

RESOURCE POTENTIAL OF THE GENUS *SORBUS* L. PLANTS AS SOURCE OF METABOLITES WITH ANTI-INFLAMMATORY ACTIVITY

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The work is devoted to determining the anti-inflammatory ability of the genus *Sorbus* L. plants and establishing correlations between the biological activity of plants and the phenolic compounds content and composition. Common plant species in Ukraine, known in traditional medicine for various curative and preventive properties, are a valuable, but insufficiently studied source of biologically active compounds. The rationale for the relevance of research aimed at identifying natural substitutes for the currently used synthetic anti-inflammatory drugs in order to get rid of the negative side effects of their use is provided. The anti-inflammatory ability of rowan plants was evaluated by the degree of inhibition of heat-induced denaturation of albumin by plant leaf extracts in comparison with the effect of the well-known synthetic drug diclofenac. A dose-dependent decrease in albumin denaturation by ethanolic extracts of the leaves of *S. domestica*, *S. torminalis*, and *S. aucuparia* plants was experimentally established. At the highest concentration of extracts, their activity in inhibiting heat-induced albumin denaturation was almost half of the maximum activity of diclofenac, which indicates a significant anti-inflammatory potential of plant extracts. Correlation analysis revealed a strong positive relationship between the degree of inhibition of heat-induced denaturation of albumin and the content of polyphenols, flavonoids and phenolic acids in plant extracts. The study of the seasonal dynamics of the total content of phenolic compounds in plant leaves showed the highest level of their accumulation in June in all species, which can serve as a criterion for selecting the best plant material. It was concluded that the ethanolic extracts of *S. domestica*, *S. torminalis* and *S. aucuparia* leaves are an available source of biologically active secondary metabolites, potentially suitable for the development of natural anti-inflammatory drugs. *Key words*: rowan species, plant extracts, biologically active substances, anti-inflammatory activity, polyphenols, flavonoids, phenolic acids.

Ресурсний потенціал рослин роду *Sorbus* L. як джерела метаболітів із протизапальною активністю. Ляшенко О.В., Хроміх Н.О., Лихолат Ю.В.

Робота присвячена визначенню протизапальної здатності рослин роду *Sorbus* L. та встановленню кореляційних зв'язків між біологічною активністю рослин і вмістом та складом фенольних сполук. Поширені в Україні рослинні види, відомі у традиційній медицині різноманітними лікувально-профілактичними властивостями, є цінним, проте недостатньо вивченим джерелом біологічно активних сполук. Надано обґрунтування актуальності досліджень, спрямованих на виявлення природних замінників використовуваних наразі синтетичних протизапальних препаратів задля позбавлення від негативних побічних ефектів їх застосування. Протизапальну здатність рослин горобини оцінювали за ступенем інгібування теплової денатурації альбуміну екстрактами листя рослин у порівнянні з ефектом відомого синтетичного препарату диклофенак. Експериментально встановлено дозово-залежне зниження денатурації альбуміну етаноловими екстрактами листя рослин *S. domestica*, *S. torminalis* і *S. aucuparia*. За найвищої концентрації екстрактів їх активність щодо інгібування теплової денатурації альбуміну становила майже половину від максимальної активності диклофенаку, що свідчить про значний протизапальний потенціал рослинних екстрактів. Кореляційний аналіз виявив сильний позитивний зв'язок між ступенем інгібування теплової денатурації альбуміну та вмістом поліфенолів, флавоноїдів і фенольних кислот у рослинних екстрактах. Дослідження сезонної динаміки загального вмісту фенольних сполук у листі рослин показало найвищий рівень їх накопичення у червні в усіх видів, що може слугувати критерієм відбору рослинної сировини. Зроблено висновок про перспективність етанолових екстрактів листя *S. domestica*, *S. torminalis* і *S. aucuparia* як доступного джерела біологічно активних вторинних метаболітів, потенційно придатних для розробки натуральних протизапальних препаратів. *Ключові слова*: види горобини, рослинні екстракти, біологічно активні речовини, протизапальна активність, поліфеноли, флавоноїди, фенольні кислоти.

Formulation of the problem. One of the modern trends in the human health protection is the elimination of synthetic compounds from food and the use of natural plant extracts as the raw materials for the medicines production [1]. The most valuable are the extracts of traditional medicinal plants, which contain biologically active substances in perfect synergy, which can promise to obtain a therapeutic effect without undesirable consequences. The use of bioactivity of plants is becoming common because of the harmfulness and toxicity of some synthetic drugs. In particular, the unwanted side effects of some effective synthetic non-steroidal

anti-inflammatory drugs can manifest in the form of heartburn, rash, and gastrointestinal bleeding [2]. The above determines the expediency of research aimed at identifying the anti-inflammatory activity of plant extracts with the potential for practical application of the results for the development of medicinal products.

