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STUDY OF ORGANIC ACIDS IN SHCHAVNAT (*RUMEX PATIENTIA* L. × *RUMEX TIANSHANICUS* LOSINSK) HERB EXTRACT BY GC/MS METHOD

Actuality. *Shchavnat (Rumex patientia L. × Rumex tianshanicus Losinsk) is a valuable fodder, vegetable, food, and medicinal plant developed by scientists from the Department of Cultural Flora of the M. M. Hryshko National Botanical Garden in Ukraine through the crossbreeding of spinach sorrel and Tianshan sorrel. Shchavnat is a source of protein, vitamins, organic and fatty acids, amino acids, macro- and microelements, lipids, and carotenoids, and it has a high caloric value. It is recommended in the form of dietary supplements*

for iron-deficiency anemia, vitamin C and A deficiency, atherosclerosis, and chemical intoxication. Considering shchavnat's wide range of biological activity, it is appropriate to obtain and study phytosubstances based on the raw materials of this plant.

The aim of the research *was to establish the qualitative composition and determine the quantitative content of individual organic acids in the extract of shchavnat herb using the GC/MS method.*

Materials and methods. *The extract from shchavnat herb was obtained using the maceration method with a forced flow of the extractant, employing 70% ethanol as the solvent at a raw material to solvent ratio of 1:5. The herb of shchavnat was collected in 2023 at the research plots of the Department of Cultural Flora of the M. M. Hryshko National Botanical Garden of the National Academy of Sciences of Ukraine.*

The organic acids in the obtained extract of shchavnat herb were identified, and their quantitative content was determined using the GC/MS method.

Results and discussion. *The qualitative composition and quantitative content of organic acids in the extract of shchavnat herb were determined using the GC/MS method. Five organic acids were identified: oxalic, succinic, malic, citric, and cis-aconitic acids. The obtained extract had a high content of citric acid (34.70 μg/mL). The content of malic and succinic acids in the extract of shchavnat herb was somewhat lower, amounting to 14.63 μg/mL and 11.65 μg/mL, respectively. The quantitative content of oxalic (3.04 μg/mL) and cis-aconitic (2.97 μg/mL) acids was significantly lower.*

Conclusion. *The results of the research indicate that the extract of shchavnat herb contains organic acids. The following organic acids were identified in the obtained extract: citric, malic, succinic, oxalic, and cis-aconitic acids. Among them, citric, malic, and succinic acids were dominant. The obtained research results can serve as a basis for further pharmacological studies and be used in the development of medicines and dietary supplements based on shchavnat.*

Key words: *shchavnat, Rumex patientia L. × Rumex tianshanicus Losinsk, herb, extract, organic acids, GC/MS.*

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ДОСЛІДЖЕННЯ ОРГАНІЧНИХ КИСЛОТ У ВИТЯЖЦІ З ТРАВИ ЩАВНАТУ (*RUMEX PATIENTIA* L. × *RUMEX TIANSHANICUS* LOSINSK) МЕТОДОМ ГХ/МС

Актуальність. *Щавнат (Rumex patientia L. × Rumex tianshanicus Losinsk) – цінна кормова, овочева, харчова та лікарська рослина, яка була виведена вченими відділу культурної флори Національного ботанічного саду імені М.М. Гришка НАН України шляхом схрещування шпинатного та тянь-шанського щавлю. Щавнат є джерелом білка, вітамінів, органічних та жирних кислот, амінокислот, макро- та мікроелементів, ліпідів та каротиноїдів і має високу калорійність. Його рекомендують у вигляді дієтичних добавок при залізодефіцитній анемії, дефіциті вітамінів С і А, атеросклерозі та хімічній інтоксикації. Ураховуючи широкий спектр біологічної активності щавнату, доцільно отримати та вивчити фітосубстанції на основі сировини цієї рослини.*

Мета дослідження. *Установити якісний склад та визначити кількісний уміст індивідуальних органічних кислот у витяжці з трави щавнату методом ГХ/МС.*

Матеріал і методи. *Витяжку з трави щавнату одержували методом мацерації з примусовою подачею екстрагента, використовуючи 70% етанол як екстрагент у співвідношенні сировини до екстрагента – 1:5. Траву щавнату було зібрано у 2023 р. на дослідних ділянках відділу культурної флори Національного ботанічного саду ім. М.М. Гришка НАН України.*

Органічні кислоти в отриманій витяжці з трави щавнату були ідентифіковані, та визначено їх кількісний уміст методом ГХ/МС.

