Determination of biologically active compounds (costunolide and dehydrocostuslactone) in the leaves of some forms of laurel noble (sweet bay)

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Abstract. Laurel noble (sweet bay) leaves are widely used for food (aromatic seasoning, preservative) and medicinal (antibacterial, anti-inflammatory, hepatoprotective) purposes. The plant is actively cultivated and has many ecological forms. The main sesquiterpene lactones in laurel leaves are costunolide and dehydrocostuslactone. The content of these substances varies significantly depending on the ecological-geographical, climatic, edaphic and other factors. Several methods have been developed and used to quantify these sesquiterpene lactones in laurel leaves. They differ significantly in the cost of equipment and materials required for research, the duration of the analysis, economic costs, and the accuracy of the results. The article presents the results of studying the content of costunolide and dehydrocostuslactone in the leaves of some forms of sweet bay, cultivated on the southern coast of the Crimean Peninsula using capillary electrophoresis methods, reliably confirmed by the results of HPLC analysis of the leaves of the studied forms of sweet bay. Studies have shown that both methods give comparable results. At the same time, the HPLC method is characterized by a higher accuracy of results and a lower determination error, and the method of capillary electrophoresis allows a study to be carried out with lower economic costs using simpler methods. Both methods are pharmacopoeial and are recommended for the standardization of medicinal plant materials.

1. Introduction

Costunolide and dehydrocostuslactone (Fig. 1) are sesquiterpene lactones found in some plants. Among them are species with food and medicinal value: Saussurea lappa, Magnolia sieboldii, Mangolia grandiflora, Mikania guaco, Laurus nobilis, L. novocanariensis, Aucklandia lappa.

Figure 1. Structure of costunolide (A) and dehydrocostuslactone (B)
These two lactones have been very actively studied in the last decade. According to the PubMed textual database of medical and biological publications, more than 120 articles have been devoted to the study of these compounds over the past 5 years. For costunolide and dehydrocostuslactone, a variety of biological and pharmacological activities have been described. Among them such activities as antibacterial [1, 2], antioxidant [3, 4], hepatoprotective [5, 6], anticholinesterase [7], antiinflammatory [8] activity, the ability to inhibit ethanol absorption [9] and angiogenesis [10].

But the largest number of pharmacological studies is associated with the antiinflammatory [11, 12] and anticancer [13-15] activities of costunolide and dehydrocostuslactone. In recent years many studies of these compounds have appeared, including clinical [2, 15].

The results obtained by various authors and research groups make it possible to consider costunolide, dehydrocostuslactone and plants containing them as promising sources for the creation of new drugs and biologically active additives [16]. The food and medicinal plant sweet bay was chosen as the object of the study.

Laurel is grown as a decorative species in USA, Russia and other countries (such as Turkey, Algeria, Morocco, Portugal, Spain, Italy, France, Russia and Mexico [17-19]). A variety of ecological, geographical, soil, climatic and agrotechnological factors affecting the growing conditions and development of both wild and cultivated laurel plants determines its pronounced morphological variability. So, only for the southern coast of Crimea the following decorative forms of laurel are known: "Angustifolia" – narrow lanceolate leaves; "Aurea" – leaves with a golden color; "Eriobotrifolia" – broad-lanceolate leaves, similar to the leaves of Eriobotrya japonica; "Grandiflora" – large flowers, up to 1-1.2 cm in diameter; "Latifolia" – broad-oblong leaves; "Ligustrifolia" – leaf-like leaves of Ligustrum japonicum; "Macrocarpa" – large fruits, up to 2 cm long, 1.3 cm wide; "Microcarpa" – fruits are small, up to 0.5-0.7 cm long and 0.8-1 cm wide; "Microphylla" – leaves are small, up to 0.5-0.7 cm long and 2-3 cm wide; "Multiflora" – numerous flowers, up to 2 cm long and 1.5 cm wide; "Olivaeformis" – olive-like fruits, up to 2 cm long and 1.5 cm wide; "Ovalifolia – leaves are oblong; "Pallidus" – leaves are yellowish-green; "Pedunculata" – leaves are oblong-lanceolate with long petioles; "Rotundifolia" – leaves are round or almost round; "Salicifolia" – leaves are oblong and narrow, somewhat similar in shape to Salix viminalis; "Undulata" – oblong-lanceolate leaves, strongly wavy along the edge [20]. The chemical composition of sweet bay is rich and diverse: carbohydrates, lipids, proteins, dietary fiber, sesquiterpene lactones, phenolic compounds, tannins [21, 22].

