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POLYPHENOLS CONTENT, ANTIOXIDANT CAPACITY, AND ANTIMICROBIAL POTENTIAL OF INTRODUCED FRUIT PLANT *PRINSEPIA UNIFLORA* BATALIN

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This work deals with the studying antioxidant ability and the polyphenols accumulation of introduced fruit plant *Prinsepia uniflora* Batalin in order to reveal the low spread species having great secondary metabolites content and bioactivity. The urgency of the task of enriching the plant resource base for a balanced diet, the creation of functional foods and potential sources of new drugs substantiated by the results of research in recent years. The relevance of this task for the steppe zone having an insufficient variety of the fruit plant species with health-promoting properties was argued. High polyphenols content in *Prinsepia uniflora* fruits with predominant accumulation in the peel compared to the pulp was established. However, total antioxidant capacity was greater in the fruit pulp, indicating the possible contribution of some other secondary metabolites in the fruit antioxidant potential along with the polyphenols. Analysis of *Prinsepia uniflora* leaf extracts revealed twice the levels of polyphenols and antioxidant capacity in young leaves compared to mature ones. In general, the predominant accumulation of polyphenols in the leaves, while the greater antioxidant potential in the fruits was established. Thus, the results of the work testify in favor of the suitability of both the generative and vegetative organs of *Prinsepia uniflora* plants as a rich source of diverse secondary metabolites including polyphenols. Significant antimicrobial activity of both plant fruit and leaf extracts was experimentally shown by disc-diffusion assay. The greatest inhibition of colony growth was exhibited against Gram-positive bacterial strain *Staphylococcus aureus* B904, Gram-negative *Erwinia dissolvens* 170, and clinical fungal strain *Candida albicans* as well. Revealed range of the antimicrobial activity indicates the viability of *Prinsepia uniflora* fruits and leaves as a potential source of active compounds for the creation of new drugs. It was concluded that the introduced *Prinsepia uniflora* plants are able to realize the genetic potential for the accumulation of secondary metabolites and can enrich the phytodiversity of the steppe zone, serving as a potent source of biologically active compounds. *Key words:* secondary metabolites, antioxidants, antimicrobial activity, non-traditional fruit plants, *Prinsepia uniflora* Batalin.

Уміст поліфенолів, антиоксидантна здатність та антимікробний потенціал інтродукованої плодової рослини *Prinsepia uniflora* batalin. Лихолат Ю.В., Хромих Н.О., Дідур О.О., Скляр Т.В., Дрегваль О.А., Лихолат О.А.

Дана робота присвячена вивченню антиоксидантної здатності та накопичення поліфенолів інтродукованої плодової рослини *Prinsepia uniflora* Batalin з метою виявлення малопоширених видів, що мають значний уміст вторинних метаболітів та біологічну активність. Надано обґрунтування актуальності завдання збагачення рослинно-ресурсної бази для збалансованого харчування людини, створення функціональних продуктів харчування та потенційних джерел нових лікарських засобів, яке базується на результатах досліджень останніх років. Аргументовано актуальність цього завдання для степової зони з недостатнім розмаїттям видів плодових рослин, які мають оздоровчі властивості. Встановлено високий уміст поліфенолів у плодах *Prinsepia uniflora* з переважним накопиченням у шкірці порівняно з м'якоттю плодів. Однак загальна антиоксидантна здатність була більшою в м'якоті плодів, що вказує на можливий внесок деяких інших вторинних метаболітів у антиоксидантний потенціал плодів разом з поліфенолами. Аналіз екстрактів листя *Prinsepia uniflora* виявив у молодому листі вдвічі більший рівень поліфенолів та антиоксидантної здатності порівняно зі зрілими. У цілому встановлено, що у листках переважає накопичення поліфенолів, а у плодах – більший антиоксидантний потенціал. Отже, результати роботи свідчать про придатність як генеративних, так і вегетативних органів рослин *Prinsepia uniflora* як багатого джерела різноманітних вторинних метаболітів, у тому числі поліфенолів. Експериментально показано значну антимікробну активність екстрактів плодів і листя рослин за допомогою диск-дифузійного аналізу. Найбільше пригнічення росту колоній було проти грам-позитивного штаму бактерій *Staphylococcus aureus* B904, грам-негативного *Erwinia dissolvens* 170, а також клінічного штаму грибів *Candida albicans*. Виявлений діапазон антимікробної активності вказує на можливість плодів і листя *Prinsepia uniflora* бути потенційним джерелом активних речовин для створення нових лікарських засобів. Зроблено висновок, що інтродуковані рослини *Prinsepia uniflora* здатні реалізувати генетичний потенціал із накопичення вторинних метаболітів та збагатити фіторізноманіття степової зони, виступати як потужне джерело біологічно активних сполук. *Ключові слова:* вторинні метаболіти, антиоксиданти, антимікробна активність, нетрадиційні плодові рослини, *Prinsepia uniflora* Batalin.

