RESEARCH ARTICLE **(Open Access)**

Assessment of Saponins Stability in Liquid Extracts of *Hedera helix* and *Primula veris* by Using High Performance Thin Layer Chromatography Method

REZARTA SHKRELI[1]*, KLITON VIDE[2]

[1]Department of Pharmacy, Faculty of Medical Sciences, ALDENT University, Tirana, Albania

[2]Central Laboratory of Albanian Armed Forces, Tirana, Albania

*****Corresponding author: Rezarta Shkreli; rezarta.shkreli@ual.edu.al

Abstract

In recent years, the interest in the herbal medicine use has increased worldwide. Herbal therapy has several advantages over synthetic medicine, and the fact of being inexpensive with minimal side effects has accelerated the demand for plant products. The aim of the study was to determine the contents of Hederacoside C in *Hedera helix* leaves and Primula acid 1 in *Primula veris* roots as well as the stability of extracts under different storage conditions. Four different extraction methods were performed: simple maceration, condenser-reflux maceration, ultrasound maceration and maceration with magnetic mixer. The extracts were stored for two weeks under different conditions of temperature, humidity and packaging containers. Quantitative assessments of Hederacoside C and Primula acid 1 were performed immediately after extraction as well as after storage period, by using CAMAG Linomat 4. The highest amount of extracted saponins resulted from extracts obtained from the condenser-reflux maceration method: 2.97% for Hederacoside C and 5.82% for Primula acid 1. The losses of Hederacoside C for 2 weeks of storage in different conditions were for the following: for maceration with magnetic mixer method - in refrigerated condition, 8.1%; maceration method - in room condition, 10.2%; condenser-reflux method - in coloured glass package, 7.7% and for maceration method - under high temperature and humidity, 27.4%. The losses of Primula acid 1 for two weeks of storage in different conditions have resulted in the following: maceration with condenser-reflux method in refrigerated condition, 4.7%; maceration method - in room condition, 12.3%, condenser-reflux method - in coloured glass package, 7.7%, and maceration method stored in high temperature and humidity condition, 21.6%. The stability of the active principles were affected by storage conditions, so it is recommended to store them in a cool and dark place. This conclusion is significant and may provide guidance on the phytochemicals used in oral form-dosage formulations.

Keywords: *hedera helix* extract, *primula veris* extract, Hederacoside C, Primula acid 1, stability.

1. Introduction

Active components in herbal remedies are beneficial to human health [1]. These active ingredients protect plants from harm and illnesses while also contributing to their scent, flavor, and color. They are classified as phytochemicals in science, and they include saponins, flavonoids, glycosides, tannins, alkaloids, and terpenoids [2]. Phytochemicals have been scientifically proven to provide health benefits for humans throughout time. Saponins can be found in a wide range of plants. Saponins are amphiphilic molecules with one or more hydrophilic sugar parts and a lipophilic steroidal or triterpenic element in their structure. Saponins containing one, two, or more sugar chains are classified as monodesmosidic, bidesmosidic, or polydesmosidic saponins [3]. The evergreen woody

*Corresponding author: Rezarta Shkreli; E-mail: rezarta.shkreli@ual.edu.al
(Accepted for publication 04.12.2021)
***ISSN**: 2218-2020, © Agricultural University of Tirana*

liana *Hedera helix* (ivy) is endemic to Western Asia and Europe. The juvenile leaves are palmate with 3-5 lobes, whereas the mature leaves are cordate, rhomboid, or ovate-lanceolate. The plant's flowers are tiny and greenish-yellow, with umbels measuring 3-5 cm in diameter. In many places, *Hedera helix* is a popular ornamental plant [4].

The active principles responsible for the medicinal use are: triterpenoid saponins (2.5-6%), the bidesmosidic glycosides of hederagenin and oleanolic acid (hederacoside C, B, D, E, F, G, H, I) and the monodesmoside α-hederin. Other groups of biological compounds are represented by phenolics (flavonoids, anthocyanins, coumarins, and phenolic acids), amino acids, steroids, vitamins, volatile and fixed oils, β-lectins, and polyacetylenes [5]. Antifungal, hepatoprotective, antioxidant, hypoglycaemic, cytotoxic, spasmolytic, secretolytic, anti-inflammatory, antibacterial, and antimutagenic are some of *Hedera helix* L.'s pharmacological activities [6]. The German Commission E has approved ivy leaves for the treatment of respiratory catarrhs and symptoms of chronic inflammatory bronchial diseases [7].