Результати дослідження. *За допомогою методу ГХ/МС було встановлено якісний склад та визначено кількісний уміст органічних кислот у витяжці з трави щавнату. Ідентифіковано п'ять органічних кислот: щавлеву, бурштинову, яблучну, лимонну та цис-аконітову. В отриманій витяжці визначено високий уміст лимонної кислоти – 34,70 мкг/мл. Уміст яблучної та бурштинової кислот у витяжці з трави щавнату був дещо меншим і становив 14,63 мкг/мл і 11,65 мкг/мл відповідно. Кількісний уміст таких кислот, як щавлева (3,04 мкг/мл) та цис-аконітова (2,97 мкг/мл), був значно меншим.*

Висновок. *Результати дослідження свідчать, що витяжка з трави щавнату містить органічні кислоти. В отриманій витяжці були ідентифіковані такі органічні кислоти: лимонна, яблучна, бурштинова, щавлева та цис-аконітова. Серед них домінували лимонна, яблучна та бурштинова кислоти. Отримані результати досліджень можуть бути основою для подальших фармакологічних досліджень та використовуватися під час розроблення лікарських засобів та дієтичних добавок на основі щавнату.*

Ключові слова: *щавнат, Rumex patientia L. × Rumex tianshanicus Losinsk, трава, витяжка, органічні кислоти, ГХ/МС.*

Introduction. Topicality. The search for and research of new plants and the development of medicines and dietary supplements based on them are relevant for modern pharmacy.

Herbal medicines are widely used in traditional medicine in many countries around the world (Budniak, 2023). Due to their beneficial properties, plant-based remedies constitute a significant portion of the total number of medicinal products used in modern medicine (Hudzenko, 2012). Herbal medicinal products have an advantage over synthetic ones, as they rarely cause side effects and are well-tolerated by patients of different ages (Budniak, 2024).

In the 1990s, scientists from the Department of Cultural Flora of the M. M. Hryshko National Botanical Garden in Ukraine conducted breeding work by crossbreeding spinach sorrel (*Rumex patientia* L.) and Tianshan sorrel (*Rumex tianschanius* Losinsk). The resulting hybrid successfully passed all necessary tests and was included in the State Register of Plant Varieties of Ukraine (Rakhmetov, 2006; Bazhay-Zhezherun, 2014).

Shavnath (*Rumex patientia* L. × *Rumex tianshanicus* Losinsk) is a valuable fodder, vegetable, food, and medicinal plant. It plays an important role as a bioenergy plant, with its biomass used as a raw material for the production of bio-oil, biogas, bioethanol, or solid biofuel (Rolinec, 2018). In 2005, shavnath was registered in the European Union as an energy plant. Shavnath has pleasant taste qualities and can be used in dietary nutrition (Rakhmetov, 2008). It is a source of plant-based biologically valuable plant-based protein, macro- and microelements, vitamins, organic acids, lipids, amino acids, carotenoids, fatty acids, and has high caloric value. The most valuable components of shavnath are the high content of ascorbic acid and carotene in its leaves (Bazhay-Zhezherun, 2014).

Shavnath is recommended in the form of dietary supplements for iron deficiency anemia, chemical intoxication, atherosclerosis, vitamin C and A deficiencies, as well as for other diseases. Over 30 recipes have been developed for public catering, including cold appetizers, first and second courses, desserts, and beverages based on shavnath (Silka, 2017).

After conducting an analysis of the pharmaceutical market for medicinal products registered in our country, it was established that there are no medicinal products

based on shavnath raw materials in Ukraine (State register of medicines of Ukraine, http://www.drlz.com.ua; Compendium. Medicines, https://compendium.com.ua).

