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Evaluation of antifungal activity of "green" *Solidago canadensis* extracts

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The flowering aerial parts of the local invasive *Solidago canadensis* were collected in the vicinity of Lviv (Western Ukraine) during flowering in July and August 2024, leaves and inflorescences were separated and dried. Total polyphenols from crushed raw materials were extracted with distilled water, 20 %, 60 %, and 96 % aquatic ethanol solution under reflux condition and maceration. The content of total polyphenols in extracts was determined with a Folin–Ciocalteu reagent and with gallic acid as standard by spectrophotometric method. The strains of unicellular fungi have been used from the Microbial Culture Collection of Ivan Franko National University of Lviv, and method of diffusion in agar for anticandidal investigation. The purpose of the work was to analyze the antifungal activity of green extracts of leaves and inflorescences of *S. canadensis*, obtained by maceration and reflux methods. The most important results were obtained for aquatic-ethanol extracts. For all extracts obtained from aerial parts of *S. canadensis* were find a high content of total polyphenols (TPC): in leaves from 3.54±0.04 to 8.55±0.003 mg•g-1 of dry weight (DW) in gallic acid equivalent (GAE) depend on extragent and method of extraction; in inflorescences extracts TPC ranged from 4.15 to 17.35 mg•g-1 DW in GAE. Leaf extracts prepared with aqueous ethanol concentrations of 20%, 60% and 96% showed a zone of growth inhibition (ZGR) of fungi of 7.3–28.0 mm. Extracts of inflorescences had antifungal activity against investigated strains and diameter of ZGR ranged from 8.3 to 31.0 mm. Strong correlations have been found between the ZGR of the fungi *Kluyveromyces marxianus, Cutaneotrichosporon curvatus, Candida parapsilosis* and the content of polyphenols in the studied extracts. The activity of *S. canadensis* leaf and inflorescences extracts that we found will contribute to further more detailed study of their properties as antifungal.

Key words*: Solidago canadensis L., polyphenolics, antifungal activity*

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Introduction

Antimicrobial resistance, which remains one of the greatest health threats in the 21st century, is the leading cause of death worldwide. This problem is constantly increasing, as noted in a recent study, highlighting the global burden of antibiotic resistance and the need for urgent action to overcome it (GBD 2021). Medicinal plants that contain a variety of biologically active substances can be a powerful source of antimicrobial compounds with minimal toxicity and significant pharmacological effects. The genus *Solidago* L. (Compositae: Asteraceae) includes about 140 species, which are distributed worldwide (Semple, Beck, 2021), particularly in Europe (Marksa et al., 2020). Some of them are considered native, some are invasive or hybrids (Marksa et al., 2020). Several *Solidago* spp. are growing in Ukraine and use as medicinal (Grodzinsky D.M., 1992; Nosal', 1992**)**. Recently, populations of the invasive species *S. canadensis* have significantly increased in Ukraine (Dvirna, 2015; Кузярін та ін., 2020). Simultaneously with the possible negative impact of invasive species on the natural environment, in particular, displacement of local species, etc. (EPPO Lists, 2023), such plants can become medicinal if complexes of substances with biological activity are found in their composition.

Various groups of phenolic compounds, saponins, essential oils, monoterpenes and sesquiterpenes, and tannins etc. have been reported in the raw materials of *Solidago* spp. (Apati et al., 2002; Woźniak et al., 2018; Marksa et al., 2020; Poljuha et al., 2024; Radušienė et al., 2024). Antibacterial and other types of pharmacological activity of *Solidago* spp. are known (Mishra et al., 2010; Kołodziej et al., 2011; Krüzselyi et al., 2021; Baglyas et al., 2022; Baglyas et al., 2023). The leaves and inflorescences of native, invasive, and hybrid *Solidago* spp. have high antioxidant activity (Marksa et al., 2020), which usually contributes to antimicrobial activity.