The topical application includes *Hedera*-saponin complex (hederacoside C, B and α-hederin) which has been effective in the treatment of liposclerosis ("cellulitis") [8].

Cowslip (*Primula veris L.*,) is small, long-lived perennial, growing wild in temperate Europe and Asia [9]. *Primula veris* produces a rosette of leaves that can reach a height of 20-30 cm. Cowslip flowers are bright yellow with orange patches at the edges of each lobe, and they bloom in an umbel-like inflorescence at the top of the stalks. An orange ring may be seen in the center of these blooms [10]. *Primula veris* has been used medicinally for a long time. It is listed as a source of *Primula* roots in the fifth edition of the European Pharmacopeia [11], the British Herbal Pharmacopeia [12], and Pharmacopee Française [13]. *Primula* raw ingredients are said to come from *P. veris*. Triterpene saponins, as well as phenolic compounds such as flavonoids (approximately 3% in flowers), phenolic glycosides, and phenolic acid, are the most active components in *Primula* roots and flowers [14, 15]. Referring to EMA, *Primula* flowers roots are used against bronchitis, coughs, and catarrhs of the respiratory tract and also to treat nervousness, headache, or rheumatism [13, 14]. Saponins are responsible for secretolytic and expectorant activity. In turn, phenolic compounds, present especially in *Primula* flowers, reveal antioxidant, antimicrobial, and cytostatic properties [16, 17].

Because of the natural complexity and inherent unpredictability of the chemical ingredients of plant-based pharmaceuticals, establishing quality control measures is rather difficult [18]. Herbal medicine quality assurance is a critical aspect and a basic requirement for the herbal drug industry and other drug development organizations.

The aim of the study was to determine the contents of Hederacoside C in *Hedera helix* leaves and Primula acid 1 in *Primula veris* roots, as well as the stability of extracts under different storage conditions, by using the High Performance Thin Layer Chromatography (HPTLC) Method.

2. Materials and Methods

2.1. Materials and instruments

In this study were use dried *Hedera helix* leaves as well as dried *Primula veris* root, that were collected in different areas of Albania.

Hederacoside C and Primula acid 1 analytical standards were purchased from Sigma-Aldrich Products (Germany). Instruments used were pre-coated silica gel plates PolygranSilG/UV254 (Macherey – Nagel, Germany) – TLC, and pre-coated silica gel GF254 plates (E.Merck) – HPTLC, CAMAG TLC Scanner 3 equipped with CAMAG Linomat 5 sample applicator, CAMAG twin development chambers.

2.2. Sample's preparation

All of the extracts were obtained from chopped plant leaves and plant roots. Four types of extracts for each plant were prepared for HPTLC identification and quantification analyses, with the organic solvent: methanol. Methanol extracts were prepared by four different methods: simple

maceration, reflux-condenser maceration, ultrasound maceration and magnetic mixer maceration. Thirty mg of dried ivy leaves were accurately weighed, initially soaked for 5 minutes, then added to methanol to a volume of 30.00 mL, and left for one hour in four different maceration processes [19, 20].

The samples were filtered and the HPTLC method was used to evaluate them. The eight extracts were stored for two weeks in different conditions of temperature, humidity, and packaging containers (room condition, 27.2ºC & relative humidity 38.2% in coloured and uncoloured containers; refrigerated condition, 2º-8ºC; and stress condition, 40ºC & relative humidity 65%). Quantitative assessment of Hederacoside C and Primula acid 1 were performed immediately after extraction as well as after storage period, by using CAMAG Linomat 5 according to the method of Bezruk et al [21] and Coran et al [22]. The mobile phase for Hederacoside C determination was composed by anhydrous formic acid/acetone/methanol/ethyl acetate (4:20:20:30 v/v); and the mobile phase for Primula Acid 1 determination was composed by ethyl acetate/ water/ formic acid (5:1:1 v/v).

Standard and all eight extracts were applied in the quantity of 3 µl (this procedure was repeated three time for each type of extract) on the same pre-coated silica gel HPTLC plate. The results obtained after the densitometric analysis were used to make the calibration curve and to determine the saponins content in our extracts, as well. The regression coefficient, relative standard deviation, slope and intercept on the Y-axis were calculated by the software.