Considering that shavnath is still understudied, further research and investigation of extracts and phytosubstances obtained from this plant remain relevant.

The aim of the study was to establish qualitative composition and determine the quantitative content of individual organic acids in extract of shchavnat herb using the gas chromatography/mass spectrometry (GC/MS) method.

Materials and methods of the study.

Plant material

The plant material used for the research was the herb of shchavnat (*Rumex patientia* L. × *Rumex tianshanicus* Losinsk), which was collected in 2023 during the flowering period at the research plots of the Cultural Flora Department at the M. M. Hryshko National Botanical Garden of the National Academy of Sciences of Ukraine (Kyiv). The herb was dried in a warm-air convection dryer at a temperature of 40°C and stored in paper bags in a dry place (Slobodianiuk, 2022).

Obtaining the extract

To obtain the extract from shchavnat herb, 70 % ethanol was used as an extractant at a raw material to extractant ratio of 1:5, and the maceration method with forced extractant flow was applied.

Extraction of organic acids

An aliquot of the shchavnat herb extract (1000 µL) was evaporated to dryness using a rotary evaporator at a temperature of 40°C. To the dry residue, 600 µL of methanol and 300 µL of 50% sulfuric acid were added and thoroughly mixed. Methylation of organic acids was carried out for several hours at 60°C. After methylation, the mixture was cooled to room temperature, and 500 µL of chloroform and 500 µL of 6.0% potassium carbonate solution were added and thoroughly mixed. The chloroform phase was used for chromatographic analysis.

Chromatographic conditions.

GC/MS analysis of organic acids was performed using a gas chromatograph Agilent 6890N with a mass detector 5973 inert (Agilent Technologies, USA). Samples were analyzed using an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm, Agilent Technologies, USA). The evaporator temperature was set at 250°C, and the interface temperature at 280°C. The separation was carried out in temperature programming mode: the initial temperature of 70°C was held for 1 minute, then increased at a gradient of 5°C/min to 220°C and held for 1 minute, followed by an increase at a gradient of 10°C/min to 300°C. The final temperature was held for 5 minutes. A 1 µL sample was injected in split mode with a flow ratio of 1:50. Detection was performed in SCAN mode in the range of 38–400 m/z. Helium was used as the carrier gas at a constant flow rate of 1.0 ml/min (Budniak, 2021).

Identification of organic acids was carried out by comparing the retention times of standards (oxalic, maleic, succinic, benzoic, itaconic, malic, benzeneacetic, α-ketoglutaric, salicylate, p-formylbenzoic, *cis*-aconitic, vanillic, gentisic, citric, and isocitric acids) with the the National Institute Standard and Technology (NIST 2008) database.

Results and discussion.

A total of five organic acids, namely oxalic, succinic, malic, citric, and *cis*-aconitic acids, were identified and quantified in the extract of shchavnat herb using the GC/MS method (fig. 1).

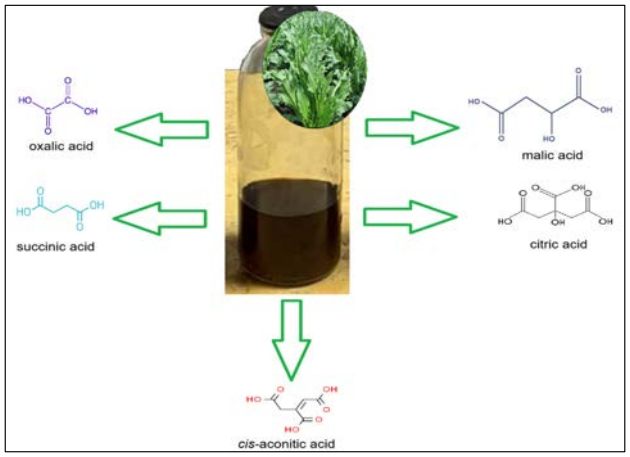

Fig. 1. Organic acids identified in the extract of shchavnat (*Rumex patientia* L. × *Rumex tianshanicus* Losinsk) herb

In plants, the content of organic acids varies with seasonal, diurnal, species, and varietal changes, affecting both the total content and qualitative composition.