Extracts from *Solidago* spp. roots inhibited the plant pathogenic fungi *Fusarium avenaceum* and *Bipolaris sorokiniana* (Krüzselyi et al., 2021). In recent years, there has been a significant increase in fungal diseases caused by the yeast-like fungi *Candida* (Mohamed et al., 2019). The crude aqueous extract of *S. virgaurea* was a potent inhibitor of *Candida albicans* biofilm formation, preventing fungal adhesion to surfaces and disrupting the extracellular matrix, although *in vitro* these extracts were not inhibitors of fungal growth (Chevalier et al., 2019). Studies of the antifungal activity of plant extracts remain relevant and open new prospects for their search among *Solidago* spp. The antifungal properties of aqueous-ethanolic extracts of *S. canadensis* have not been thoroughly studied.

The aim of this study was to prepare extracts and quantify the content of polyphenols in the leaves and inflorescences of *S. canadensis* from wild populations in Western Ukraine, to study the antifungal activity of these extracts, and to predict the importance of this plant material for possible use as a source of antifungal activity. The object of the research was the antifungal properties of aqueous and aqueous-ethanolic extracts from the leaves and inflorescences of *S. canadensis* L.

Material and Methods

Sample collection of *Solidago canadensis* was performed from wild populations in Lviv district, Ukraine in 2024. The botanical identification of taxa was performed following to the morphological description of Plant Identifier and voucher plant sample.

30-40 centimeter flowering tops were cut, dissected into inflorescences and leaves, and dried at 25 °C in the shade. Dried plants materials were then stored in paper bags until research. Dry inflorescences and leaves were separately ground in a mechanical mill, sieved through a sieve with a diameter of 3 mm and used to extracts preparation. The work used "green extraction", that is, solvents (extractants) were used - water and aqueous ethanol (AE) of various concentrations, which are non-toxic and extract a wide range of biologically active compounds, in particular of a phenolic nature. AE extracts of inflorescences and leaves were prepared with 20%-, 60%- and 96% aqueous ethanol by the reflux method at 60-80° C for 30 min and by the maceration method in the dark at 25° C for 14 days. Extraction was carried out in accordance with the requirements of the State Pharmacopoeia of Ukraine: (ratio of sample: extractant = 1:20 (mass, g/volume, ml). After cooling or infusing, each extract was filtered through a paper filter. The extracts thus obtained were used in the experiment. Extracts were prepared by methods in accordance with the requirements of the State Pharmacopoeia of Ukraine (2015).

The total polyphenol content (TPC) in extracts was determined according to the method with Folin-Ciocalteu reagent as described by Chew et al. (2011) and Yavorska et al. (2023). 1 ml of the test extract was mixed with 1 ml of diluted Folin-Ciocalteu reagent (10 times); incubated at room temperature for 4 min. Then 0.8 ml of 7.5% (w/v) sodium carbonate solution was added. All this was thoroughly mixed for 5 s and stored in the dark at room temperature for 2 h. To measure the extinction, a ULAB 102UV (measuring range 190–1100 nm) was used and measured at a wavelength of 650 nm. A control sample was prepared identically by replacing 1 ml of extract with 1 ml of bidistilled water.

The antifungal effect of the extracts was detected by diffusion into a dense medium, in which 0.2 ml of aqueous and aqueous-ethanolic extract from the wells diffused into agar. Approximately 20 ml of nutrient medium (Sabureau agar) was poured into sterile Petri dishes. After the medium solidified, the Petri dishes were inoculated with prepared thick suspensions standardized to 0.5 McFarland. 4–5 wells with a diameter of 6 mm were cut with a flamed drill, maximally spaced from each other. The dishes with extracts in the wells were placed in a thermostat at a temperature of 28±1 °C for two days.

