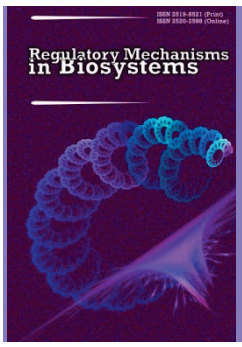

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Antibacterial and nematicidal activities of extracts from plants of the Asteraceae family

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Drugs based on plants of the Asteraceae family are broadly used against microorganisms that are pathogenic to people and animals. However, their potentials in this aspect have not been fully researched. In our in vitro experiment, we tested alcohol tinctures of 12 species of plants of the Asteraceae family against 10 species of bacteria, one species of fungi, and the larval stages of three species of nematodes – parasites of ruminants (L_1 *Muellerius capillaris*, L_{1-3} *Strongyloides papillosus*, L_3 *Haemonchus contortus*). The growth inhibition zone larger than 8 mm was observed after using ethanol extracts of the aboveground parts of several plants against the colonies of various microorganisms: *Solidago canadensis* showed activity against five species – *Klebsiella pneumoniae*, *Proteus mirabilis*, *Shigella flexneri*, *Clostridium perfringens*, and *Candida albicans*; *Cyclachaena xanthiifolia* was effective against five species – *Escherichia coli*, *P. mirabilis*, *Enterococcus faecalis*, *C. perfringens*, and *C. albicans*); *Jurinea arachnoidea* inhibited four species – *K. pneumoniae*, *P. mirabilis*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*); *Scorzoneroides autumnalis* acted against four species – *P. mirabilis*, *Sh. flexneri*, *C. perfringens*, and *C. albicans*; *Helichrysum arenarium* demonstrated activity against four species – *P. mirabilis*, *Sh. flexneri*, *E. faecalis*, and *Listeria monocytogenes*; and extracts from the fruits of *Echinops ritro* were active against four species – *K. pneumoniae*, *P. mirabilis*, *Staphylococcus aureus*, and *B. subtilis*, while extracts from the flowers of *Echinops ritro* were effective against four microorganisms – *K. pneumoniae*, *P. mirabilis*, *Sh. flexneri*, and *P. aeruginosa*. However, we observed no expressed nematocidal action of ethanol extracts of the studied species of plants. After 24 h exposures to 0.1% solutions of those extracts, over 95% of the nematode larvae of ruminants were found to be vital. Nonetheless, as a result of the study, those plants were recognized the most promising for further in vivo research of antibacterial activity. During the search for antibacterial and antifungal activities, the following plants were found to be the less promising: the aboveground parts of *Artemisia austriaca*, *Lactuca serriola*, *Ambrosia artemisiifolia*, *Solidago virgaurea*, roots of *Artemisia vulgaris*, *Echinops ritro*, *Lactuca serriola*, *Solidago canadensis*, *Ambrosia artemisiifolia*, *Scorzoneroides autumnalis*, and leaves of *Echinops ritro*.

Keywords: growth inhibition zone; bacterial colonies; multi-resistant strain; parasitic nematode.

Introduction

Medicinal plants are valuable sources of pharmaceuticals all around the globe. Plant raw material remains a valuable resource for combating serious diseases worldwide. Over recent years, the number of infectious diseases and antibiotic resistance has significantly increased, becoming a dire therapeutic problem. Extracts from medicinal plants exert various biological effects, such as antimicrobial, anti-inflammatory, antioxidant, and anti-parasitic (Boyko et al., 2020; Boyko & Brygadyrenko, 2021). Compounds obtained from medicinal plants are effective against bacteria, fungi, and viruses. They can inhibit the development of protozoans through mechanisms other than those of the antimicrobial drugs currently in use. This may have a substantial clinical effect during treatment of resistant bacterial strains (Isgor & Geven, 2018; Khamraeva & Bussmann, 2023).

With the increasing globalization of the food economy, there is a growing need to extend the shelf life and enhance the safety of food products to access global markets. Traditionally, this has been achieved by adding synthetic antimicrobial and antioxidant compounds, which is now posing a contradiction to the rising consumer demand for "fresh" products with minimal processing (Albayrak & Hamzaoglu, 2010). Therefore, producers are currenly considering natural approaches to fight deterioration and enhance food quality. A cheap and stable source of plant compounds can provide manufacturers with cheap natural agents that can help exending the shelf life. Using plant-based alternatives to synthetic antioxidants in treated food products is becoming increasingly important in the food industry.