Due to its valuable chemical composition, water extracts of bay leaves are used as a nutrient enrichment in order to activate the fermentative activity of yeast cells; hydroalcoholic extracts of bay leaves are used as a source of natural antioxidants to stabilize carotenoids in the development of technology for carotenoid biologically active additives in the form of fine powders; oil extract of sweet bay on the basis of unrefined sunflower oil allows to stabilize microbiological processes in the production of functional food products [23].

From the point of view of the requirements for the standardization of pharmaceutical substances, including medicinal herbal raw materials, the search for an adequate method for the quantitative determination of active compounds is urgent. Preliminary studies, the results of which have already been published [24, 25], made it possible to propose high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) as alternative methods for analyzing the content of costunolide and dehydrocostuslactone in pharmaceutical substances.

The aim of this work was to study the content of costunolide and dehydrocostuslactone in the leaves of some forms of laurel cultivated on the southern coast of the Crimean Peninsula.

2. Methods
Dried leaves of some forms of laurel, collected on the southern coast of the Crimean peninsula in 2017, were used as objects of research. Costunolide and dehydrocostuslactone manufactured by Sigma were used as standard samples, with an active ingredient content of at least 97% and 98%, respectively.
For the quantitative determination of costunolide and dehydrocostuslactone by HPLC, a Stayer HPLC chromatographic system (Akvilon, Russia) was used. A detailed description of this system and the method for determining the analyzed sesquiterpene lactones are given in the article [23].

Capillary electrophoresis was also used for the quantitative determination of costunolide and dehydrocostuslactone. The work was carried out on a Kapel 105 capillary electrophoresis system (Lumex group of companies, Russia). A detailed description of the determination method is presented in [24].

3. Results
It was previously established that double extraction with methanol at a feed-to-extractant ratio of 1:250 provides the maximum yield of analytes (costunolide and dehydrocostuslactone).

A typical chromatogram of the extract from the leaves of Laurel noble is shown in Fig. 2.

![Figure 2. Chromatogram of methanol extract from the leaves of laurel noble](image)

An electrophoretogram of the extraction of laurel noble leaves from the leaves is shown in Fig. 3.

![Figure 3. Electropherogram of an extract from the leaves of laurel noble](image)
4. Discussion
The quantitative determination of the studied sesquiterpene lactones in the test samples was carried out by comparison with standard samples.

The results of the quantitative determination of costunolide and dehydrocostuslactone in the leaves of some forms of laurel collected in the Republic of Crimea in terms of absolutely dry raw materials are presented in Table 1.

| Type of plant                                      | Content, % ±SD          |
|---------------------------------------------------|-------------------------|
|                                                   | Costunolide  | Dehydrocostuslactone |
|                                                   | HPLC         | CE           | HPLC       | CE           |
| Laurus nobilis (eriobotroliolia)                  | 0.773±0.043  | 0.751±0.042  | 0.396±0.024 | 0.435±0.027  |
| Laurus nobilis (broadleaf with corrugated leaf blade) | 0.895±0.059  | 0.863±0.057  | 0.312±0.022 | 0.314±0.024  |
| Laurus nobilis f. angustifolia                    | 0.395±0.024  | 0.384±0.024  | 0.070±0.005 | 0.072±0.006  |
| Laurus nobilis f. salisifolia (willowleaf)        | 0.488±0.027  | 0.453±0.026  | 0.055±0.004 | 0.054±0.005  |
| Laurus nobilis f. aureus                          | 0.498±0.028  | 0.491±0.027  | 0.022±0.002 | 0.024±0.002  |
| Laurus nobilis f. angustifolia (with corrugated leaf blade) | 0.152±0.009  | 0.149±0.009  | 0.087±0.007 | 0.093±0.008  |

Thus, the highest content of costunolide sesquiterpene lactone is characteristic of the broad-leaved form of laurel with a corrugated leaf blade. The lowest content of this substance is found in the form of angustifolia laurel with a corrugated leaf blade. The highest content of dehydrocostuslactone was found in the eriohotrolic form of laurel, and the lowest in Laurus nobilis f. aureus.

Earlier, a method was developed for the densitometric analysis of costunolide and dehydrocostuslactone in medicinal plant raw materials [28]. However, the error of determination using this method is more than 10%.

The results obtained using the methods developed earlier by us allow a more accurate quantitative determination of the main sesquiterpene lactones in the leaves of laurel noble.

The data obtained by the method of capillary electrophoresis reliably confirm the results of HPLC analysis of costunolide and dehydrocostuslactone in the leaves of the studied forms of laurel noble, which indicates their reliability.