Chromatography method has developed in its significance and use, and it has become a well-known type of analysis in instrumental analytical chemistry [23]. High-performance thin-layer chromatography (HPTLC) is a robust, simple, rapid, and efficient tool in quantitative analysis of compounds [24]. HPTLC is still increasingly finding its way in pharmaceutical analysis in some parts of the world. With the advancements in the stationary phases and the introduction of densitometers as detection equipment, the technique achieves for given applications a precision and trueness comparable to high-performance liquid chromatography (HPLC) [25].

3. Results and Discussion

High Performance Thin Layer Chromatography, HPTLC method was performed for qualitative and quantitative determination of hederacoside C and primula acid 1 in plant materials as well as in prepared extracts.

The data used to make the calibration curves for hederacoside C are shown in figure 1, and for primula acid 1, in figure 2. The HPTLC calibration curve of hederacoside C is shown in figure 3 and the HPTLC calibration curve of primula acid 1 is shown in figure 4.

Substance: hederacoside C @ 205 nm

Regression via area: Linear Y = 42.04 + 0.3432 * X r = 0.99976 sdv = 1.72

Track	Vial	Rf	Amount	Height	X(Calc)	Area	X(Calc)	SampleID/Remark
1	1							Not used
2	2	0.49	502.00 ng			214.29		
3	2	0.49	1.004 µg			392.56		
4	2	0.50	2.008 µg			719.16		
5	2	0.50	3.012 µg			1081.59		

Figure 1. Calibration curve data for hederacoside C

Substance: primula saponin @ 254 nm

Regression via area: Polynomial Y = 28.01 + 96.95 * X + -0.4901 * X2 r = 0.99971 sdv = 1.83

Track	Vial	Rf	Amount	Height	X(Calc)	Area	X(Calc)	SampleID/Remark
1	1							Not used
2	2	0.26	5.028 µg			467.00		
3	2	0.26	10.06 µg			996.11		
4	2	0.27	20.11 µg			1791.80		
5	2	0.27	30.17 µg			2497.55		
6	2	0.28	40.22 µg			3107.47		
7	2	0.28	50.28 µg			3680.80		

Figure 2. Calibration curve data for primula acid 1

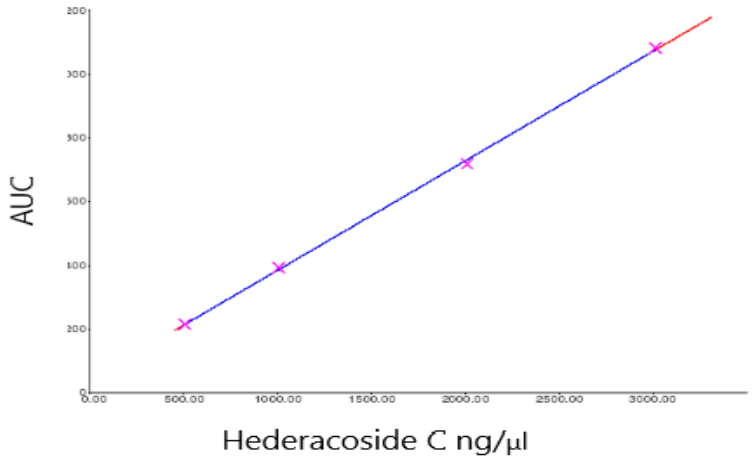

Figure 3. The HPTLC calibration curve for hederacoside C
Y = 42.04 + 0.3432 X presented by R = 0.99976 and SD = 1.72

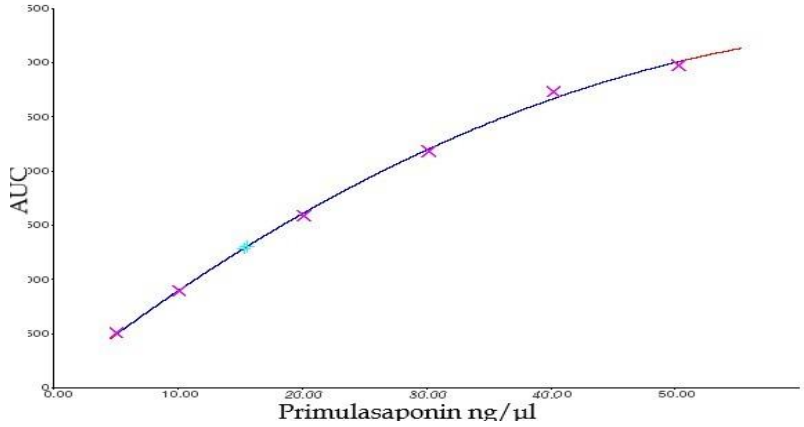

Figure 4. The HPTLC calibration curve for primula acid 1
Y = 28.01 + 96.95 X + 0.4901 X2 presented by R = 0.99971 and SD = 1.83