Two-day-old yeasts were used as test cultures: *Kluyveromyces marxianus* ATCC 4922 (formerly *Candida pseudotropicalis* VKM Y-922), *Cutaneotrichosporon curvatus* ATCC 10567 (formerly *Candida curvata* VKM Y-2230), *C. parapsilosis* ATCC 22019, *Saitozyma flava* ATCC 10656 (formerly *Candida flavus* VKM Y-331), *Papiliotrema laurentii* ATCC18803 (formerly *Cryptococcus laurentii* VKM Y-743) from the culture museum of the Department of Microbiology of the Ivan Franko National University of Lviv. Test cultures of fungi were grown on glucose-peptone agar (Sabureau medium) for 48 h in a thermostat at a temperature of 28±1 °C. After cultivation, the diameter of the zone of growth retardation (ZGR) was measured with a ruler. Fluconazole (150 mg) and extractants (20%, 60%, 96% aqueous ethyl alcohol) were used as controls. To assess antifungal activity, the criteria according to Cappelli et al. (2021) were used: the diameter of the ZGR was up to 10 mm and the fungus was considered resistant to the extract; 11–12 mm – moderately sensitive; more than 13 mm – sensitive, more than 20 mm – highly sensitive.

Statistical analysis of the results was performed using the Microsoft Office Excel 2010 software package. The statistical program: Jamovi 2.3.21 was used for correlation analysis of the obtained data. The scales were consistent with the normal distribution. To test the hypothesis of the relationship between the diameter of the ZGR and the content of TPC, Pearson's linear pairwise correlation was used. The experiments were conducted in five replicates.

Results

The favorable pre-validation characteristics obtained by other authors confirmed that the spectrophotometric procedure with Folin-Ciocalteu reagent is a valuable tool in the analysis of polyphenols in plants (Grubesić et al., 2005), the results of which are presented in Table 1.

Table 1. The content of total polyphenols in *Solidago canadensis* extracts, M±σ, n=5

Plant material	Solvent, method of extraction	TPC, mg·g^{-1} DW in gallic acid equivalent
Leaves	20% AE, M	4.13±0.34
	60% AE, M	3.54±0.04
	96% E, M	4.67±0.58
	Distilled H_2O, R	–
	20% AE, R	6.20±0.003
	60% AE, R	8.08±0.63
	96% E, R	8.55±0.003
Inflores-cences	20% AE, M	4.15±0.16
	60% AE, M	8.64±0.35
	96% E, M	17.36±0.41
	Distilled H_2O, R	6.56±0.35
	20% AE, R	6.61±2.71
	60% AE, R	9.92±1.63
	96% E, R	6.03±0.26

Note: AE – aqueous ethanol, E – ethanol; R – reflux method, M – method of maceration

The results showed the variety of TPC between different upground plant parts (leaves: up to 8.55±0.003 and inflorescences: up to 17.36±0.41 mg·g^{-1} DW in gallic acid equivalent). Both extraction methods revealed significant differences in total phenolic content among the investigated species. Comparing the two extraction methods, AE extracts showed significantly higher TPC values than water extracts. Detected ranges of TPC are in agreement with some earlier studies, although with slight quantitative differences.

Studies of the antifungal activity of extracts from the inflorescences and leaves against *K.marxianus*, *C. curvatus*, *C. parapsilosis*, *S. flava*, and *P. laurentii* using the well method made it possible to establish that these extracts differently inhibited the studied yeast cultures (Table 2).

Antifungal properties of extracts from the leaves in relation to the test cultures indicate that they mostly suppress the indicated yeast cultures (Table 2).

Aqueous extracts from the leaves did not show any effect on the tested fungal cultures. And the extracts made with 20- and 60-% AE from the leaves by the reflux method showed the least inhibitory effect on the culture of *P. laurentii* (ZGR: 7.9 and 9.7 mm). Most of the tested extracts significantly affected the tested fungal cultures, which was manifested by high ZGR values, sometimes more than 20.0 mm (Table 2). The tested fungal cultures were mostly sensitive to the investigated “green” extracts of S. canadensis inflorescences (see Table 2).

Aqueous extracts had no inhibitory effect on the tested fungi.