The Asteraceae family, comprising over 1,600 genera with over 23,000 species that are widespread in different climates and regions around the globe, is the largest family of flowering plants (Bohm & Stuessy, 2001). The diversity and variety of this family highlights the significant importance of some well-known species that have been used since ancient times as a source of food or spices, as well as for medicinal purposes (Chiavari-Frederico & Wietzikoski Lovato, 2020). Several classes of compounds from Asteraceae species have been studied and tested for biological activity, and have been reported to show medicinal potential (Silvério & Sousa, 2013; Zazharskyi & Brygadyrenko, 2020a). Of those compounds, a special attention is paid to polyphenols, especially flavonoids, which make plants valuable in the pharmaceutical, cosmetic, and food industries. This is because of their valuable medical properties, such as antioxidant, anti-inflammatory, antifungal, and antibacterial effects. Despite the global spread of Asteraceae plants and their potential as sources of antimicrobial and antioxidant agents, the bioactivity of a number of species in this family has not been studied. Therefore, in this article we present our continued research on antibacterial drugs in plant extracts in the context of

the spread of polyresistant bacterial strains, which are hard to treat (Zazharskyi & Brygadyrenko, 2020a). We have already found antiparasitic and antimicrobial effects in extracts based on some Asteraceae plants (Boyko & Brygadyrenko, 2016a; Zazharskyi & Brygadyrenko, 2020b). However, our previous studies of aqueous extracts of plants of this family in similar concentrations found no pronounced nematocidal properties against invasive nematode larvae, in particular *Strongyloides papillosus* and *Haemonchus contortus* (Boyko & Brygadyrenko, 2016b, 2019).

The objective of this study was to evaluate the antibacterial and nematocidal actions of 12 alcohol extracts against 11 species of microorganisms and three nematode larvae that typically parasitize ruminants. Until now, those species of plants were poorly studied for antimicrobial activity, and they can have a great potential in modern human and veterinary medicines.

Material and methods

The leaves and shoots of 12 species of plants were collected in the territory of the Botanical Garden of Oles Honchar Dnipo National University (Boyko & Brygadyrenko, 2019), dried at room temperature, fragmented, weighed, and kept in 70% ethyl alcohol for 10 days, and then filtered. We took 10 grams of dry fragmented plants per 100 g of 70% ethyl alcohol. Then, to assess the antibacterial properties, 0.1 mL of those filtered alcohol extracts was transferred onto paper disks of 6 mm diameter. The disks were dried in sterile conditions at the temperature of 10 ºC in a microbiological safety cabinet HR1200-IIA2-D (China).

Table 1
Taxonomic composition of 11 species of microorganisms we studied

Phylum, division	Family	Species, strains
Pseudomonadota	Enterobacteriaceae	*Escherichia coli* 055 ATCC 8739
		Klebsiella pneumoniae ATCC 13883
		Proteus mirabilis ATCC 14153
		Shigella flexneri GISK 232054
	Pseudomonadaceae	*Pseudomonas aeruginosa* ATCC 15442
Bacillota	Enterococcaceae	*Enterococcus faecalis* ATCC 29212
	Listeriaceae	*Listeria monocytogenes* ATCC 19112
	Staphylococcaceae	*Staphylococcus aureus* ATCC 25923
	Bacillaceae	*Bacillus subtilis* ATCC 6633
	Clostridiaceae	*Clostridium perfringens* ATCC 13124
Ascomycota	Saccharomycetaceae	*Candida albicans* ATCC 2091

The antibacterial activities of plant tinctures were assessed using the disk diffusion method in agar. From the daily cultures of ethanol strains of microorganisms, we prepared weighed amounts according to the standard of opacity of bacterial suspension, equaling 0.5 units of density according to McFarland (McF) 1.5 x 10^8 CFU (colony-forming units), which we determined using a densitometer (Densimeter II, Table 1). The obtained weighed amount was transferred into Muller-Hinton agar (Himedia, India, 2023), with subsequent cultivation in a TCO-80/1 Thermostat (the New Technologies and Marketing Factory of Medical Equipment Ukraine, 2015) for 24 h at the temperature of 37 ºC. On top of the inoculations, we put disks (n = 8) saturated with the corresponding ethanol tinctures of the 12 species of plants (Table 2).

