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COMPARISON OF COMPONENTS PROFILE IN HERBAL RAW MATERIAL, EXTRACT AND PHARMACEUTICALS OF *HEDERA HELIX*

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The aim. To investigate both the profile of components and possible difference among herbal raw materials, semi-products and pharmaceuticals of *Hedera helix* for determination of main standardisation markers.

Materials and methods. Investigation of components profile has been performed using the Shimadzu Nexera X2 chromatographic system coupled with a diode-area detector. The ACE C 18 column (250 mm×4.6 mm with particle size 5 µm) was used for the separation of components. 0.1 % acetic acid and acetonitrile were used as mobile phase A and B, respectively. Studies have been performed on the leaves, dry extract and capsules of *H. helix*.

Results. The determined profile had no significant variation among samples. It has been presented by 19 various components, such as phenolic acids, flavonoids and triterpene saponins. However, kaempferol, nicotiflorin and *t*-cinnamic acid were not found in the leaf raw material. Hederacoside C might be highlighted as the main marker of raw materials and products of *H. helix* due to its significant amount in comparison to other components. Its amount was in the range of 64,80 % up to 71,46 % of the total content of components. Moreover, according to some pharmacological studies, hederacoside C is responsible for pharmaceutical usage of *H. helix* pharmaceuticals. Nevertheless, it is not recommended to standardize the plant-based medicines by one marker, since the pharmaceutical activity of such dosage forms is defined by synergism action of all constituents. Except for hederacoside C significant amounts in comparison to other components were found for chlorogenic acid and 4,5-dicaffeoylquinic acid about 5 % and 3 % respectively. Though the latter was found in small concentrations in leaves (0,058 %). This sample had a much higher amount of 3,5-dicaffeoylquinic acid, but in the case of extract and capsules, its content was lower 1,55 % and 0,66 % respectively. Thus, chlorogenic acid has been chosen as a second marker due to its high concentration in all samples and some pharmaceutical activities, such as antioxidant and anti-inflammatory effects.

Conclusions. It was found, that standardisation of *H. helix* products is preferably to perform with determination both hederacoside C and chlorogenic acid. These components were dominant among all components; besides they possess a wide range of pharmaceutical effects. Hence, quantification of hederacoside C and chlorogenic acid is necessary to ensure the high quality of *H. helix* pharmaceuticals.

Keywords: ivy leaf, components profile, pharmaceuticals, extracts, HPLC

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1. Introduction

Plant-based medicines have a significant part of the pharmaceutical market in the whole world. It is a well-known fact that plant extracts show some advantages over synthetic pharmaceuticals due to the synergism of plant constituents [1]. However, the standardisation of herbal medicines is usually determined by macroscopic and microscopic examination with the following assay of the main biomarker [2, 3]. In particular, the quality of *H. helix* leaves, as well as medicines, are confirmed by quantification of hederacoside C. Nevertheless, medicinal plants contain various groups of biologically active components with different pharmacological effects [4]. Hence, the pharmacological activity is often caused by the synergic effect of phytoconstituents mixture [5]. Therefore, standardisation of plant-based medicines with a couple of biomarkers might provide higher quality and efficacy [6]. Another crucial moment is to choose the markers which can be suitable for the assessment of both raw materials and pharmaceuticals.

The aim of the research was to determine the profile of raw materials and medicines of *H. helix* and found components that can be used as markers for the standardisation.

2. Planning (methodology) of research

The State Pharmacopoeia of Ukraine requires the determination of hederacoside for the assessment of *H. helix* products [7, 8]. However, it is known that plant-based medicines contain various components that might show the synergism of their pharmaceutical activities. Hence standardisation of such pharmaceuticals only by one marker is not recommended. The present study included the following stages: analysis of *H. helix* leaves, extract, and capsules by HPLC and comparison of their profiles to determine the possible markers for standardisation.

3. Material and methods

Analysis has been performed using according to Gluschenko, Mykhailenko and Bezruk [9–11] with Shimadzu Nexera X2 chromatographic system coupled with

the diode-array detector. Column ACE C18 (250 mm × 4,6 mm with particle size 5 μm) was used in the analysis. 0.1 % acetic acid and acetonitrile were used as mobile phase A and B, respectively. A linear gradient was as follows: 0–8 min, 5–15 % B; 8–30 min, 15–20 % B; 30–48 min, 20–40 % B; 48–58 min, 40–50 % B; 58–65 min, 50 % B; 65–66 min, 50–95 % B at a flow rate of 1 ml/min. The column oven was at 25°C. The injection of the test solution (standard solution) was 10 μl.

Test solution of leaves. The solution was prepared as described in Bezruk et al. [11]. Briefly, precisely weighed 1.0 g of *H. helix* leaves were extracted in triplicate with 15 ml of methanol by ultrasound bath for 15 minutes. Obtained supernatants were combined and made up to 50 ml with methanol.

Test solution for capsules. 400 mg precisely weighted capsule powder were placed into 20.0 mL volumetric flask, added 15 mL of methanol and sonicated for 20 minutes on the ultrasonicated bath at the ambient temperature. After the solution was let cool, it was made up to volume with the same solution.

Test solution for dry extract. 200 mg precise weighted extract were placed into 20.0 mL volumetric flask, add 15 mL of methanol and sonicated for 20 minutes on the ultrasonicated bath at the ambient temperature after the solution was let cool, it was made up to volume with the same solution.