Formulation of the problem. High intake of the antioxidant-rich foods, especially plant-based foods, can provide the better human health and functional longevity. Enrichment of products with the polyphenols and other plant secondary metabolites with significant antioxidant capacity has a beneficial effect on the health-related variables, including the cardiovascular system and brain function. This trend suggests the availability

of plant materials both as part of the human diet and for obtaining a complex of non-nutrient secondary metabolites and creating functional food with an increased content of the biologically active compounds. In addition, some secondary plant metabolites have shown high antimicrobial activity, including inhibition of the growth of antibiotic-resistant pathogens. The foregoing explains the steady interest of researchers in identifying the antioxidant properties of little studied plants, including autochthonous and alien species.

The aim of the work was to determine the secondary metabolites accumulation, antioxidant potential and biological activity of leaves and fruit extracts of the introduced low spread plant *Prinsepia uniflora* Batalin, which is known in traditional medicine in northwest China for its therapeutic effects.

The relevance of research. Introduced plant species with a rich composition of the secondary metabolites expanse the phytodiversity and plant raw material base in the steppe zone of Ukraine, which has an insufficient range of the fruit and berry crops. However, the implementation of the processes of the biologically active compounds accumulation by the introduced plants is determined not only by the species-specific genetic properties, but is largely influenced by growing conditions, and therefore needs to be comprehensively studied. In addition, the known biological activity of secondary metabolites synthesized by the introduced plants in the new environments also needs confirmation.

Connection of the article with important scientific and practical tasks. Achieving the goal of the work was associated with the following tasks: determination of the total content of polyphenols and total antioxidant capacity in the leaves and fruits of introduced *Prinsepia uniflora* plants, as well as testing the antimicrobial potential of the extracts from leaves and fruits of *Prinsepia uniflora* plants.

Analysis of recent research and publications. The discovery of potential plant sources of biologically active compounds suitable for the strengthening food security by the development of nutritional balanced natural products and the functional food or the creation of new drugs was declared as one of the urgent problems in recent years [1; 2]. In this line, various aspects of plant secondary metabolism have been the subject of recent research. It was established that the secondary metabolites content in the fruits is determined by the course of their biosynthesis, transformation and accumulation during fruit ripening [3]. At the same time, plant growth conditions can also influence the outcome the synthesis process of bioactive compounds. For example, the altitude-dependent variations of anthocyanin content and profile throughout fruit development and ripening of highbush blueberry cultivars were found [4].

The secondary metabolism was observed in different parts of plant organism, including the vegetative organs of fruit plants, which contributed to the replenishment of the plant material base. In particular, it was confirmed the use of leaves and other wastes of plant cultivation as a potential source of polyphenols on the base of study results of the blackberry leaves [5], the rowan fruit peel [6], and the antioxidant activity and phytochemical composition of the methanolic extracts from leaves and flowers of *Crataegus azarolus* [7]. The rational use of plant materials has become an important aspect in recent years, in line with which the novelty technologies for polyphenols recovery from agro-food by-products were proposed [8]. The current approach to the effective use of the health-improving properties of plant secondary metabolites combines several areas, including the saturation of the diet with a variety of plant products and the development of functional food with an increased content of biologically active compounds. According to this trend, G. Khomych et al [9] reported the creation of food products with targeted biological action using natural ingredients of raw materials with probiotic properties. The limitation of the artificial flavors and colors content by the replacing them with appropriate components derived from the fruits processing was proposed [10].

Apart the dietary and functional food aspects, the potent of secondary metabolites as a source for creation of new drugs has become a promising area of research. The positive effect of flavonoids on learning, memory and neurocognitive performance was established [11]. Polyphenolic extracts from different plants showed antioxidant the antihyperglycemic activities [12] as well as vascular-protective ability [13]. Plants of the species *Prinsepia uniflora* Batalin used in traditional Chinese medicine for the treatment of eye diseases [14], and is known as the source of alkaloid galactosides [15].

Highlighting previously unsolved parts of the general problem to which this article is devoted. This work is due to insufficient knowledge of the secondary metabolites chemical composition, antioxidant capacity and biological activity of *Prinsepia uniflora* plants.

The novelty of the study. For the first time, the features of the polyphenols and antioxidant activity distribution in the vegetative and generative organs of *Prinsepia uniflora* plants was studied. The need for a differentiated study of the fruit peel and pulp activity is substantiated. The antimicrobial potential of *Prinsepia uniflora* leaf and fruit extracts was examined for the first time.