Table 2
Parts of the 12 species of plants we used to prepare ethanol extracts and the data on antibacterial activities of Asteraceae plants

Species	Used part of plant	Most important literature sources about medical properties of plant
Artemisia vulgaris L.	galls	Liu & Kikvidze (2020)
	root	Khezrilu Bandli & Mohammadkhani (2017), Khamraeva & Bussmann (2023)
Artemisia austriaca Jacq.	shoots and leaves, root	Rafiri & Dikane (2024)
Jurinea arachnoidea Bunge	shoots and leaves	Szukala & von Raab-Straube (2019)
	root	Mirtadzadini & Naderi (2022)
Echinops ritro L.	leaves	Li & Huang (2019)
	fruits	Balabanova & Gevrenova (2023)
	flower	Isgor & Geven (2018)
	root	Şapci & Vural (2018), Meirong (2024)
Lactuca serriola L.	shoots and leaves	Chadha & Florentine (2021)
	root	Kim & Lee (2022), Shukurlu & Vitalini (2023)
Solidago canadensis L.	shoots and leaves	Zhang & Wan (2017), Kato-Noguchi & Kato (2022)
	root	Gong (2019)
Ambrosia artemisiifolia L.	shoots and leaves	Comtois & Boucher (2018), Han & Lv (2021), Zhong-Shi (2023)
	root	Božić (2018), Savić (2021)
Solidago virgaurea L.	shoots and leaves	Kołodziej (2008), Fursenco & Ancuceanu (2020)
	root	Tămaş & Mogosan (2021)
Scorzoneroides autumnalis L.	shoots and leaves	Ingimundardóttir & Andersson (2024)
	root	Cruz-Mazo & Narbona (2009)
Helichrysum arenarium (L.) Moench	shoots and leaves	Pljevljakušić & Šavikin (2018), Kramberger et al. (2021), Judzentiene & Garjonyte (2022)
Cyclachaena xanthiifolia (Nutt.) Fresen.	shoots and leaves	Kurdyukova (2019), Nikolić & Masin (2024), Turalin & Childibayeva (2024)
Achillea millefolium L.	shoots and leaves	Saraç & Demirbaş (2021), Ayrom & Suleymanova (2022), Zöngür (2023)

As a positive control we used disks with 10 µg of ampicillin trihydrate (Himedia Laboratories Pvt. Limited, Mumbai, Maharashtra, India), a semi-synthetic broad-spectrum antibiotic (Valle et al., 2015). Twenty four hours later, the growth of the cultures was measured using a zone scale for reading the sizes of growth inhibition zones in microorganisms (Antibiotic Zone Scale-C, model PW297, India) and the software TpsDig2 (F. James Rohlf, USA, 2016). The data in the tables are presented as x ± SD (mean ± standart deviation).

To assess the nematocidal properties of the plants, from 10% ethanolic extracts (100 g of 70% ethanol/10 g of plant), we prepared their 0.1% solutions (0/1 mL 10% extract/10 mL of H_2O).

The feces were collected from cattle that had been naturally infected with the nematodes *Muellerius capillaris* (Mueller, 1889), *Strongyloides papillosus* (Wedl, 1856), and *Haemonchus contortus* (Rudolphi, 1803) Cobb, 1898). The larvae *S. papillosus* and *H. contortus* were cultivated for 10 days at the temperature of 18–22 °C. The Baermann test was used to separate the nematode larvae from feces (Zajac, 2011). The nematodes were identified according to the morphological features (Van Wyk et al., 2004, 2013). The larvae obtained using the Baermann test were centifuged in water at 1,500 rpm for 4 min.

Then, 0.1 mL of sediment containing larvae was treated with 1 mL of each ethanolic extract solution in 1.5 mL centrifuge test tubes with 24 h exposure in five repetitions at 22 °C. After this period, we counted live and dead larvae (immobile, with signs of damage to the integrity of the intestine).

Results

The alcohol extracts of the plants we used inhibited the growth of the individual strains of microorganisms from the families Enterobacteriaceae, Pseudomonadaceae, Enterococcaceae, Listeriaceae, Staphylococcaceae, Bacillaceae, Clostridiaceae, and Saccharomycetaceae (Tables 3–5). Against *E. coli*, inhibiting effects were exerted by *A. vulgaris* and the aboveground part of *C. xanthiifolia* (15.2 and 12.6 mm, hereinafter, the average radius of growth inhibition zone is given in mm). Somewhat lower efficiency than *A. vulgaris* and *C. xanthiifolia*, but high antibacterial

properties, was exerted by the aboveground parts of *A. artemisiifolia* (10.4), *S. virgaurea* (10.5), and *A. millefolium* (10.5). The isolates of *E. coli* were resistant to the alcohol extracts from *A. austriaca*, *J. arachnoidea*, *E. ritro*, *L. serriola*, *S. canadensis*, *S. autumnalis*, and *H. arenarium*. Ampicillin in the control was observed to be ineffective against *E. coli* (Table 3).