The aim of the work was to estimate the anti-inflammatory ability and its correlation with the polyphenols content in leaf extracts of the genus *Sorbus* L. plants, widespread in the flora of Ukraine.

The relevance of research. Plants are the important source of health-protective substances for humans,

including the *Sorbus* species, which are known in ethnopharmacology, but remain an underestimated and little-studied in terms of their different therapeutic effects' confirmation [3]. The anti-inflammatory activity was shown today in a few *Sorbus* species by the in vitro assays [4; 5], which indicates the promising direction of such researches.

Connection of the article with important scientific and practical tasks. Achievement of the research objective will improve the understanding the relationship between the health-promoting effects of plants and the phytochemical compounds that may be responsible for the biological activity, and will also expand the plant resource for the creation of therapeutic agents.

Analysis of recent research and publications. Plants are a traditional source of biologically active compounds that have served humans for a long time. So, it is quite expected that even today about 30% of pharmaceuticals worldwide are made from plant sources [1]. More than 250 species of the genus *Sorbus* L. grow in different climatic zones and are well known for their ethnomedicinal importance, but are underestimated today [3]. In Ukraine, the most common autochthonous species are *S. domestica*, *S. torminalis* and *S. aucuparia*. Recent studies confirmed several beneficial medical effects of the extracts derived from different parts of *Sorbus* plants. Anti-inflammatory ability was exhibited by the water extracts of *S. commixta* stem bark [4] and the methanol-water leaf extracts of *S. domestica* [5].

Inflammation is believed to be a normal protective response of living cells of the body to damage or injury, which is necessary as a way to limit and localize irritation, infection, and pathogens [6]. In turn, the body's response to inflammation causes a pathological condition accompanied by redness, heat, swelling and pain and can reduce physiological capabilities [2]. It has been established that inflammation is mainly caused by the formation of free radicals, so the effect of antioxidants may have a protective role in inflammatory processes [7]. In particular, the numerous health-promoting properties of phenolic compounds were associated [8] precisely with their high antioxidant potential, since the presence and distribution of numerous hydroxyl groups in the chemical structure of polyphenols makes them excellent antioxidants. According to the known data [2], inflammation accompanies the course of many diseases, during which the denaturation of intracellular proteins can lead to tissue damage. Therefore, the ability of a substance to inhibit protein denaturation means the potential for anti-inflammatory activity.

Highlighting previously unsolved parts of the general problem to which this article is devoted. This work is caused by the limited knowledge about the anti-inflammatory ability of plant extracts of various species of the genus *Sorbus* L.

The novelty of the study. The comparative studying the anti-inflammatory potential of leaf extracts of

the most common *Sorbus* species in the steppe zone of Ukraine was carried out for the first time.

Methodological and general scientific significance. Analysis of correlation between anti-inflammatory activity and polyphenols, flavonoids and phenolic acids content of leaf extracts of *Sorbus* species reflect a relationship between plant biological activities and phytoconstituents which can be responsible for these effects.

Description of the study. Plant material was collected during 2023 growing season from trees of *S. domestica*, *S. torminalis* and *S. aucuparia* in the botanical garden of Oles Honchar Dnipro National University. Washed, dried and crushed leaves were used for the crude extracts preparing. All measurements of polyphenols content and biological activity were carried out in threefold repetition. The results were expressed as mean \pm standard deviation ($\bar{x} \pm SD$). Correlations between biological activities and polyphenols were considered significant at $P \leq 0.05$.

Research methods. Method of cold maceration in 96% ethanol (1:10, w/v) was used to prepare leaf extracts followed by extractant removing at 45 °C with a rotary evaporator IKA® RV 10 (Germany), and dry mass was stored at 4°C.

The anti-inflammatory activity was estimated by the ability to inhibit heat-induced albumin denaturation according to [9] with a slight modification. Tested leaf extracts at concentrations of 800 $\mu\text{g/ml}$, 400 $\mu\text{g/ml}$, and 200 $\mu\text{g/ml}$ were prepared by dissolving evaporated dry mass in ethanol. Fresh chicken egg albumin (5% aqueous solution) served as a protein source. The reaction mixture (1 ml of extract, 1.5 ml of phosphate buffer, and 0.2 ml of albumin) was incubated for 15 min at 37°C, then kept at 70°C for 15 min. After cooling, the optical density of the mixture was measured at 660 nm. Negative control contained distilled water instead of extract; reagent-control did not contain albumin. As a positive standard, anti-inflammatory drug diclofenac sodium was used. The degree of inhibition of albumin denaturation (as a percentage of complete denaturation in the negative control) was calculated using the formula:

$$\text{Percentage inhibition (\%)} = 100 - [(A_{\text{extract}} - A_{\text{reagent-control}}) / A_{\text{negative control}}] \times 100.$$