Methodological and general scientific significance. Analysis of the relationship between polyphenols content and the antioxidant activity of the vegetative and generative organs of *Prinsepia uniflora* reflect a systematic assessment of plant genetic potential implementation in a new environment. The antimicrobial efficacy of the leaves and fruits of the plant contributes to understanding of the plant-pathogen interaction.

Description of the study. Plant material was collected in 2021 in the Botanical Garden of Oles Honchar Dnipro National University, where plants of *Prinsepia uniflora* Batalin (*Rosaceae* family) were introduced

above 60 years ago. Ripe fresh fruits but air-dried leaves were used for the crude extracts obtaining. All measurements of content, antioxidant and biological activity of the extracts were carried out in fivefold repetition.

Research methods. The isopropanol-water (80:20, v/v) extracts from the leaves and fruits (separated peel and pulp) were prepared. Briefly, 2.0 g of ground plant material with 20 mL of isopropanol kept for 24 hours at room temperature with shaking followed by filtration. Total polyphenols content, and total antioxidant capacity were determined in the crude extracts obtained. For the antimicrobial assays, crude extracts were evaporated, and solid residue was dissolved in 600 μL of isopropanol.

Content of polyphenols was determined according to Singleton et al [16] with Folin-Ciocalteu reagent, which was added to reaction mixture containing 0.2 mL of plant extract, 0.2 mL of distilled water, and 0.8 ml of sodium carbonate solution. The reaction mixture was incubated for 40 minutes at 45 °C and cooled. The absorbance was measured using spectrophotometer at 726 nm; the results were calculated on a calibration graph and expressed as mg gallic acid (GA) equivalents per 100 g (mg GA/100 g).

Evaluation of total antioxidant capacity of fruit and leave extracts was carry out in accordance with Prieto et al [17] using the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). After the samples incubation at 95 °C for 90 min, absorbance at 695 nm was measured against blank; the results were calculated using a calibration graph prepared on the solutions of ascorbic acid (AA), and expressed as mg of ascorbic acid equivalents (mg AA/100 g).

Antimicrobial activity of the fruit and leaf extracts were tested using the disc diffusion method [18]. The test strains of microorganisms were from the culture collection of Microbiology, Virology and Biotechnology Department of Oles Honchar DNU. Of these, there were collection Gram-negative bacterial strain *Erwinia dissolvens* 170, Gram-positive strain *Staphylococcus aureus* B904, and clinical fungus *Candida albicans*. In each case, Petri plates containing meat-peptone agar (MPA) medium were seeded with suspension of microorganisms. Plant extracts were applied on the sterile paper discs (6 mm diameter) which were placed on the agar surface; plates incubated at 37 °C for 24 h. Antibiotics ofloxacin and itraconazole were used as the positive control for tested bacterial and fungal strains, respectively. Antimicrobial activity of plant extracts was expressed as the inhibition zone diameter (mm) around the discs along with disc diameter.

Study results showed differences between fruit peel and pulp in total polyphenols content and antioxidant activity (Table 1).

Polyphenols accumulation in the peel of *Prinsepia uniflora* fruits was 1.7 times higher in comparison with the index of fruit pulp ($P < 0.001$). Similar distribution of polyphenolic compounds were found in our research in several fruit plants including barberry, yoshtaberry, and rowan fruits [6]. Therefore, study results indicate the general pattern of the polyphenols fullness in fruit peel, and need the peel using as rich resource of secondary metabolites. At the same time, total antioxidant activity of the fruit peel achieved only 0.89 % of pulp level, that suppose the possible contribution of some other secondary metabolites in the fruit antioxidant potential along with the polyphenols. Yang leaves of *Prinsepia uniflora* plants accumulate the highest polyphenols content that was 3.3 times bigger as compared to mature leaves ($P < 0.001$). Similarly, total antioxidant activity of yang leaves exceeded 2.1 times the index of the mature leaves. These findings confirmed the suitability of both vegetative and generative parts of *Prinsepia uniflora* plants as the promising resource of polyphenols and other secondary metabolites.

The results of disc-diffusion assay showed prominent inhibition activity of *Prinsepia uniflora* leaf and fruit crude extracts against all tested bacterial and fungal strains (Fig. 1).

Colony growth inhibition of *Erwinia dissolvens* (Gram-negative strain) by plant extracts was more significant when compared to *Staphylococcus aureus* (Gram-positive strain). Clinical fungal strain *Candida albicans* showed the highest sensitivity to the action of leaf and fruit pulp extracts of *Prinsepia uniflora*. According to results of inhibition zones measurement, effect of both leaf and fruit peel and pulp extracts against *Erwinia dissolvens* was comparable with antibiotic ofloxacin action (Table 2).