Against the epizootic strain *K. pneumoniae*, a high antibacterial activity and competition with ampicillin were exhibited by the ethanol extracts from the galls and roots of *A. vulgaris* (11.3 and 13.5), aboveground parts of *J. arachnoidea* (10.6) and *S. canadensis* (14.6), flowers of *E. ritro* (16.5), and root of *S. virgaurea* (12.6). Moderate inhibition of growth of the colonies of *K. pneumoniae* was displayed by the extracts from the fruits of *E. ritro* (8.5) and aboveground part of *L. serriola* (5.2). At the same time, we should note the complete absence of reaction of this strain (resistance and absence of inhibition of growth of bacterial colonies) to ethyl extracts from the aboveground parts of *A. austriaca*, *H. arenarium*, *C. xanthiifolia*, *A. millefolium*, leaves and roots of *E. ritro*, roots of *L. serriola*, *S. canadensis*, and aboveground parts and roots of *A. artemisiifolia* and *S. autumnalis*. At the same time, ampicillin in the control group was ineffective against *K. pneumoniae*. High sensitivity of *P. mirabilis* was observed to 9 alcohol extracts: the aboveground parts of *A. austriaca* (14.7), *J. arachnoidea* (10.6), *L. serriola* (15.1), *S. canadensis* (10.4), *S. autumnalis* (10.2), *H. arenarium* (20.6), *C. xanthiifolia* (12.6), *A. millefolium* (12.3), root of *J. arachnoidea* (10.3), and fruits and flowers of *E. ritro* (13.4; 17.4). The bacterium showed moderate sensitivity to two species of plants: the root of E. ritro (8.3) and aboveground part of *A. artemisiifolia* (5.3). The polyresistant strain *P. mirabilis* was not sensitive to the extracts from the galls and roots of *A. vulgaris*, leaves of *E. ritro*, roots of *L. serriola*, *S. canadensis*, *A. artemisiifolia*, *S. autumnalis*, and aboveground parts and roots of *S. virgaurea*. Also, alcohol extracts from plants displayed high antibacterial action towards *Sh. flexneri*, particularly the roots of *J. arachnoidea* (10.3), flowers and roots of *E. ritro* (10.5, 10.2), and aboveground parts of *S. canadensis* (12.5), *S. autumnalis* (10.3), and *H. arenarium* (11.6). The strain *Sh. flexneri* was resistant to the extracts from the galls and root of *A. vulgaris*, aboveground parts of *A. austriaca*, *S. virgaurea*, *A. millefolium*, leaves and flowers of *E. ritro*, and roots of *L. serriola*, *S. canadensis*, *A. artemisiifolia*, *S. virgaurea*, and *S. autumnalis*.

Table 3

Antibacterial effect of ethanol extracts of plants on *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Shigella flexneri* (x ± SD, n = 8)