Total polyphenols content was determined according to [10], measuring at 726 nm the optical density of the reaction mixture (plant extract, Folin-Chiocalteu reagent and sodium carbonate), cooled after standing for 40 minutes at 45°C. Results were expressed in gallic acid (GA) equivalents. The total content of flavonoids was determined using the aluminum chloride method [11] by the optical density of the mixture (extract, acetate buffer and AlCl_3 solution) at 425 nm, and the results were expressed in rutin (Ru) equivalents. The content of phenolic acids was determined according to [8] with Arnov's reagent, measuring the optical density of the mixture at 490 nm and expressing the results in caffeic acid (CA) equivalents.

Study results of crude leaf extracts of *Sorbus* plants showed variability of polyphenols accumulation in plant leaves during the vegetative period (Table 1).

The capacity of tested ethanol leaf extracts of *Sorbus* species to inhibit heat-induced denaturation of albumin varied from $12.56 \pm 0.02\%$ (*S. torminalis* extracts at 200 $\mu\text{g/ml}$) to $47.73 \pm 0.11\%$ (*S. domestica* extracts at 800 $\mu\text{g/ml}$). The highest albumin denaturation decrease was shown by *S. domestica* extracts, which in concentration of 800 $\mu\text{g/ml}$ exceeded the appropriate activities of *S. torminalis* and *S. aucuparia* by 2.50% and 12.13%. The inhibiting ability of plant extracts was comparable with anti-inflammatory activity of the reference drug diclofenac sodium, which inhibited albumin denaturation by 89.14%, 78.64%, and 42.46%, respectively in the concentrations of 800 $\mu\text{g/ml}$, 400 $\mu\text{g/ml}$, and 200 $\mu\text{g/ml}$. The maximal albumin denaturation inhibiting achieved by tested leaf extracts at 800 $\mu\text{g/ml}$, were close to diclofenac activity at 200 $\mu\text{g/ml}$, which indicate the prominent anti-inflammatory potential of *S. domestica*, *S. torminalis* and *S. aucuparia* leaf extracts.

Study results are consistent with data [9] about the *B. racemosa* methanol leaf extract inhibition of heat albumin denaturation by 65.29%, whereas diclofenac activity was 94.9% of inhibition. Similar result was exhibited by *Cycas pectinata* methanol leaf extract [2] in different concentrations (62.5, 125, 250, and 500 $\mu\text{g/mL}$), which caused 20.96–38.12% decreasing protein denaturation compared to diclofenac

sodium (65.40–83.50%). Crude root extracts of *Berberis orthobotrys* produced 93.30% inhibition of protein denaturation at 800 $\mu\text{g/ml}$, while diclofenac sodium at the same concentration brought about 99.19% suppression of protein denaturation [13].

Correlation analysis established the relationship between the polyphenols, flavonoids, and phenolic acids content and bioactivity of the extracts (Table 2).

The strong positive correlation was revealed between phenolic compounds content in the tested extracts and their ability to decrease heat-inducing albumin denaturation, which confirm the known [12; 14] polyphenols significance for bioactivity of the genus *Sorbus* plants. At the same time, the obtained correlation coefficients also indicate the contribution of non-phenolic compounds in decrease of albumin denaturation, which require further studying.

The results obtained indicate promising leaf extracts of *S. domestica*, *S. torminalis* and *S. aucuparia* as the natural source of anti-inflammatory activity. Taking into account the ethnomedical data [15] about the use of preparations made from leaves *S. domestica* and *S. aucuparia* plants as a natural remedy for gastrointestinal problems, one can expect the absence of undesirable side effects of the use of extracts.

Conclusion

1. Ability of leaf ethanol extracts of *S. domestica*, *S. torminalis* and *S. aucuparia* to inhibit the heat-induced albumin denaturation had a dose-dependent character, and at the highest concentration might be comparable

Table 1

Seasonal changes of phenolic compounds content in leaves of *Sorbus* plants

Plant species	Total polyphenols content, mg GA/g DW				
	May	June	July	August	September
<i>S. domestica</i>	$31,4 \pm 0,4$	$40,7 \pm 0,2$	$37,7 \pm 0,9$	$29,6 \pm 1,0$	$27,6 \pm 0,2$
<i>S. torminalis</i>	$12,6 \pm 0,4$	$16,5 \pm 0,2$	$9,4 \pm 0,4$	$15,9 \pm 0,9$	$13,5 \pm 0,4$
<i>S. aucuparia</i>	$23,1 \pm 0,3$	$26,9 \pm 0,4$	$21,7 \pm 0,3$	$18,4 \pm 0,8$	$16,9 \pm 0,2$