Strong correlations (r≥0.7) have been found between the ZGR of the fungi *K. marxianus, C. curvatus, C. parapsilosis* and the content of polyphenols in the studied extracts (Table 3). No correlations were found between the studied biologically active compounds and growth inhibition zones for *S. flava* and *P. laurentii*. A highly reliable relationship was established between the phenolic compounds of *S. canadensis* leaf extracts: 60% reflux and 20% maceration, as well as flowers: 60% reflux and zones of growth inhibition of *C. curvatus*.

Table 2. Effect of *Solidago canadensis* inflorescences and leaves extracts on fungi

Plant extract, ethanol concentration in the extractant in %, extraction method		Test cultures, diameter of growth retardation zone, mm				
		Kluyvero-myces marxianus	*Cutaneo-trichosporon curvatus*	*Candida parapsilosis*	*Saitozyma flava*	*Papiliotrema laurentii*
Control, fluconazole		24.3±0.5	35.4±0.3	35.6±2.1	30.2±1.2	35.0±1.3
Leaves	Aqueous, R	8.7±0.5	6.2±0.3	9.1±0.3	7.7±0.5	6.8±0.2
	AE 20, R	21.0±0.5	7.3±0.3	22.7±0.5	15.0±0.5	9.7±0.3
	AE 60, R	17.0±0.5	14.0±0.5	20.7±0.3	12.7±0.3	7.9±0.5
	E 96, R	9.3±0.2	20.4±0.5	25.5±0.2	11.7±0.3	21.2±0.5
	AE 20, M	28.0±0.4	16.0±0.5	19.7±0.7	9.6±0.3	23.3±0.7
	AE 60, M	19.3±0.5	14.3±0.3	8.5±0.5	25.2±0.5	12.6±0.3
	E 96, M	17.7±0.3	19.0±0.5	27.7±0.3	14.3±0.3	11.7±0.3
Inflorescences	Aqueous, R	6.3±0.3	6.3±0.3	6.3±0.3	7.3±0.3	6.3±0.3
	AE 20, R	22.3±0.3	23.3±0.3	19.0±0.0	10.7±0.3	12.7±0.3
	AE 60, R	15.3±0.3	19.1±0.5	8.7±0.3	8.3±0.3	14.0±0.3
	E 96, R	31.0±0.5	26.3±0.5	24.6±0.5	30.2±0.4	19.7±0.7
	AE 20, M	22.3±0.3	23.0±0.5	16.3±0.1	12.7±0.3	10.7±0.3
	AE 60, M	7.3±0.2	21.1±0.5	10.7±0.3	14.0±0.4	13.3±0.3
	E 96, M	27.6±0.5	27.6±0.5	22.7±0.3	17.0±0.5	15.3±0.3

Note: AE – aqueous ethanol, E – ethanol; R – reflux method, M – method of maceration; the table grid where the extracts inhibited the test cultures are marked in color.

Applying a two-factor variance analysis to determine the share of the influence of extracts and extraction methods, it was established (Fig. 1) that the ZGR of the studied yeast cultures are most influenced by: when using 20% aqueous ethanol for the preparation of inflorescence extracts – the culture itself (96.9%, $p < 0.001$), and leaf extracts – the culture (70.8); when using 60% aqueous ethanol for the preparation of inflorescence and leaf extracts – the culture (60.8 and 56.4%, respectively); for 96% aqueous ethanol extracts of inflorescences, the culture is important (61.0%), and for aqueous ethanol extracts of leaves – the unaccounted factors (50.8%).

Thus, extracts from the leaves and inflorescences of *S. canadensis* made with aqueous-ethanol of various concentration showed a high inhibitory effect on various fungi strains.