Species	*E. coli*		*K. pneumoniae*		*P. mirabilis*		*Sh. flexneri*	
	test	control	test	control	test	control	test	control
Artemisia vulgaris L., galls	15.23 ± 1.71*	1.56 ± 0.38	11.34 ± 1.62*	1.12 ± 0.25	0 ± 0	21.37 ± 3.32	0 ± 0	16.21 ± 1.58
– root	0 ± 0	1.22 ± 0.26	13.47 ± 1.33*	0 ± 0	0 ± 0	21.23 ± 2.27	0 ± 0	18.43 ± 1.57
Artemisia austriaca Jacq.								
– shoots and leaves	0 ± 0	1.27 ± 0.24	0 ± 0	0 ± 0	14.74 ± 1.62*	21.32 ± 2.21	0 ± 0	16.38 ± 2.14
Jurinea arachnoidea Bunge,								
– shoots and leaves	2.44 ± 0.24	1.43 ± 0.37	10.62 ± 1.57*	1.54 ± 0.45	10.63 ± 1.77*	21.31±2.35	1.29 ± 0.13	17.30 ± 2.63
– root	0 ± 0	0 ± 0	2.38 ± 0.21	1.60 ± 0.42	10.26 ± 1.41*	21.62 ± 2.34	10.33 ± 1.28*	16.15 ± 2.11
Echinops ritro L., leaves	0 ± 0	2.21 ± 0.33	0 ± 0	0 ± 0	0 ± 0	21.40 ± 2.26	0 ± 0	16.22 ± 2.25
– fruits	0 ± 0	1.59 ± 0.18	8.53 ± 1.45*	1.26 ± 0.31	13.35 ± 1.52*	21.17 ± 3.11	0 ± 0	17.36 ± 2.14
– flower	0 ± 0	0 ± 0	16.51 ± 1.79*	1.71 ± 0.48	17.74 ± 1.43*	20.29 ± 2.18	10.52 ± 1.76*	16.30 ± 2.27
– root	0 ± 0	2.17 ± 0.25	0 ± 0	0 ± 0	8.28 ± 1.41*	21.44 ± 2.74	10.15 ± 1.52*	18.31 ± 2.16
Lactuca serriola L., shoots and leaves	0 ± 0	1.46 ± 0.32	5.26 ± 0.62	1.22 ± 0.17	15.10 ± 1.26*	21.53 ± 3.41	2.65 ± 0.31	15.93 ± 1.73
– root	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	21.11 ± 2.43	0 ± 0	16.51 ± 1.68
Solidago canadensis L.,								
– shoots and leaves	0 ± 0	2.24 ± 0.33	14.55 ± 1.73*	1.18 ± 0.13	10.42 ± 1.34*	20.26 ± 2.29	12.53 ± 1.17*	18.36 ± 2.52
– root	0 ± 0	1.28 ± 0.21	0 ± 0	1.13 ± 0.25	0 ± 0	21.50 ± 2.33	0 ± 0	18.41 ± 2.50
Ambrosia artemisiifolia L.,								
– shoots and leaves	10.43 ± 1.47*	1.59 ± 0.22	0 ± 0	1.57 ± 0.44	5.29 ± 0.37	21.22 ± 2.17	2.26 ± 0.37	16.53 ± 2.17
– root	0 ± 0	1.27 ± 0.24	0 ± 0	1.25 ± 0.14	0 ± 0	20.91 ± 2.11	0 ± 0	16.43 ± 2.64
Solidago virgaurea L., shoots and leaves	10.56 ± 1.20*	2.14 ± 0.31	2.40 ± 0.21	0 ± 0	0 ± 0	21.52 ± 2.19	0 ± 0	17.33 ± 2.51
– root	0 ± 0	2.15 ± 0.28	12.64 ± 1.31*	1.21 ± 0.36	0 ± 0	20.66 ± 2.40	0 ± 0	18.11 ± 1.93
Scorzoneroides autumnalis L.,								
– shoots and leaves	0 ± 0	1.61 ± 0.12	0 ± 0	0 ± 0	10.23 ± 1.51*	21.23 ± 2.18	10.31 ± 1,74*	18.66 ± 2.21
– root	0 ± 0	0 ± 0	0 ± 0	1.19 ± 0.11	0 ± 0	21.37 ± 2.81	0 ± 0	18.32 ± 2.94
Helichrysum arenarium (L.) Moench,								
–shoots and leaves	2.22 ± 0.36	2.12 ± 0.26	0 ± 0	1.10 ± 0.29	20.55 ± 1.79*	21.62 ± 2.14	11.58 ± 1.73*	16.92 ± 2.46
Cyclachaena xanthiifolia (Nutt.) Fresen.,								
– shoots and leaves	12.61 ± 1.74*	1.43 ± 0.27	0 ± 0	1.66 ± 0.13	12.64 ± 1.35*	20.88 ± 2.13	2.25 ± 0.37	16.74 ± 2.38
Achillea millefolium L., shoots and leaves	10.45 ± 1.79*	1.54 ± 0.13	0 ± 0	1.17 ± 0.22	12.26 ± 1.73*	21.55 ± 2.91	0 ± 0	16.41 ± 1.73

Note: * – disks with 10.0 μg of ampicillin were used for all bacteria as positive control.

Table 4

Antibacterial effects of ethanol extracts of plants on *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Listeria monocytogenes* (x ± SD, n = 8)

Species	*P. aeruginosa*		*E. faecalis*		*L. monocytogenes*	
	test	control	test	control	test	control
Artemisia vulgaris L., galls	10.36 ± 1.52*	1.83 ± 0.44	0 ± 0	1.33 ± 0.39	0 ± 0	40.51 ± 3.66
– root	2.21 ± 0.23	1.42 ± 0.27	0 ± 0	1.28 ± 0.27	0 ± 0	40.18 ± 3.73
Artemisia austriaca Jacq., shoots and leaves	5.24 ± 0.75	1.17 ± 0.21	0 ± 0	0 ± 0	2.26 ± 0.32	40.32 ± 3.85
Jurinea arachnoidea Bunge, shoots and leaves	10.78 ± 1.46*	1.45 ± 0.32	0 ± 0	0 ± 0	0 ± 0	40.66 ± 4.41
– root	8.63 ± 1.42	1.74 ± 0.46	0 ± 0	0 ± 0	0 ± 0	40.21 ± 4.33
Echinops ritro L. leaves	2.23 ± 0.44	1.33 ± 0.34	0 ± 0	1.42 ± 0.31	0 ± 0	30.44 ± 3.62
–fruits	0 ± 0	1.68 ± 0.37	0 ± 0	0 ± 0	2.35 ± 0.43	31.29 ± 3.83
– flower	12.52 ± 1.71*	1.53 ± 0.28	0 ± 0	1.29 ± 0.29	2.52 ± 0.28	30.72 ± 3.54
– root	0 ± 0	1.71 ± 0.43	0 ± 0	0 ± 0	0 ± 0	30.51 ± 3.77
Lactuca serriola L. shoots and leaves	10.81 ± 1.63*	1.55 ± 0.21	2.28 ± 0.24	0 ± 0	0 ± 0	31.55 ± 3.26
– root	0 ± 0	1.46 ± 0.24	10.42 ± 1.36*	1.40 ± 0.24	0 ± 0	32.33 ± 3.11
Solidago canadensis L., shoots and leaves	0 ± 0	1.71 ± 0.28	0 ± 0	0 ± 0	0 ± 0	30.28 ± 3.43
– root	0 ± 0	1.54 ± 0.22	0 ± 0	1.35 ± 0,22	0 ± 0	30.12 ± 3.34
Ambrosia artemisiifolia L., shoots and leaves	0 ± 0	1.65 ± 0.36	6.21 ± 0.37	1.46 ± 0.32	0 ± 0	40.16 ± 3.32