The chromatogram of hederacoside C standard is presented in figure 5 and the chromatogram of primula acid 1 standard is presented in figure 6.

The mean value of hederacoside C and primula acid 1 contents in dried drugs are shown in table 1.

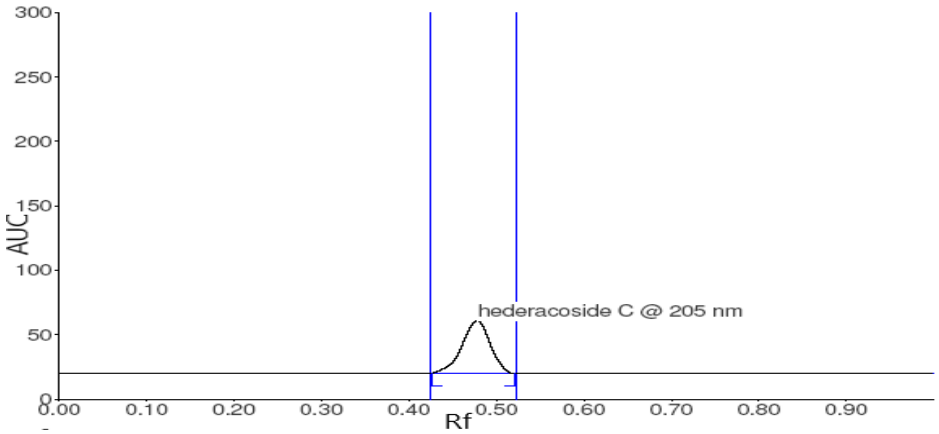

Figure 5. HPTLC chromatogram of hederacoside C standard

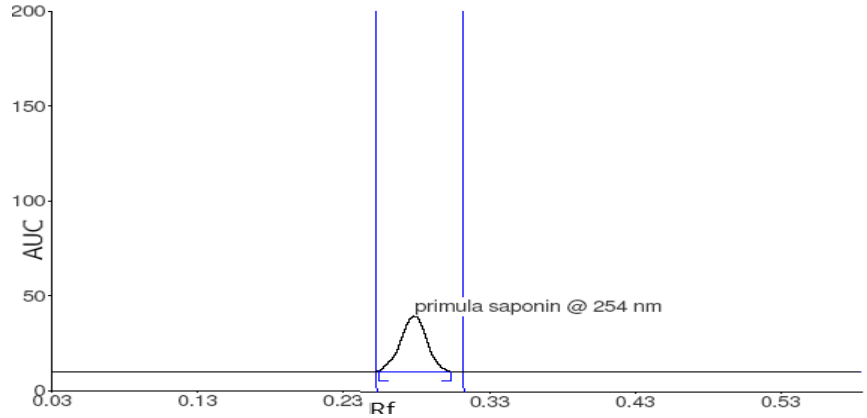

Figure 6. HPTLC chromatogram of primula acid 1 standard

Table 1. Hederacoside C and Primula acid 1 content in dried drug (g/100g)

The analyzed sample	Simple Maceration	Condenser-reflux maceration	Ultrasound maceration	Magnetic mixer maceration
Hederacoside C (%) ± SD (g/100g in dried drug)	2.853 ± 0.068	2.974 ± 0.083	2.782 ± 0.072	2.681 ± 0.061
Primula Acid 1 (%) ± SD (g/100g in dried drug)	3.71 ± 0.076	5.82 ± 0.087	4.11 ± 0.103	5.07 ± 0.095

The highest amount of both extracted hederacoside C as well as primula acid 1 was obtained by the condenser-reflux maceration: respectively 2.97% and 5.82%; whereas the lowest content of extracted hederacoside C was by maceration with magnetic mixer, 2.68% and of extracted primula acid 1 was by simple maceration, 3.71%.