Organic acids play a significant role in the biochemical processes of mammals, particularly in the Krebs cycle, where they contribute to the formation of adenosine triphosphate, the primary source of cellular energy (Dunn, 2023).

Organic acids exhibit a wide range of effects on the human body and are commonly utilized in medical practice for their antiseptic, detoxifying, and choleretic properties (Krch, 2017; Panchal, 2021).

The results of the determination of the component composition of organic acids in the extract of shchavnat herb are presented in the table, and the chromatograms of the organic acids of the studied object are shown in fig. 2.

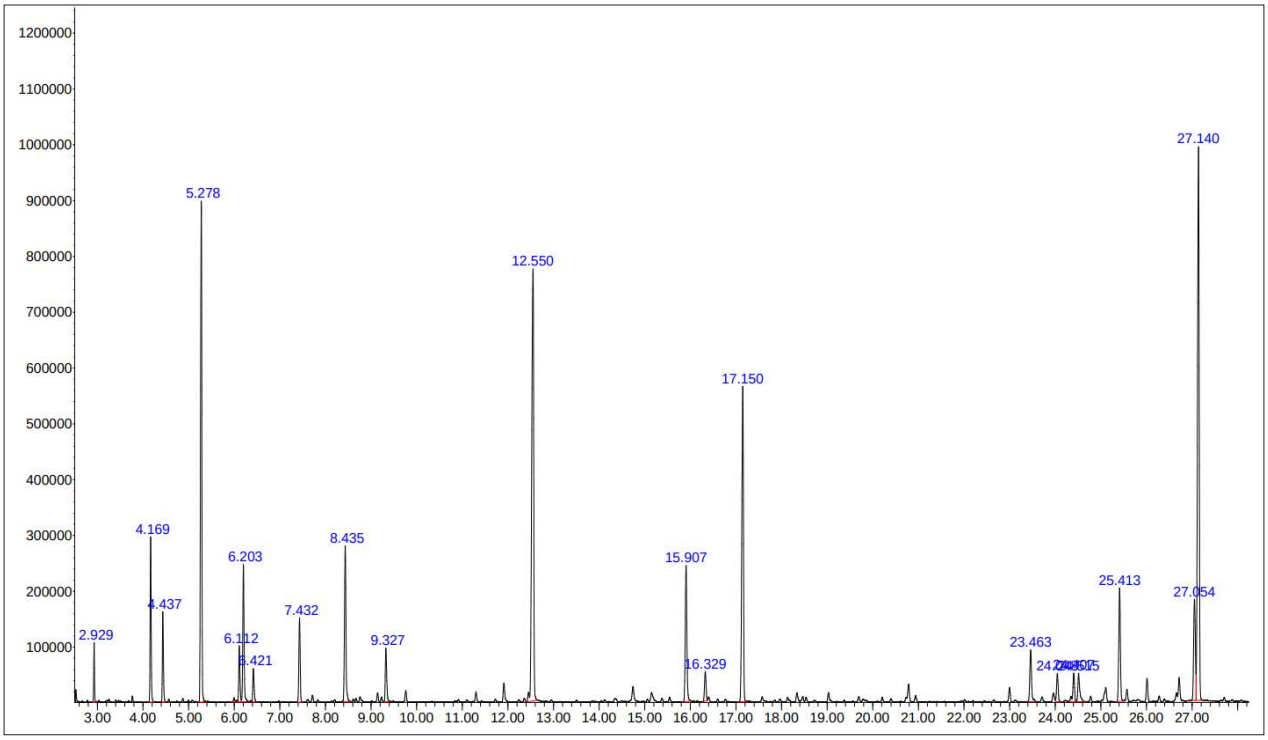

Fig. 2. GC/MS chromatogram of organic acids in the extract of shchavnat (*Rumex patientia* L. × *Rumex tianshanicus* Losinsk) herb