Different concentrations of *Prinsepia uniflora* leaf and fruit extracts did not show a clear dose dependence of the inhibitory effect on colony growth of the tested strains. Moreover, low concentration of leaf and peel extracts exhibited greater effect against *Erwinia dissolvens*, as well as pulp extract against *Staphylococcus aureus*. Antifungal activity of all tested

Table 1

Secondary metabolites features of Prinsepia uniflora fruits and leaves

Index	Fruits		Leaves		P
	peel	pulp	yang	mature	
Total polyphenols content	646.96 ± 6.79	371.92 ± 5.54	933.6 ± 8.41	407.4 ± 8.31	< 0.001
Total antioxidant capacity	1968.48 ± 22.17	2198.73 ± 29.81	2594.8 ± 63.80	1229.3 ± 27.70	< 0.001

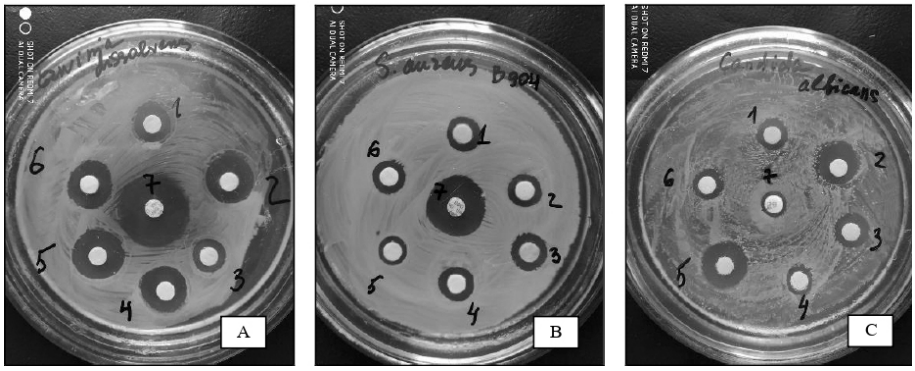

Fig. 1. Effect of Prinsepia uniflora extracts on E. dissolvens (A), St. aureus (B), and C. albicans (C): 1, 2 – leaves; 3, 4 – peel; 5, 6 – pulp; 7 – antibiotic (positive control)

Table 2

Inhibition zones diameter (mm) caused by *Prinsepia uniflora* extracts

Plant extracts concentration	*Erwinia dissolvens* 170	*Staphylococcus aureus* B904	*Candida albicans* (clinical strain)
Leaf (50 μg/μL)	12.28 ± 0.18	11.32 ± 0.22	9.63 ± 0.13
Leaf (25 μg/μL)	15.73 ± 0.20	10.52 ± 0.10	13.31 ± 0.08
Peel (50 μg/μL)	10.63 ± 0.23	11.44 ± 0.14	10.55 ± 0.13
Peel (25 μg/μL)	14.85 ± 0.21	11.4 ± 0.13	8.5 ± 0.14
Pulp (50 μg/μL)	16.3 ± 0.12	9.21 ± 0.22	14.02 ± 0.07
Pulp (25 μg/μL)	14.48 ± 0.17	10.54 ± 0.19	9.44 ± 0.17
Positive control	22.86 ± 0.17	19.24 ± 0.18	7.87 ± 0.12

plant extracts, especially pulp higher concentration, against clinical strain *Candida albicans* exceeded the ability of antibiotic itraconazole. Further research is need to study the patterns of antimicrobial activity of different concentrations of *Prinsepia uniflora* leaf and fruit extracts.

Conclusion.

1. High total polyphenols content along with great antioxidant capacity of both the generative and vegetative parts of *Prinsepia uniflora* plants indicate this low spread species suitability as a rich source of diverse secondary metabolites. Predominant polyphenols accumulation in the fruit peel, but greater total antioxidant capacity in the fruit pulp allows the possible contribution of some other secondary metabolites in the general fruit antioxidant potential apart the polyphenols.

2. Prominent antimicrobial activity of *Prinsepia uniflora* fruits and leaf crude extracts exhibited against both Gram-positive and Gram-negative bacteria, and antibiotic-resistant fungal strain as well confirm the promising use of this plant extracts as a potential source of active compounds for the creation of new drugs.

3. The introduced plants of low spread fruit species *Prinsepia uniflora* are able to realize the genetic potential for the accumulation of secondary metabolites in a new environment and can enrich the phytodiversity of the steppe zone, serving as a promising source of biologically active compounds.

Prospects for the use of research results. The discovery of *Prinsepia uniflora* high polyphenols content and antioxidant potential as well opens up prospects for obtaining a rich complex of secondary metabolites when this introduced species is cultivated in the steppe zone. Antimicrobial ability of the plant extracts may be useful in addressing the problem of bacterial and fungal drug resistance.

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