Table 2. Hederacoside C & Primula Acid 1 content in extracts (g/100 ml) and its losses (%) after 2 weeks of storage in refrigerated condition

The analyzed sample	Simple Maceration	Condenser-reflux maceration	Ultrasound maceration	Magnetic mixer maceration
Hederacoside C (%) ± SD after storage in refrigerated condition (g/100 ml extract)	0.7840 ± 0.0161	0.7753 ± 0.0142	0.7319 ± 0.0115	0.7391 ± 0.0169

Loss of Hederacoside C (%) ± SD after 2 weeks of storage in refrigerated condition	8.4 ± 0.245	13.1 ± 0.169	12.3 ± 0.106	8.1 ± 0.147
Primula Acid 1 (%) ± SD after storage in refrigerated condition (g/100 ml extract)	0.2393 ± 0.0051	0.3698 ± 0.0058	0.2501 ± 0.0042	0.3081 ± 0.0087
Loss of Primula Acid 1 (%) ± SD after 2 weeks of storage in refrigerated condition	4.8 ± 0.193	4.7 ± 0.102	7.4 ± 0.152	8.9 ± 0.310

The lowest loss percentage of saponins after 2 weeks of storage in refrigerated condition was: for hederacoside C, in the extract obtained by magnetic mixer method, 8.1% and for primula acid 1, in the extract obtained by condenser-reflux maceration method, 4.7%.

Table 3. Hederacoside C and Primula acid 1 content in extracts (g/100 ml) and its losses (%) after 2 weeks of storage in room condition and coloured/uncoloured container

Methods of extraction	**Simple Maceration**	**Condenser-reflux maceration**	**Ultrasound maceration**	**Magnetic mixer maceration**
Hederacoside C (%) after storage in room condition & uncoloured container (g/100 ml extract)	0.7677 ± 0.0161	0.7456 ± 0.0115	0.6852 ± 0.0101	0.6772 ± 0.0164
Loss of Hederacoside C (%) after 2 weeks of storage in room condition & uncoloured container	10.2 ± 0.119	16.8 ± 0.081	18.1 ± 0.124	16 ± 0.163
Hederacoside C (%) after storage in room condition & coloured glass package (g/100 ml extract)	0.7762 ± 0.0164	0.8271± 0.0100	0.6810 ± 0.0105	0.7013 ± 0.0148
Loss of Hederacoside C (%) after 2 weeks of storage in room condition & coloured glass package	9.2 ± 0.124	7.7 ± 0.163	18.6 ± 0.205	13 ± 0.189
Primula Acid 1 (%) after storage in room condition & uncoloured container (g/100 ml extract)	0.2206 ± 0.0057	0.3340 ± 0.0060	0.2253 ± 0.0065	0.2873 ± 0.0035
Loss of Primula Acid 1 (%) after 2 weeks of storage in room condition & uncoloured container	12.3 ± 0.123	13.9 ± 0.264	16.6 ± 0.278	15 ± 0.194
Primula Acid 1 (%) after storage in room condition & coloured glass package (g/100 ml extract)	0.2300 ± 0.0050	0.3580 ± 0.0109	0.2473 ± 0.0053	0.3036 ± 0.0059

Loss of Primula Acid 1 (%) after 2 weeks of storage in room condition & coloured glass package	8.5 ± 0.213	7.7 ± 0.153	8.5 ± 0.192	10.2 ± 0.175

The lowest loss percentage of saponins after 2 weeks of storage in room condition and uncoloured container was for hederacoside C as well as for primula acid 1 in the extracts obtained by maceration method, 10.2% and 12.3%, whereas, in coloured glass package was 7.7% for hederacoside C as well as for primula acid 1 obtained by condenser reflux maceration method.