Discussion

Fungi often maintain symbiotic relationships with their hosts, but they can cause mucosal infections in healthy individuals and systemic/life-threatening infections in immunocompromised individuals. Fungal pathogenesis is a multifaceted process involving a variety of mechanisms and pathways. *Candida* spp. are

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Table 3. Results of correlation analysis of inhibition zone diameter with phenolic content in *Solidago canadensis* leaf and inflorescence extracts (n=3, Pearson's r≥0.7)

Plant extract, ethanol concentration in the extractant in %, extraction method		*Kluyveromyces marxianus*	*Cutaneo-trichosporon curvatus*	*Candida parapsilosis*	*Saitozyma flava*	*Papiliotrema laurentii*
Leaves	20, R	-0.866	X	+0.866	X	X
	60, R	-0.866	+1.000*	X	X	X
	96, R	+0.933	+0.629	X	X	X
	20, M	X	+1.000*	X	X	X
	60, M	-0.693	+0.971	+0.971	X	X
	96, M	X	-0.974	+0.956	X	X
Inflorescences	20, R	+0.756	X	X	X	X
	60, R	+1.000*	X	X	X	X
	96, R	X	X	X	X	X
	20, M	-0.500	X	X	X	X
	60, M	+0.693	X	+0.693	X	X
	96, M	-0.995	X	+0.585	X	X

Note: X – there is no correlation, - – negative correlation, + – positive correlation; R – reflux method, M – method of maceration; *p<0.001.

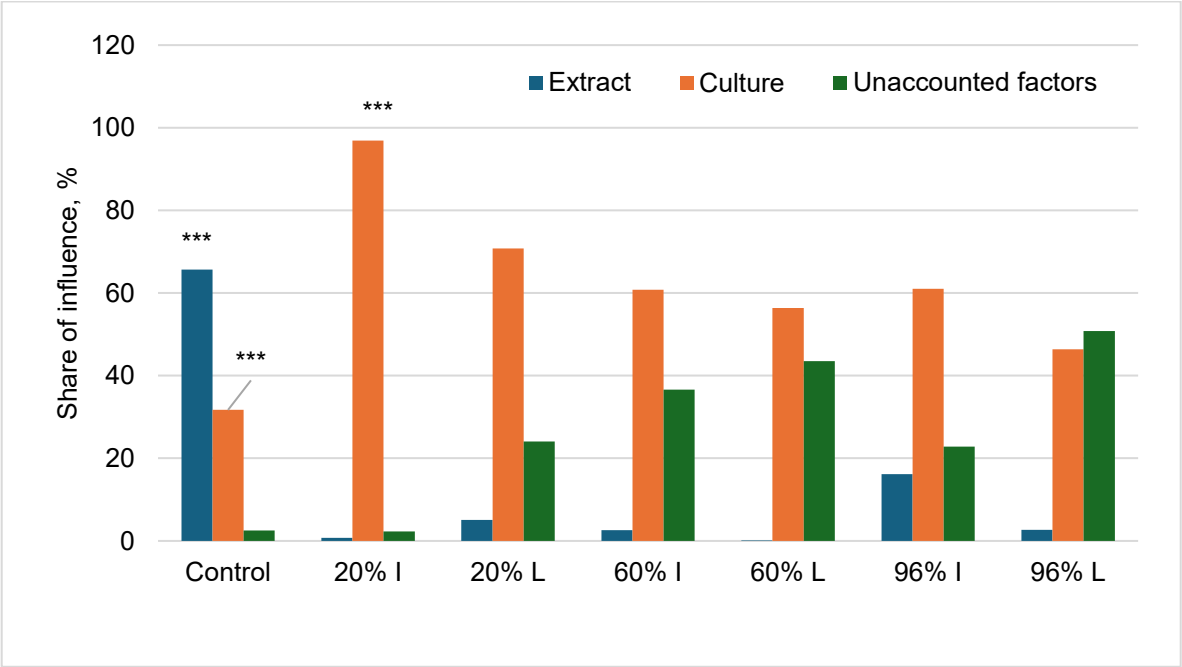

Figure 1. The share of influence of extracts and extraction methods on zones of yeast growth retardation, %; I – inflorescences, L – leaves (*** – p < 0.001)

facultative anaerobes, and therefore more often affect organs and tissues with a high oxygen content. *Candida* pathogenicity is mediated by a number of virulence factors, the most important of which are adherence to host surfaces, including medical devices, biofilm formation, and secretion of hydrolytic enzymes (eg, proteases, phospholipases, and hemolysins) (Silva et al., 2012).