Table

The results of the organic acids determination in the extract of shchavnat (*Rumex patientia* L. × *Rumex tianshanicus* Losinsk) herb

N	Retention time, min	Common name of organic acid (IUPAC)	Molecular formula	Quantitative content (μg/mL)
1	2.93	Oxalic (ethanedioic) acid	$C_2H_2O_4$	3.04
3	6.2	Succinic (butanedioic) acid	$C_4H_6O_4$	11.65
5	8.44	Malic (2S)-2-hydroxybutanedioic) acid	$C_4H_6O_5$	14.63
6	16.33	*cis*-aconitic (*Z*)-prop-1-ene-1,2,3-tricarboxylic) acid	$C_6H_6O_6$	2.97
7	17.15	Citric (2-hydroxypropane-1,2,3-tricarboxylic) acids	$C_6H_8O_7$	34.70

As shown in Table, the highest content of citric acid (34.70 μg/mL) was found in the extract of shchavnat herb.

This acid is a common plant metabolite, a natural component, and the most universally and widely used organic acid in pharmaceuticals and the food industry compared to other organic acids. Citric acid and its salts are used in industry for chelation, derivatization, and as a buffering agent and pH regulator (Crolla and Kennedy, 2001; Celik, 2014).

The next most abundant identified organic acids are malic and succinic acids, with their content in the extract of shchavnat herb being 14.63 μg/mL and 11.65 μg/mL, respectively.

Malic acid is known as the "most ideal food acidity agent". In low concentrations, malic acid positively affects the human body by improving appetite, boosting immunity, promoting collagen synthesis, and having anti-inflammatory, anti-edematous, and laxative effects. Due to its antioxidant properties, it is widely used in the food industry, medicine, pharmaceuticals, and cosmetics. In cosmetics, it is included in deep-cleaning and moisturizing products, as well as in formulations aimed at wrinkle reduction and stimulating collagen synthesis. In pharmaceuticals, malic acid is used in the production of drugs and dietary supplements. Additionally, it extends product shelf life by reducing the presence of microorganisms. Malic acid is an essential

organic acid and a low-calorie food additive (Chi, 2016; Nazarko, 2022).

Succinic acid is a precursor to a significant number of biologically active compounds and is particularly important in the accumulation of succinate, a mitochondrial metabolite. During ischemia, succinic acid controls reperfusion injury by regulating the production of mitochondrial reactive oxygen species (Khvorost, 2023). The application of succinic acid, which is an intermediate product of the Krebs cycle, stimulates various physiological and biochemical processes in plants. The effect of this acid is observed even at relatively low concentrations and is explained not only to the activation of photosynthetic processes but also to the intensive synthesis of reduced forms of amino acids. Moreover, succinic acid can modify enzyme activity, increase seed germination and the productivity of certain plant species, stimulate growth processes, and enhance the synthesis of ascorbic acid.

The content of oxalic acid in the extract of shchavnat herb was lower, amounting to 3.04 μg/mL. Oxalic acid is characterized by prolonged action and metabolic stability. Oxalic acid has the ability to selectively target malignant cells, leading to the apoptosis of cancer cells, while not affecting healthy cells in the body. This acid is used in the production of a therapeutic anticancer drug (Khvorost, 2023).

Another organic acid found in the extract of the studied plant was *cis*-aconitic acid. Pinto de Oliveira et al. established that this acid has significant anti-inflammatory effects in monosodium urate-induced arthritis in mice and antigen-induced arthritis. This, in turn, suggests its potential for the treatment of inflammatory joint diseases in humans.

Conclusions

1. The results of the research indicate that the extract of shchavnat (*Rumex patientia* L. × *Rumex tianshanicus* Losinsk) herb contains organic acids.

2. The qualitative composition and quantitative content of organic acids were studied using the GC/MS method. Five organic acids were identified in the extract of shchavnat herb, namely citric, malic, succinic, oxalic, and *cis*-aconitic. Among them, citric (34.70 μg/mL), malic (14.63 μg/mL) and succinic (11.65 μg/mL) acids were dominant.

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