
LOQ	11.94 ug/mL

3.2.1. Precision

Precision was found to be good for the given three concentrations (100, 150, 300 µg/mL) as RSD values are less than 2% as shown in Table 4.

Table 4 Results of instrument and method precision for the quantification of hederacoside C in the SDE of ivy leaf

Concentration (ug/ml)	**Instrument Precision RSD (%)**	**Method Precision RSD (%)**	
		Interday	**Intraday**
100	0.248	0.748	0.412
150	0.307	0.513	0.364
300	0.742	0.693	0.324

3.2.2. Accuracy and Recovery

A known amount of standard was added to the sample of known concentration at 80, 100, and 120% of standard and analyzed in triplicate, and percent recovery was calculated against each level (mentioned in Table 5) and found in the acceptance limit (95%-105%).

Table 5 Results of analytical method accuracy for the quantification of hederacoside C in the SDE of ivy leaf

Concentration of standard added (mg)	RSD (%)	Recovery (%)
80	0.19	98.91
100	0.25	100
120	0.23	99.53

3.2.3. Ruggedness and Robustness

Ruggedness was evaluated by the change of analyst, and percent RSD was less than 1%. Robustness was evaluated by changing the source of acetonitrile, a distance of mobile phase, and column saturation time. Method was found to be robust as the RSD was less than 2%.

3.2.4. LOQ and LOD

The limits of quantification and limit of detection were determined as follows: LOD = 3.3 (SD_y/S), LOQ = 10 (SD_y/S). The LOD and LOQ of triterpene saponins were found to be 3.93 and 11.94 ug/mL, respectively.

4. Conclusion

Hedera helix is a rich source of triterpene saponins. The pharmacological activity of the plant is attributed to the presence of these compounds. Many studies have been performed and developed for the determination of hederacoside C (triterpene saponin). However, none of the studies reported employed spray-dried extract of the plant. For this reason, this study reports a method for the quantification of triterpene saponins named hederacoside C by HPLC from the spray-dried extract of *Hedera helix* (ivy leaf). The content of triterpene saponins was found to be 17.61%. The analytical procedure was validated as per ICH guidelines. The analytical method developed was precise, accurate, specific, reproducible and robust. Hence, it is concluded that the developed method can be employed in routine pharmaceutical analysis of ivy leaf spray-dried extract as it is simple and fast.

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References

[1] BOZUKOV V. Family Araliaceae in the Bulgarian macrofossil flora. *Phytologia Balcanica*, 2018, 24(1): 3-10. http://www.bio.bas.bg/~phytolbalcan/PDF/24_1/PhytolBalcan_24-1_01_Bozukov.pdf

[2] BEKAR M., & GÜNEROĞLU N. Utilization of Living Walls in Urban Ecosystems. In: EFE R., CÜREBAL İ., GAF A., and TOTH B. (eds.) *Enviromenthal Sustainability and Landscape Management*. St. Kliment Ohridski University Press, Sofia, 2016: 467-482.

[3] OKERMAN A. Combating the "Ivy Desert": The Invasion of Hedera helix (English Ivy) in the Pacific Northwest United States. *Restoration and Reclamation Review*, 2000, 6(4): 1-10. https://conservancy.umn.edu/bitstream/handle/11299/59738/1/6.4.Okerman.pdf

[4] FRIDLENDER A., & PECH N. *About a tetraploid ivy in Sicily: from autochthonous Hedera to horticultural-invasive-hybrid package?*, 2019. https://www.biorxiv.org/content/10.1101/751743v1

[5] YOUNIS A., RIAZ A., SABI-UL-HASAN N. M., and HAMEED M. Response of Hedera helix (English Ivy) to various salinity levels. *Journal of Horticultural Science and Biotechnology*, 2014, 18: 1-6.