5. Conclusions
The possibility of using both HPLC and capillary electrophoresis in the analysis of costunolide and dehydrocostuslactone has been shown. At the same time, using the example of some forms of laurel leaves by both methods, results comparable in quantitative content were obtained.

References
[1] Lee H K, Song H E, Lee H B and et al 2014 PloS one vol 9 (4) p e95550
[2] Luna-Herrera J, Costa M C, Rodrigues A I and Castilho P C 2007 Journal of Antimicrobial Chemotherapy vol 59 (3) pp 548–552
[3] Seo M S and Choi E M 2012 Immunopharmaccol Immunotoxicol vol 34 pp 810–814
[4] Cheong C U, Yeh C S, Hsieh Y W and et al 2016 Molecules vol 21 (7) pp 898
[5] Matsuda H, Shioda H, Ninomiya K and Yoshikawa M 2002 Alcohol and Alcoholism vol 37 pp 121–127
[6] Wang Y, Zhang X, Zhao L and et al 2017 Journal of Surgical Research vol 220 pp 40–45
[7] Ferreira A, Proença C, Serralheiro M L and Araujo E M 2006 Journal of ethnopharmacology vol 108 (1) pp 31–37
[8] Lee B K, Park S J, Nam S Y and et al 2017 *Journal of Ethnopharmacology* vol 213 pp 256–261
[9] Yoshikawa M, Shimoda H, Uemura T, Morikawa T and Kawahara Y 2000 *Bioorganic and Medicinal Chemistry* vol 8 pp 2071–2077
[10] Jeong S J, Itokawa T, Shibuya M and et al 2002 *Cancer letters* vol 187 (1) pp 129–133
[11] Zheng H, Chen Y, Zhang J and et al 2016 *Chem-biol interactions* vol 250 pp 68–77
[12] Seo C S, Lim H S, Jeong S J and Shin H K 2015 *Molec Med reports* vol 12 (5) pp 7789–7795
[13] Zhuge W, Chen R and Vladimir K 2018 *Cancer Lett* vol 1 (412) pp 46–58
[14] Peng Z, Wang Y and Fan J 2017 *J Sci Rep* vol 24 (7) p 41254
[15] Lin X, Peng Z and Su C 2015 *International journal of molecular sciences* vol 16 (5) pp 10888–10906
[16] Konovalov D A and Nasukhova N M 2014 *Pharmacy and Pharmacology* vol 2 2 (3) pp 23–33
[17] Barla A, Topcu G, Oksuz S, Tumen G and Kingston D G I 2007 *Food Chem* vol 104 (4) pp 1478–1484
[18] Fang F, Sang S, Chen K Y, Gosslau A, Ho C-T and Rosen R T 2005 *Food Chem* vol 93 (3) pp 497–501
[19] Ivanoic J, Misin D, Ristic M, Pesic O and Zizovic I 2010 *Journal of the Serbian Chemical Society* vol 75 pp 395–404
[20] Kharchenko A L 2003 *Uchenye zapiski Tavricheskogo natsionalnogo universiteta im V I Vernadsky Series "Biology"* vol 16 (55) pp 3223–3228
[21] Nasukhova N M, Logvinenko L A, Kharchenko A L and Konovalov D A 2017 *Pharmacy and Pharmacol* vol 5 (3) pp 200–221
[22] Roshchina V V, Melnikova E V, Gordon R Ya, Konovalov D A and Kuzin A M 1998 *Electronic journal of information technology in construction* vol 358–360 pp 20–23
[23] Potapov V O 2019 *Materials of the VII international scientific-practical conference "Innovations and energy technologies"* (Ukraine, Odessa) 9-13 April pp 137–139
[24] Senchenko S P, Nasukhova N M, Agova L A and Konovalov D A 2015 *Pharmacy and Pharmacology* vol 1 (8) pp 46–49
[25] Senchenko S P, Nasukhova N M, Agova L A and Konovalov D A 2014 *Bulletin of the Volgograd State Medical University* vol 4 (52) pp 18–20
[26] Senchenko S P, Agova L A, Bobrovs'kii I N, Nasukhova N M and Konovalov D A 2016 *Medical Bulletin of the North Caucasus* vol 11 (4) pp 529–532
[27] Senchenko S P, Nasukhova N M, Agova L A and Konovalov D A 2016 *Chemical Pharmaceutical Journal* vol 50 (5) pp 39–41
[28] Vijayakannan R, Karan M, Dutt S, Jain V and Vasisht K 2006 *Chromatographia* vol 63 pp 277–281