Table 4. Hederacoside C and primula acid 1 content (g/100 ml) in extracts and its losses (%) after 2 weeks of storage in stress condition

The analyzed sample	Simple maceration	Condenser-reflux maceration	Ultrasound maceration	Magnetic mixer maceration
Hederacoside C (%) ± SD after storage in refrigerated condition (g/100 ml extract)	0.6213 ± 0.014	0.6290 ± 0.012	0.5767 ± 0.012	0.5549 ± 0.013
Loss of Hederacoside C (%) ± SD after 2 weeks of storage in stress condition	27.4 ± 0.286	29.5 ± 0.301	30.9 ± 0.311	31 ± 0.329
Primula Acid 1 (%) ± SD after storage in refrigerated condition (g/100 ml extract)	0.1973 ± 0.022	0.2702 ± 0.032	0.1855 ± 0.018	0.2310 ± 0.014
Loss of Primula Acid 1 (%) ± SD after 2 weeks of storage in stress condition	21.6 ± 0.451	30.3 ± 0.311	31.4 ± 0.254	31.7 ± 0.301

The lowest percentage of both hederacoside C and primula acid 1 losses, for 2 weeks of storage in stress condition have resulted in extracts obtained from maceration method, 27.4% and 21.6%.

The summary of percentage of hederacoside C and primula acid 1 losses are shown in figure 7 and figure 8.

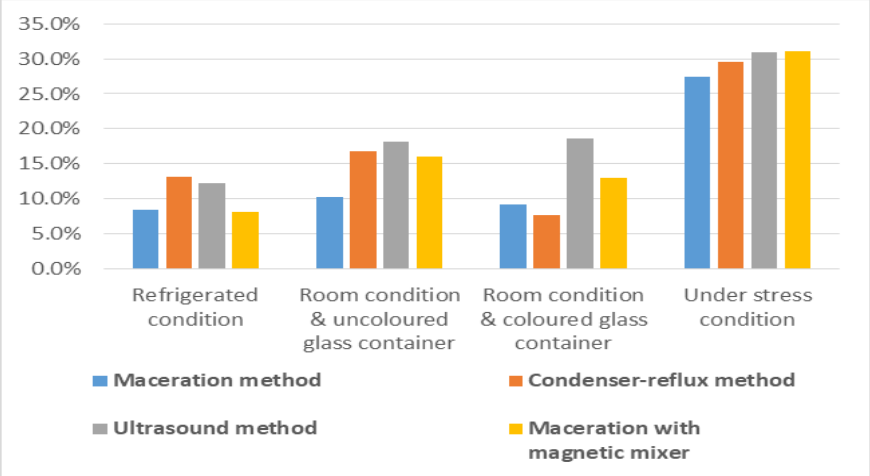

Figure 7. Percentage of hederacoside C losses (%) after different storage condition

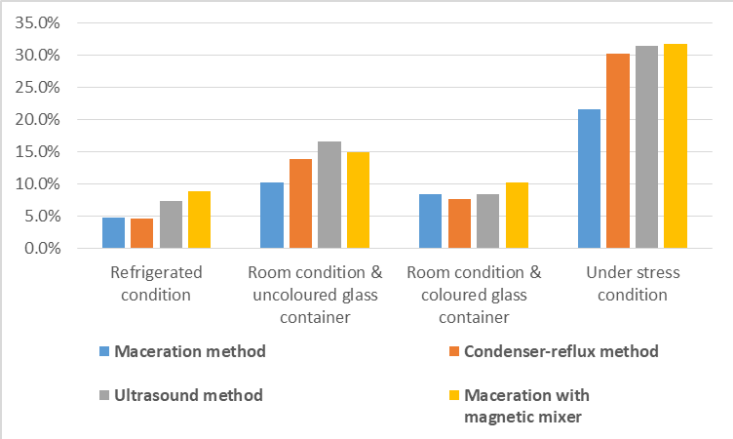

Figure 8. Percentage of primula acid 1 losses after different storage condition

The high-pressure thin layer chromatography method used in our study was simple and sensitive; it was not necessary to purify the extracts of both saponins. The results shown in our study identified the active principles in our initial eight extracts, as well as in extracts after the period of storage. HPTLC method resulted to be effective in qualitative and quantitative determination of hederacoside C as well as primula acid 1. It was possible, through this simple method, to determine the stability of the active principles in different storage conditions as well as to make a comparative assessment about the effectiveness of extraction methods.