Species	*P. aeruginosa*		*E. faecalis*		*L. monocytogenes*	
	test	control	test	control	test	control
– root	0 ± 0	1.33 ± 0.21	0 ± 0	0 ± 0	0 ± 0	40.18 ± 3.73
Solidago virgaurea L., shoots and leaves	0 ± 0	1.47 ± 0.33	10.77 ± 1.25*	1.24 ± 0.23	0 ± 0	31.83 ± 3.27
– root	0 ± 0	1.54 ± 0.26	8.64 ± 1.32	1.31 ± 0.26	0 ± 0	30.25 ± 3.42
Scorzoneroides autumnalis L., shoots and leaves	0 ± 0	1.48 ± 0.27	0 ± 0	0 ± 0	0 ± 0	32.14 ± 3.55
– root	0 ± 0	1.52 ± 0.25	0 ± 0	1.20 ± 0.24	0 ± 0	31.22 ± 3.31
Helichrysum arenarium (L.*)* Moench, shoots and leaves	0 ± 0	1.35 ± 0.32	11.70 ± 1.44	1.33 ± 0.25	10.74 ± 1.31	40.13 ± 4.66
Cyclachaena xanthiifolia (Nutt.) Fresen., shoots and leaves	2.25 ± 0.37	1.56 ± 0.21	12.63 ± 1.51	1.38 ± 0.31	0 ± 0	39.29 ± 4.51
Achillea millefolium L., shoots and leaves	0 ± 0	1.43 ± 0.36	0 ± 0	0 ± 0	0 ± 0	39.44 ± 4.75

Note: see Table 3.

Table 5

Antibacterial effects of ethanol extracts of plants on *Staphylococcus aureus*, *Bacillus subtilis*, *Clostridium perfringens*, and *Candida albicans* (x ± SD, n = 8)