K. marxianus also known as *C. kefir* is a rarely documented fungal pathogen, mostly this is a pathogen that appears in patients with malignant tumors (Dufresne et al., 2014).

C. parapsilosis often causes fungal bloodstream infections, especially in critically ill newborns and immunocompromised individuals. Biofilm formation significantly increases the risk of infection during the use of indwelling catheters and other medical devices, and also complicates treatment, as the cells in the biofilm exhibit reduced sensitivity to antifungal drugs. The presence of a biofilm may be an important clinical factor influencing the choice of further therapeutic strategy (Gómez-Molero et al., 2021).

The genus *Cryptococcus* is characterized by globular or elongated yeast-like cells, or blastoconidia, which reproduce by budding (Kurtzman et al., 2011**).** The main pathogenic species are *Cryptococcus neoformans* and *C. gattii* that cause opportunistic infection, especially in immunocompromised and groups such as those with haematopoietic malignancy, solid transplant patients and therapy for rheumatoid arthritis (Gibson, Johnston, 2015).

Cryptococcus albidus and *Cryptococcus laurentii* have also occasionally been implicated in human infection. Several environmental and human isolate species of *Cryptococcus* have been identified to cause invasive infections in humans, leading to high morbidity (Singh et al., 2017). Due to the limited number of reported cases, there is no validated standard treatment for *C. laurentii* infection (Thompson et al., 2023).

The main therapeutic agents for the treatment of candidiasis are antifungal agents, although the optimal therapy for some fungal illnesses has not been fully determined (Bush 2020). Increasing use of broad-spectrum antibiotics, an ever-expanding range of immunosuppressive disease states and treatments (e.g. for cancer and following solid organ transplantation), and advances in intensive care medicine have led to rising incidence of invasive candidiasis over the past two decades (Murphy, Bicanic, 2021). An increasing number of *Candida* species that are resistant to first line antifungal treatments (azoles or echinocandins) are being identified (Pfaller et al., 2011; Castanheira et al., 2013), particularly in high antifungal use settings thereby almost eliminating all current treatment options (Healey et al., 2016). Yeast infections that are resistant to fluconazole and other synthetic antifungal agents require alternative antifungal treatment. Natural products, both in the form of pure phytocompounds and standardized plant extracts, provide unlimited opportunities for new pharmaceutical developers due to their unparalleled chemical diversity (Mishra et al., 2020).

All aqueous-ethanolic extracts of leaves and inflorescences of *S. canadensis* prepared and studied in this work had high anti-fungal activity against all tested fungi, although lower compared to the reference antibiotic fluconazole.

There are few conducted research studies about the antifungal effect of the vegetal extract and EO of *S. canadensis*, such as Elshafie et al. (2019), who reported that the essential oil (EO) of *S. canadensis* showed promising antifungal activity against some post-harvest phytopathogenic fungi (*Monilinia fructicola, Botrytis cinerea, Aspergillus niger* and *Penicillium expansum*).

The content of phenolic compounds in the leaves we found is comparable to that obtained by other researchers (Deng et al., 2015).

Identifying antifungal properties in plants against these and other pathogenic yeasts remains a critically important task. Yeast may be susceptible to varying degrees of exposure to the *S. canadensis* extracts we investigated. And we attribute this to the BAS content, including phenolic compounds. The observed results were supported by previous studies as the presence of phenols, flavonoids, in the leaf and inflorescence of *S. canadensis* was reported by Woźniak et al. (2018). Crude AE extracts of *S. candensis* were found to be rich in phenolic content, but correlative analysis suggests that there are other groups of compounds, which significantly affect their antifungal activity.