Studies on the high-performance thin layer chromatography method used in the determination of chemical components in *Hedera helix* extract or its products have been mainly focused on the analysis of hederacoside C. Shawki E. et al. [26] determined the chemical profile of content in *Hedera helix* subspecies using HPTLC imaging analysis. Final results expressed an average concentration of the Hederacoside C compound (mg/100 g) of dry weight of different *Hedera helix L*. subspecies leaf powder varied from 17.85 ± 0.97 to 20.7 ± 1.09. Havlíkováa L. et al [27] determined the amount of Hederacoside C in different type of dried extracts by using the HPLC method, the content varied from the range 8.3 mg to 18.4 mg in 100 g of the dried extract. In another study, the contents of hederacoside C in ivy leaves extracts as well as in syrups and capsules were determined by HPTLC method [28]. There is one comparative report on the quantitation of hederacoside C by HPTLC, HPLC and UPLC methods. The content of Hederacoside C in different dried extracts varied from 16.10 to 19.83 %. In this study, the difference between HPTLC and HPLC method were determined to be from 2.51 to 3.19%.

Műller A. et al described a suitable chromatographic method for determining the bioactive components, saponins and phenolic glycosides, found in *Primula elatior* and *Primula veris*; the total saponin content was highest in *P. veris* roots (max. 14.9%), the aerial parts or *P. elatior* contained less amounts [29]. Dedić M. et al. performed high pressure liquid chromatography for the quantitative determination of primula acid 1, and the content of primula acid 1 calculated by the equation of the calibration curve was 0.2793 mg per gram of extract [30]. HPTLC method had the lowest expenses and it was identified as a less greenish method, but HPLC remains the more sensitive, safe and widely used method.

4. Conclusion

In our study, four methods of saponin extraction resulted to be effective, the active principles, were identified among the extracts and their amounts varied from 0.8043% to 0.8922% for hederacoside C and 0.2516% to 0.3881 % for primula acid 1. All four methods are easily and appropriate as sources of both active components. Based on the results obtained by this survey we recommend the

condenser-reflux method as the most effective method of extraction.

The highest stability of hederacoside C has resulted from the maceration with magnetic mixer method for extracts in refrigerated condition; condenser – reflux method for extracts in room condition; and simple maceration method in coloured glass container for extracts under stress condition.

The highest stability of primula acid 1 have resulted from the maceration with condenser-reflux method for extracts in refrigerated condition and coloured glass container; maceration method for extracts in room condition and under stress condition.

The conclusion is that the active principle`s stability is affected by storage conditions, and it is recommended to store them in a cool and dark place. The results of this study may provide guidance on the phytochemicals used in oral form-dosage formulations.

5. Acknowledgements

In this study we are grateful to the staff of Central Laboratory Armed Forces, specially to K.V. for the help and support during the analyses with HPTLC Scanner.

6. References

1. **WHO guidelines on good herbal processing practices for herbal medicines**. 2018. Annex 1. pg 83-84.
2. Hussein Rehab A. et al: **Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants**. Herbal Medicine. DOI: 10.5772/ intechopen.76139. pg 20-24.
3. Hussien, S.A.; Awad, Z.J. **Isolation and characterization of triterpenoid saponin hederacoside C. present in the leaves of Hedera helix L. cultivated in Iraq**. Iraqi J. Pharm. Sci. 2014, 23, 33–41.
4. Gruenwald, J., Brendler, T., Jaenicke, C. **PDR for Herbal Medicines**. Medical Economics Company, Montvale, 2000.
5. Yulia L., et al. **Hedera helix as medicinal plant**. Herba Polinica, 2012; 56 (1).
6. EMA/HMPC/325716/2017.**Committee on Herbal Medicinal Products** (HMPC), 21 November 2017.
7. Blumenthal M. **Herbal Medicine Expanded Commission E. Monographs**. 1st ed. Austin 2000: 215-218.
8. Facino RM, et al. **Anti-elastase and anti-hyaluronidase activities of saponins and sapogenins from Hedera helix, Aesculus hippocastanum, and Ruscus aculeatus: factors contributing to their efficacy in the treatment of venous insufficiency**. Arch Pharm (Weinheim) 1995. 328(10): 720-724.
9. M. Wichtl. **Herbal Drugs and Phytopharmaceuticals,**A Handbook of Practice on a Scientific Basis, CRC Press,Stuttgart, Germany, 3rd edition, 2004.
10. K. Kalm et al. **"Morph-specific variation of floral traits associated with reciprocal herkogamy in natural populations of Primula vulgaris and Primula veris,"** Plant Systematics and Evolution, 2007, vol. 268, no. 1-4, pp. 15–27.
11. European Pharmacopoeia, **"Primula root (Primulaeradix),"** in European Directorate for the Quality of Medicines and Health Care (EDQM), pp. 2310-2311, Council of Europe, Strasbourg, France, 5th edition, 2006.
12. **British Herbal Pharmacopoeia,** Primula veris, British Herbal Medicine Association, London, UK, 1974.
13. **Pharmacopee Francaise**, L'Adapharm, 10th edition, 1988
14. EMA (European Medicines Agency), **"Assessment report on Primulaveris L. and/or Primulaelatior (L.) Hill, flos,"** EMA/HMPC/136583/2012, 2012.
15. EMA (European Medicines Agency), **"Assessment report on Primulaveris L. and/or Primulaelatior (L.) Hill, radix,"** EMA/ HMPC/113577/2012EMA, 2012.
16. N. Demir, A. A. Gungor, H. Nadaroglu, and Y. Demir, **"The antioxidant and radical scavenging activities of Primrose (Primula vulgaris),"** European Journal of Experimental Biology, vol. 4, pp. 395–401, 2014.