[6] DEMIRCI B., GOPPEL M., DEMIRCI F., and FRANZ G. HPLC profiling and quantification of active principles in leaves of Hedera helix L. *Die Pharmazie - An International Journal of Pharmaceutical Sciences*, 2004, 59(10): 770-774. https://www.ingentaconnect.com/content/govi/pharmaz/2004/00000059/00000010/art00006

[7] STAUSS-GRABO M., ATIYE S., WARNKE A., WEDEMEYER R., DONATH F., and BLUME H. Observational study on the tolerability and safety of film-coated tablets containing ivy extract (Prospan® Cough Tablets) in the treatment of colds accompanied by coughing. *Phytomedicine*, 2011, 18(6): 433-436. https://doi.org/10.1016/j.phymed.2010.11.009

[8] DHAWAN K., KUMAR S., and SHARMA A. (eds.) Passiflora incarnata Linn.: a promising anxiolytic and sedative-isolation of a benzoflavone moiety as the bioactive agent. Presentation at the 42nd Annual Meeting of the American Society of Pharmacognosy, 2001.

[9] BLUMENTHAL M. *Therapeutic guide to herbal medicines.* American Botanical Council, Austin, Texas, 1998.

[10] OZDEMIR C., SCHNEIDER L. A., HINRICHS R., STAIB G., WEBER L., WEISS J. M., and SCHARFFETTER-KOCHANEK K. Allergic contact dermatitis to common ivy (Hedera helix L.). *Der Hautarzt*, 2003, 54(10): 966-969. https://doi.org/10.1007/s00105-003-0584-4

[11] REHMAN S. U., CHOI M. S., KIM I. S., KIM S. H., and YOO H. H. An ultra-high-performance liquid chromatography-tandem mass spectrometric method for the determination of hederacoside C, a drug candidate for respiratory disorder, in rat plasma. *Journal of Pharmaceutical and Biomedical Analysis*, 2016, 129: 90-95. https://doi.org/10.1016/j.jpba.2016.06.039

[12] YU M., SHIN Y. J., KIM N., YOO G., PARK S., and KIM S. H. Determination of saponins and flavonoids in ivy leaf extracts using HPLC-DAD. *Journal of Chromatographic Science*, 2015, 53(4): 478-483. https://doi.org/10.1093/chromsci/bmu068

[13] SHAWKY E., & ABOU EL KHEIR R. M. Rapid discrimination of different Apiaceae species based on HPTLC fingerprints and targeted flavonoids determination using multivariate image analysis. *Phytochemical Analysis*, 2018, 29(5): 452-462. https://doi.org/10.1002/pca.2749

[14] KHDAIR A., MOHAMMAD M. K., TAWAHA K., AL-HAMARSHEH E., ALKHATIB H. S., AL-KHALIDI B., BUSTANJI Y., NAJJAR S., and HUDAIB M. A validated RP HPLC-PAD method for the determination of hederacoside C in Ivy-Thyme cough syrup. *International*