Conclusions

Therefore, *Solidago canadensis* green extracts of leaves and inflorescences rich in polyphenolics and, at all tested concentrations demonstrated promising *in vitro* antifungal activities against the majority of tested yeasts. There have been discovered that the studied aqueous-ethanolic extracts of leaves and inflorescences of *S. canadensis* affected the studied yeast cultures (ZGR: from 15.3 to 31.0 mm). Two-factor analysis of variance was used to determine the proportions of the effects of extracts and extraction methods on the zones of fungal growth inhibition. It was found that when using 20% aqueous-ethanol extracts of inflorescences, the proportion of the effect of yeast cultures was 96.9% ($p < 0.001$). The proportions of the effects of factors in the controls were statistically significant. Strong correlations were found between the growth retardation zones of *Kluyveromyces marxianus*, *Cutaneotrichosporon curvatus*,

Candida parapsilosis and the content of total polyphenols in the studied extracts. In general, the results of studies on the effects of extracts of leaves and inflorescences of *S. canadensis* confirm their potential antifungal effect.

It is obvious that further study of the sensitivity profiles of fungal strains to plant extracts can help in establishing directions for studying their interaction and establishing further therapeutic recommendations. The prevalence of *S. canadensis*, due to high allelopathic properties and seed productivity, provide a significant raw material base for use. Using the studied potential of *S. canadensis* makes it an excellent candidate for development in creating new antifungal drugs.

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Оцінка протигрибкової активності «зелених» екстрактів *Solidago canadensis*

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Квітучі надземні частини інвазійної рослини *Solidago canadensis* були зібрані в околицях Львова (Західна Україна) під час цвітіння в липні та серпні 2024 року, листки та суцвіття були відокремлені та висушені. Загальні поліфеноли з подрібненої сировини екстрагували «зеленими» екстрагентами – дистильованою водою, а також 20%-, 60%- та 96% водно-етанольними розчинами під час нагрівання на нагрівнику за умов кипіння та методом мацерації. Вміст загальних поліфенолів в екстрактах визначали за допомогою реактиву Фоліна–Чокальтеу спектрофотометричним методом та з використанням галової кислоти як стандарту. Штами одноклітинних грибів використовували з колекції мікробних культур кафедри мікробіології Львівського національного університету імені Івана Франка, а для антикандидозного дослідження – метод дифузії в агар. Метою роботи було аналізування антигрибкової активності зелених екстрактів листків і суцвіть *S. canadensis*, отриманих методами мацерації і рефлюксу. Найважливіші результати були отримані для водно-етанольних екстрактів. Для всіх екстрактів, отриманих з надземної частини *S. canadensis*, було виявлено високий загальний вміст поліфенолів (ЗВП): у листках від 3,54±0,04 до 8,55±0,003 мг•г-1 сухої маси в перерахунку на галову кислоту залежно від екстрагенту та методу екстракції; в екстрактах суцвіть ЗВП коливався від 4,15 до 17,35 мг•г-1 сухої маси в перерахунку на галову кислоту. Екстракти листків, виготовлені з водним етанолом концентрацій 20%-, 60%- та 96% демонстрували діаметри зони затримки росту (ЗЗР) досліджених грибів 7,3–28,0 мм. Екстракти суцвіть мали протигрибкову активність проти досліджуваних штамів, а діаметр ЗЗР коливався від 8,3 до 31,0 мм. Виявлено сильні кореляційні зв'язки між зонами інгібування грибів *Kluyveromyces marxianus, Cutaneotrichosporon curvatus, Candida parapsilosis* та вмістом поліфенолів у досліджуваних екстрактах. Виявлена нами активність екстрактів листків та суцвіть *S. canadensis* сприятиме подальшому детальнішому вивченню їх властивостей як протигрибкових засобів.

Ключові слова: *Solidago canadensis L., поліфеноли, протигрибкова активність*

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