Species	*S. aureus*		*B. subtilis*		*C. perfringens*		*C. albicans*	
	test	control	test	control	test	control	test	control
Artemisia vulgaris L., galls	0 ± 0	38.22 ± 4.46	0 ± 0	1.31 ± 0.27	0 ± 0	15.36 ± 1.62	0 ± 0	15.32 ± 1.68
– root	0 ± 0	38.37 ± 4.25	8.28 ± 1.23	1.33 ± 0.21	0 ± 0	15.42 ± 1.91	0 ± 0	15,25 ± 2.43
Artemisia austriaca Jacq., – shoots and leaves	0 ± 0	37.96 ± 4.33	10.62 ± 1.74*	1.35 ± 0.34	0 ± 0	15,47±1.64	0 ± 0	16.46 ± 1.51
Jurinea arachnoidea Bunge, – shoots and leaves	0 ± 0	37.90 ± 4.18	12.46 ± 1.63*	1.42 ± 0.38	0 ± 0	15.53 ± 1.48	0 ± 0	15.40 ± 2.27
– root	0 ± 0	38.2 ± 4.31	0 ± 0	1.34 ± 0.23	2.36 ± 0.81	15.62 ± 1.77	2.36 ± 0.81	15,23 ± 2.11
Echinops ritro L. leaves	0 ± 0	37.50 ± 4.34	0 ± 0	1,,47 ± 0.37	0 ± 0	14.81 ± 2.14	0 ± 0	14.98 ± 1.73
– fruits	16.62 ± 1.35	37.93 ± 4.45	12.51 ± 1.38*	1.21 ± 0.34	0 ± 0	15.14 ± 2.56	0 ± 0	15,31 ± 1.94
– flower	0 ± 0	37.38 ± 4.22	0 ± 0	1.35 ± 0.22	0 ± 0	15.52 ± 1.88	0 ± 0	15.66 ± 2.23
– root	0 ± 0	38.21 ± 4.36	0 ± 0	1.43 ± 0.26	0 ± 0	14.39 ± 1.72	0 ± 0	15,12 ± 2.61
Lactuca serriola L., shoots and leaves	0 ± 0	38.34 ± 4.31	0 ± 0	1.36 ± 0.33	5.32 ± 0.97	15.23 ± 2.31	5.35 ± 0.93	15.44 ± 2.32
– root	0 ± 0	37.96 ± 4.12	0 ± 0	1.24 ± 0.20	0 ± 0	15.16 ± 1.83	0 ± 0	15,83 ± 2.61
Solidago canadensis L., – shoots and leaves	0 ± 0	37.53 ± 4.37	0 ± 0	1.31 ± 0.25	16.48 ± 1.43	14.41 ± 1.76	16.43 ± 1.47	15.50 ± 1.73
– root	0 ± 0	37.88 ± 4.54	0 ± 0	1.37 ± 0.21	0 ± 0	15.22 ± 1.13	0 ± 0	14,62 ± 2.163
Ambrosia artemisiifolia L., – shoots and leaves	0 ± 0	38.25 ± 3.63	0 ± 0	1.28 ± 0.22	0 ± 0	15.47 ± 2.33	0 ± 0	14.34 ± 2.25
– root	0 ± 0	38.47 ± 4.32	9.43 ± 1.25*	1.33 ± 0.34	0 ± 0	14.66 ± 1.52	0 ± 0	15,53 ± 2.13
Solidago virgaurea L., shoots and leaves	0 ± 0	37.66 ± 4.21	0 ± 0	1.35 ± 0.38	1.57 ± 0.33	15.54 ± 1.26	0 ± 0	14.23 ± 1.78
– root	0 ± 0	38.13 ± 3.96	0 ± 0	1.29 ± 0.33	8.66 ± 1.31	15.39 ± 1.71	0 ± 0	15,64 ± 2.263
Scorzoneroides autumnalis L., – shoots and leaves	0 ± 0	38.42 ± 4.28	0 ± 0	1.42 ± 0.36	10.65 ± 1.52	14.03 ± 2.25	10.68 ± 1.54	15.61 ± 2.37
– root	0 ± 0	38.31 ± 3.35	0 ± 0	1.45 ± 0.29	0 ± 0	15.11 ± 1.84	1.53 ± 0.31	14,18 ± 2.52
Helichrysum arenarium (L.*)* Moench, – shoots and leaves	2.77 ± 0.31	37.54 ± 4.11	5.63±0.38	1.37 ± 0.35	0 ± 0	15.43 ± 1.72	0 ± 0	14.43 ± 2.68
Cyclachaena xanthiifolia (Nutt.) Fresen., – shoots and leaves	0 ± 0	38.47 ± 3.23	5.22 ± 0.46	1.44 ± 0.23	12.61 ± 1.57	15.12 ± 1.44	12.63 ± 1.57	15.14 ± 2.47
Achillea millefolium L., – shoots and leaves	8.33 ± 1.26	38.13 ± 4.22	5.49 ± 0,24	1.43 ± 0.35	0 ± 0	15.54 ± 2.36	1.48 ± 0.22	15.22 ± 2.45

Note: see Table 3.

We found complete absence of sensitivity to ampillicin in two polyresistant strains of microorganisms of the families Pseudomonadaceae and Enterococcaceae – *P. aeruginosa* and *E. faecalis* (Table 4). At the same time, the alcohol extracts of the galls of *A. vulgaris* (10.4), aboveground parts of *J. arachnoidea* (10.8) and *L. serriola* (10.8), and flowers of *E. ritro* (12.5) effectively inhibited growth of the bacteria of *P. aeruginosa*, which had moderate sensitivity to the aboveground part of *A. austriaca* (5.2) and root of *J. arachnoidea* (8.6). Four extracts – from the root of *L. serriola* (10.4), aboveground part of *S. virgaurea* (10.8), *H. arenarium* (11.7), and *C. xanthiifolia* (12.6) – had a strong inhibitory effect on growth of the colonies of *E. faecalis*. We should note that the polyresistant strain *E. faecalis* was moderately sensitive to the alcohol extracts from the aboveground part of *A. artemisiifolia* (6.2) and root of *S. virgaurea* (8.6).

A strong inhibitory effect on the colonies of *L. monocytogenes* was produced only by the alcohol extract from the aboveground part of *H. arenarium* (10.7), which can suggest high resistance of this strain.