Journal of Analytical Chemistry, 2010, 2010: 478143. https://doi.org/10.1155/2010/478143
[15] GAILLARD Y., BLAISE P., DARRÉ A., BARBIER T., and PÉPIN G. An unusual case of death: suffocation caused by leaves of common ivy (Hedera helix). Detection of hederacoside C, α-hederin, and hederagenin by LC—EI/MS-MS. *Journal of Analytical Toxicology*, 2003, 27(4): 257-262. https://doi.org/10.1093/jat/27.4.257
[16] PETERSEN M., & SIMMONDS M. S. Rosmarinic acid. *Phytochemistry*, 2003, 62(2): 121-125. https://doi.org/10.1016/S0031-9422%2802%2900513-7
[17] HADAD G. M., ABDEL SALAM R. A., SOLIMAN R. M., and MESBAH M. K. HPLC–DAD determination of seven antioxidants and caffeine in different phytopharmaceuticals. *Journal of Chromatographic Science*, 2014, 52(7): 617-623. https://doi.org/10.1093/chromsci/bmt086
[18] ABDALLAH O. M., MOTAAL A. A., and ELBAHRAWY S. A. Development of a Validated Reversed Phase HPLC-UV Method for a Simultaneous Determination of Hederacoside C and Thymol in Ivy-Thyme Cough Syrup. *Chemistry Research Journal*, 2018, 3(2): 33-41. https://chemrj.org/download/vol-3-iss-2-2018/chemrj-2018-03-02-33-41.pdf
[19] SOLLOHUB K., & CAL K. Spray drying technique: II. Current applications in pharmaceutical technology. *Journal of Pharmaceutical Sciences*, 2010, 99(2): 587-597. https://doi.org/10.1002/jps.21963
[20] NANDIYANTO A. B. D., & OKUYAMA K. Progress in developing spray-drying methods for the production of controlled morphology particles: From the nanometer to submicrometer size ranges. *Advanced Powder Technology*, 2011, 22(1): 1-19. https://doi.org/10.1016/J.APT.2010.09.011
[21] TONON R. V., BRABET C., and HUBINGER M. D. Influence of process conditions on the physicochemical properties of açai (Euterpe oleraceae Mart.) powder produced by spray drying. *Journal of Food Engineering*, 2008, 88(3): 411-418. http://dx.doi.org/10.1016/j.jfoodeng.2008.02.029

参考文:

[1] BOZUKOV V. 保加利亚大型化石植物群中的五加科。巴尔卡尼卡植物学，2018, 24(1): 3-10. http://www.bio.bas.bg/~phytolbalcan/PDF/24_1/PhytolBalcan_24-1_01_Bozukov.pdf
[2] BEKAR M., & GÜNEROĞLU N. 城市生态系统中活墙的利用。在：EFE R.、CÜREBAL İ.、GAF A. 和 TOTH B.（编辑）环境可持续性和景观管理。圣克里门特奥赫里德斯基大学出版社，索非亚，2016：467-482。
[3] OKERMAN A. 对抗“常春藤沙漠”：美国太平洋西北部常春藤（英语常春藤）的入侵。恢复和开垦审查，2000，6（4）：1-10。https://conservancy.umn.edu/bitstream/handle/11299/59738/1/6.4.Okerman.pdf
[4] FRIDLENDER A. 和 PECH N. 关于西西里岛的四倍体常春藤：从本土常春藤到园艺侵入性混合包装？，2019年。https://www.biorxiv.org/content/10.1101/751743v1
[5] YOUNIS A.、RIAZ A.、SABI-UL-HASAN N. M. 和 HAMEED M. 常春藤螺旋（英国常春藤）对各种盐度水平的反应。园艺科学与生物技术杂志，2014，18：1-6。
[6] DEMIRCI B.、GOPPEL M.、DEMIRCI F. 和 FRANZ G. HPLC 分析和定量常春藤大号. 药剂师叶片中的活性成分-国际药物科学杂志，2004 年，59(10)： 770-774。https://www.ingentaconnect.com/content/govi/pharmaz/2004/00000059/00000010/art00006
[7] STAUSS-GRABO M.、ATIYE S.、WARNKE A.、WEDEMEYER R.、DONATH F. 和 BLUME H. 对含有常春藤提取物的薄膜包衣片剂（普罗斯潘®咳嗽片剂）的耐受性和安全性的观察研究治疗伴有咳嗽的感冒。植物医学，2011, 18(6): 433-436. https://doi.org/10.1016/j.phymed.2010.11.009
[8] DHAWAN K., KUMAR S., 和 SHARMA A. (编辑。) 西番莲.: 苯黄酮部分作为生物活性剂的有前途的抗焦虑和镇静分离。在 2001 年美国生药学会第 42 届年会上的报告。
[9] BLUMENTHAL M. 草药治疗指南。美国植物委员会，德克萨斯州奥斯汀，1998 年。
[10] OZDEMIR C.、SCHNEIDER L. A.、HINRICHS R.、STAIB G. 、WEBER L. 、WEISS J.M. 和 SCHARFFETTER-KOCHANEK K. 常春藤过敏性接触性皮炎（常春藤 L.）。皮肤科医生, 2003, 54(10): 966-969。https://doi.org/10.1007/s00105-003-0584-4
[11] REHMAN S.U.、CHOI M.S.、KIM I.S.、KIM S.H. 和 YOO H.H. 一种超高效液相色谱-串联质谱法测定大鼠血浆中呼吸系统疾病候选药物常春藤苷 C。医药与生物医学分析杂志，2016，129：90-95。https://doi.org/10.1016/j.jpba.2016.06.039
[12] YU M.、SHIN Y. J.、KIM N.、YOO G.、PARK S. 和 KIM S.H. 使用高效液相色谱-爸爸测定常春藤叶提取物中的皂苷和类黄酮。色谱科学杂志, 2015, 53(4): 478-483. https://doi.org/10.1093/chromsci/bmu068
[13] SHAWKY E., & ABOU EL KHEIR R. M. 基于 HPTLC 指纹和使用多变量图像分析的目标类黄酮测定快速区分不同伞形科物种。植物化学分析, 2018, 29(5): 452-462. https://doi.org/10.1002/pca.2749
[14] KHDAIR A.、MOHAMMAD M. K.、TAWAHA K. 、AL-HAMARSHEH E. 、ALKHATIB H. S. 、AL-KHALIDI B.、BUSTANJI Y.、NAJJAR S. 和 HUDAIB M.常春藤百里香止咳糖浆中常春藤苷 C 的测定。国际分析化学杂志，2010, 2010: 478143. https://doi.org/10.1155/2010/478143
[15] GAILLARD Y. 、BLAISE P. 、DARRÉ A. 、BARBIER T. 和 PÉPIN G. 一个不寻常的死亡案例：由普通常春藤(常春藤螺旋)的叶子引起的窒息。通过液相色谱-EI/质谱-质谱检测常春藤苷 C、α-常春藤素和常春藤素。分析毒理学杂志，2003 年，27（4）：257-262。https://doi.org/10.1093/jat/27.4.257
[16] 彼得森 M. 和 西蒙兹 M. S. 迷迭香酸。植物化学, 2003, 62(2): 121-125. https://doi.org/10.1016/S0031-9422%2802%2900513-7
[17] HADAD G. M.、ABDEL SALAM R. A.、SOLIMAN R. M. 和 MESBAH M. K. 高效液相色谱-爸爸测定不同植物药物中的七种抗氧化剂和咖啡因。色谱科学杂志, 2014, 52(7): 617-623. https://doi.org/10.1093/chromsci/bmt086
[18] ABDALLAH O. M. 、MOTAAL A. A. 和

ELBAHRAWY S. A. 开发用于同时测定常春藤百里香止咳糖浆中常春藤苷 C 和百里酚的经过验证的反相高效液相色谱-紫外方法。化学研究杂志，2018，3(2)：33-41. https://chemrj.org/download/vol-3-iss-2-2018/chemrj-2018-03-02-33-41.pdf
[19] SOLLOHUB K., & CAL K. 喷雾干燥技术：II。目前在制药技术中的应用。药学杂志，2010，99(2)：587-597。https://doi.org/10.1002/jps.21963
[20] NANDIYANTO A. B. D. 和 OKUYAMA K. 开发用于生产受控形态颗粒的喷雾干燥方法的进展：从纳米到亚微米尺寸范围。先进粉末技术，2011，22(1)：1-19. https://doi.org/10.1016/J.APT.2010.09.011
[21] TONON R. V.、BRABET C. 和 HUBIGER M. D. 工艺条件对喷雾干燥生产的巴西莓（油橄榄科市场.）粉末物理化学性质的影响。食品工程学报，2008，88(3)：411-418. http://dx.doi.org/10.1016/j.jfoodeng.2008.02.029