4. Results

Obtained results showed the presence of 19 various components, such as phenolic acids, flavonoids and triterpene saponins. All of the components were present

in each sample, except t-cinnamic acid, kaempferol and nicotiflorin which were not found in leaves. The triterpene saponins were the dominant components in all samples (Table 1–3). Their concentration was about 70 % of the total quantitative amount in each sample. Mostly, the contents of triterpene saponins were in the following order: hederacoside C > hederacoside D > α-hederin > hederasaponin B. Greunke et al. reported about the importance of α-hederin and hederasaponin B [12]. Among all constituents, hederacoside C was the dominant component. Its concentration was in the range of 63973.701±19.677 up to 149433.64±852.86 which was about 70 % of the total content of all components. Moreover, hederacoside C is essential due to its transformation into α-hederin in the human body [13]. The second greatest group was phenolic acids. They contribute to pharmaceutical effects [12]. Among them, the most significant amount was for chlorogenic acid and 4,5-dicaffeoylquinic acid about 5 % and 3 % respectively. Caffeoylquinic acid derivatives are established as being responsible for anti-inflammatory and antioxidant effects [14]. Though 4,5-dicaffeoylquinic acid was present in a small concentration in leaves (52,04±2.59 μg/g). This sample had a much higher amount of 3,5-dicaffeoylquinic acid (2904.445±143.27 μg/g), but in the case of extract and capsules, its contents were lower 1489.07±73.68 and 178.35±8.87 respectively. Thus, the chlorogenic acid can be highlighted as the second biomarker in *H. helix* due to its high amount in each sample. Also, chlorogenic acid shows anti-inflammatory activity [15] which can be beneficial in the treatment of acute respiratory diseases.

Table 1

Content of phenolic acids (mean±SD) in *Hedera helix* samples

Object	Capsules	Extract	Leaves
Gallic acid	8.70±0.34	86.31±2.65	12.30±0.15
Neochlorogenic acid	105.81±2.57	2145.15±54.52	92.104±1.41
Chlorogenic acid	494.59±14.92	10539.29±234.69	4838.402±23.21
t-Cinnamic acid	0.56±0.02	0.21±0.01	n/d
Caffeic acid	6.93±0.16	146.35±5.57	74.503±2.73
3,5-dicaffeoylquinic acid	178.35±8.87	1489.07±73.68	2904.445±143.27
3,4-dicaffeoylquinic acid	279.81±13.72	2151.32±105.76	365.402±15.02
4,5-dicaffeoylquinic acid	569.47±27.61	4700.32±219.57	52.04±2.59
Cryptochlorogenic acid	143.82±7.07	3698.14±179.36	13.576±0.627

Table 2

Content of flavonoids (mean±SD) in *Hedera helix* samples

Object	Capsules	Extract	Leaves
Quercetin	49.12±1.54	897.15±24.57	2.471±0.132
Kaempferol	7.35±0.29	133.32±2.57	n/d
Rutin	9.46±0.34	176.42±3.81	57.101±1.558
Hyperoside	403.39±16.09	7752.61±18.36	3266.802±13.364
Isoquercitrin	8.48±0.24	190.46±5.39	39.000±0.952
Nicotiflorin	16.68±0.82	54.49±2.69	n/d

Table 3

Content of triterpene saponins (mean±SD) in *Hedera helix* samples

Object	Capsules	Extract	Leaves
Hederacoside C	7447.41±253.24	149433.64±852.86	63973.701±19.677
α-hederin	555.93±19.74	10505.21±26.26	249.604±26.482
Hederasaponin B	261.14±13.01	3612.05±176.35	2762.240±109.052
Hederacoside D	946.86±46.53	26441.31±1324.07	10825.469±537.835

5. Discussions

Results of performed studies have complied with previously conducted researches [16, 17]. Yu et al. [16] found chlorogenic acid, rutin, nicotiflorin, hederacoside C, hederasaponin B and α -hederin in ivy leaf extracts using HPLC-DAD method. Except for rutin content, its results have completely corresponded to the ones in this article. Probably Yu et al. misidentified rutin instead of hyperoside. In this case, further investigations should be carried out to highlight hyperoside as a marker in *Hedera helix* products since it has a significant amount in all analysed samples.

According to Demirci et al., the most important markers for standardisation of ivy leaf are hederacoside C and α -hederin [17]. This seems logical since, as it was above mentioned, hederacoside C transforms into α -hederin which is responsible for pharmaceutical usage of *H. helix* pharmaceuticals. However, the level of α -hederin showed no stability due to some variation in content in studied samples. Thus, it is not advisable to use this component for standardisation of *H. helix* products.

In addition, Khadar et. al [18] recommended standardising medicines containing ivy leaf extract by quantification of hederacoside C. In our opinion, it is the most optimum decision due to the fact that hederacoside C was the dominant substance in all studied samples.

Study limitations. The profile of components in ivy is presented by various constituents the content of which significantly varied. Hence the determination of the main biomarkers is a complicated task. Besides during the preparations either dry extract or medicine some substance might reduce their concentration.

Prospects for further research. The obtained data might be used in the standardization and quality assurance of pharmaceuticals containing *H. helix* leaf extract.

6. Conclusions

Analysis of *H. helix* leaves, extract and capsules showed the presence of various components. Some of them possess pharmacological activities which can cause the synergism of effects. Hederacoside C and chlorogenic acid had a dominant concentration among all components. Also, both of them contribute the beneficial pharmaceutical effects to cure bronchitis and cold accompanied by cough and sputum. Hence, medicines of *H. helix* might standardize using hederacoside C and chlorogenic acid to deliver effective and safe pharmaceuticals for patients.

Conflict of interest

There are no conflicts of interest regarding this study.

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