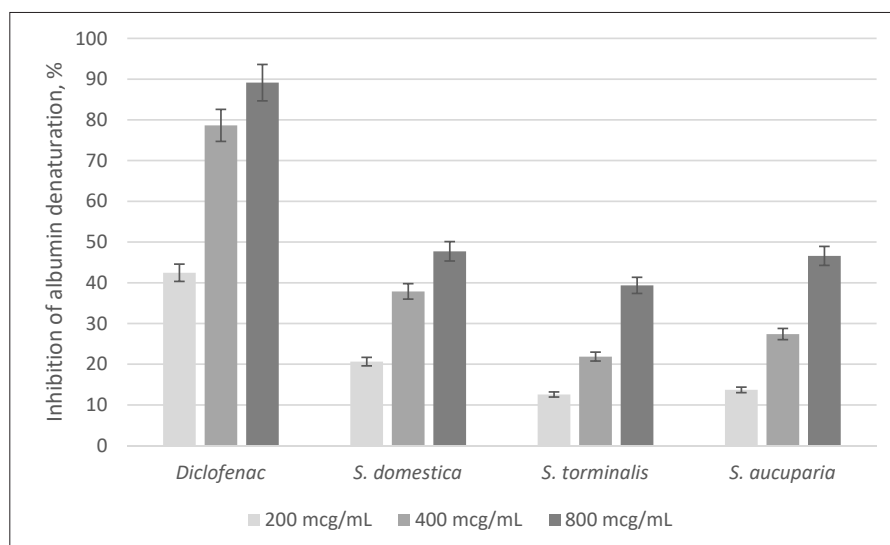


Fig. 1. Inhibition (%) of egg albumin denaturation by leaf extracts of *S. domestica*, *S. torminalis* and *S. aucuparia* as compared to diclofenac sodium

Table 2

Correlation between phenolic compounds content and denaturation inhibition ability of the ethanol leaf extracts of *Sorbus* plants

Indicator	Concentration	<i>S. domestica</i>	<i>S. torminalis</i>	<i>S. aucuparia</i>
Polyphenols content in extract	800 µg/ml	198.4 ± 0.5	79.1 ± 0.3	125.1 ± 0.5
	400 µg/ml	119.6 ± 0.3	39.4 ± 0.3	61.6 ± 0.2
	200 µg/ml	79.8 ± 0.3	28.5 ± 0.1	32.4 ± 0.1
Correlation	$y = 0,2145 \cdot x + 11,539; R^2 = 0,7617$			
Flavonoids content in extract	800 µg/ml	37.4 ± 0.3	26.6 ± 0.2	45.3 ± 0.4
	400 µg/ml	12.6 ± 0.2	10.7 ± 0.1	18.7 ± 0.2
	200 µg/ml	8.5 ± 0.1	9.3 ± 0.1	9.2 ± 0.1
Correlation	$y = 0,8642 \cdot x + 11,756; R^2 = 0,7125$			
Phenolic acids content in extract	800 µg/ml	0.69 ± 0.02	0.27 ± 0.02	0.25 ± 0.01
	400 µg/ml	0.21 ± 0.02	0.14 ± 0.01	0.13 ± 0.01
	200 µg/ml	0.17 ± 0.01	0.09 ± 0.01	0.06 ± 0.01
Correlation	$y = 58,745 \cdot x + 14,524; R^2 = 0,7127$			

with the inhibiting capacity of the known anti-inflammatory drug diclofenac sodium. Considering ethnomedical data about the health-protective properties of *Sorbus* plants, one can expect the absence of undesirable side effects of leaf extracts which are promising source for natural anti-inflammatory remedy.

2. Correlation analysis confirmed high contribution of polyphenols, flavonoids, and soluble phenolic acids content into the anti-inflammatory ability of leaf extracts of *Sorbus* species, and indicated the contribution of non-phenolic compounds as well.

3. Studying seasonal dynamics showed the highest polyphenols content in leaves of *S. domestica*, *S. torminalis* and *S. aucuparia* collected in June, which indicated the best plant material to extract the phytoconstituents having anti-inflammatory ability.

Prospects for the use of research results. The discovery of significant ability of *S. domestica*, *S. torminalis* and *S. aucuparia* ethanol leaf extracts to inhibit the heat-induced albumin denaturation opens up prospects for obtaining an available and inexpensive natural source of anti-inflammatory agents.

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