17. S. V. Tokalov, B. Kind, E. Wollenweber, and H. O. Gutzeit, **"Biological Effects of Epicuticular Flavonoids from Primula denticulata on Human Leukemia Cells,"** Journal of Agricultural and Food Chemistry, vol. 52, no. 2, pp. 239–245, 2004.
18. Jeganathan N.S., Kannan K. **HPTLC Method for Estimation of Ellagic Acid and Gallic Acid in Triphalachurnam Formulations.** Res. J. Phytochemistry 2008; 2 (1): 1-9.
19. EMA/HMPC/325716/2017. **Committee on Herbal Medicinal Products (HMPC).** 21 November 2017.
20. EMA/HMPC/104095/2012. **Committee on Herbal Medicinal Products (HMPC).** 19 September 2012
21. Bezruk I., Kotvitska A, Korzh I, Materiienko A, Gubar S, Budanova L, Ivanauskas L, Vyshnevsky I and Georgiyants V. **"Combined Approach to the Choice of Chromatographic Methods for Routine Determination of Hederacoside C in Ivy Leaf Extracts, Capsules, and Syrup".** Sci. Pharm, 88, 24; doi: 10.3390/scipharm88020024, 2020.
22. Silvia A. Coran, Stefano Mulas **"Validated determinations of saponins in primula root by high- performance- thin- layer- chromatography densitometric approach"** Journal of Pharmaceutical and Biomedical Analysis, (November 2012), vol. 70, pg.647-651.
23. Reich E, Schibli, A. **Stationary Phases for Planar Separations – Plates for Modern TLC. LC GC**. 2005; 23:58-69.
24. Attimarad M, Ahmed MKK, Aldhubaib BE, Harsha S. **High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery Symposium – HPTLC**, 2014. (PDF)
25. Srivastava M.M. **High-Performance Thin-Layer Chromatography (HPTLC).** Springer Verlag Berlin Heidelberg 2011: 32-60.
26. Shawki E. et al. **"Untargeted and targeted chemical profiling for efficacy-directed discrimination of Hedera helix L. subspecies using HPTLC-image analysis and HPTLC/MS"**. Industrial Crops and Products. November 2019. DOI: 10.1016/j.indcrop.2019.111980.
27. Havlíková L. et al. **"Rapid Determination of α-Hederin and Hederacoside C in Extracts of Hedera helix Leaves Available in the Czech Republic and Poland"**. Natural Product Communications Vol. 10 (9) 2015.
28. Bezruk I., Kotvitska A, Korzh I, Materiienko A, Gubar S, Budanova L, Ivanauskas L, Vyshnevsky I and Georgiyants V. **"Combined Approach to the Choice of Chromatographic Methods for Routine Determination of Hederacoside C in Ivy Leaf Extracts, Capsules, and Syrup".** Sci. Pharm. 2020, 88, 24; doi: 10.3390/scipharm88020024.
29. Műller et al. **"Analysis of phenolic glycosides and saponins in Primula elatior and Primula veris (primula root) by liquid chromatography, evaporative light scattering detection and mass spectrometry".** Journal of Chromatography A. 2006, Vol 1112, issue 1-2, pg 218-223.
30. Dedić M. et al. **"Chromatographic Method for the Determination of Primula acid 1content in Primula extractum fluidum"** Bulletin of Chemists and Technologists of Bosnia and Herzegovina. 2020, Vol. 5. pg 13-18.