High antibacterial activity (Table 5) against *S. aureus* was confirmed for ethanolic extract of the fruits of *E. ritro* (16.6). Also, those bacteria showed moderate sensitivity to the aboveground part of *A. millefolium* (8.3).

During our studies, we determined that some plants inhibited growth of the colonies of *B. subtilis*: alcohol extracts of the aboveground parts of *A. austriaca* (10.6), *J. arachnoidea* (12.5), and fruits of *E. ritro* (12.5). The bacteria had moderate sensitivity to the alcohol extracts from the aboveground parts of *H. arenarium* (5.6), *C. xanthiifolia* (5.2), *A. millefolium* (5.5), and roots of *A. vulgaris* (8.3) and *A. artemisiifolia* (9.4). The studied *B. subtilis* strain showed tolerance to the action of ampicillin.

Strong inhibitory effects on *C. perfringens* were produced by the extracts from the aboveground parts of *S. canadensis* (16.5), *S. autumnalis* (10.7), and *C. xanthiifolia* (12.6). The strain was observed to have moderate sensitivity to the aboveground part of *L. serriola* (5.3) and the root of *S. virgaurea* (8.7). At the same time, we should note the complete absence of reaction of this strain to the ethyl extracts of *A. vulgaris*, *A. austriaca*, *E. ritro*, *A. artemisiifolia*, *H. arenarium*, and *A. millefolium*.

We found three alcohol extracts with maximal antifungal effects (the inhibition zone larger than 10 mm, Table 5) on *C. albicans* – the aboveground parts of *S. canadensis* (16.4), *S. autumnalis* (10.7), and *C. xanthiifolia* (12.6). Also, this polyresistant strain had moderate sensitivitiy to the aboveground part of *L. serriola* (5.4) and was not sensitive to the extracts from the galls and root of *A. vulgaris*, aboveground parts of *A. austriaca*, *J. arachnoidea*, *H. arenarium*, roots of *E. ritro*, *L. serriola*, *S. canadensis*, *A. artemisiifolia*, *S. virgaurea*, and leaves, fruits, and flowers of *E. ritro*.

The study of solutions of ethanol extracts from plants of the Asteraceae family found no notable nematocidal properties towards the nematode larvae of ruminants, in particular, *M. capillaris*, *S. papillosus*, *H. contortus* in the concentration of 0.1%. According to the results, over 95% of the larvae were recorded as vital.

Discussion

The compounds produced by plants as secondary metabolites were found to be biologically active against microorganisms that are pathogenic to humans and agricultural animals. Some of the plants we tested can be

the basis for development of novel pharmaceutical drugs (Zazharskyi & Zazharska, 2024).

Singh & Ebibeni (2012) conducted a comparative assessment of antimicrobial activities of essential oils of *A. vulgaris*, extracted from fresh and dried herbs, and found insignificant statistical difference ($P < 0.40$). The authors determined that the oxidase-positive strains of *Pseudomonas* (60.0%), *Aeromonas* (53.6%), spore-forming bacilli (71.6%), *Pasteurella* (83.3%), and *Micrococcus* (66.7%) were more sensitive ($P < 0.001$) than the oxidase-negative bacteria (8.3%). Gas chromatography/mass spectrometryanalysis of essential oils revealed that eucalyptol is most dominant compound in the leaves of *A. vulgaris*, and exerted antifungal activity towards *S. oryzae* and *F. oxysporum* and antibacterial effect against *S. aureus* (Singh & Rajashekar, 2023).

High sensitivity to the extracts from *A. vulgaris* and *Artemisia abrotanum* L. (solvent – 90% ethanol) was seen in *Candida tropicalis* – a clinical strain with high sensitivity to antifungal antibiotics and synthetic antimycotics. However, the mentioned extracts inhibited the germination of spores of the strain of *Aspergillus niger*. The antifungal activity of *A. abrotanum* is associated with the presence of davanone sesquiterpenoid (Fiamegos & Tegos, 2011). Hrytsyk & Grytsyk (2021) studied the synergism of the antimicrobial action of extracts from plants of the *Artemisia* genus and erythromycin and concluded about the likely presence of inhibitors of efflux pump of macrolipids of Staphylococcus aureus in extracts from *A. vulgaris*.

Han & Shao (2023) report that the essential oil of *A. vulgaris* demonstrated a strong antimicrobial activity against *Escherichia coli* and moderate activities against *Aspergillus niger*, *Verticillium dahliaei*, and *Bacillus subtilis*. The alcohol extracts from the leaves of *A. absinthium* of second year of vegetation produced the maximal inhibition zones in the *Klebsiella* carbapenem-resistent enterobacteriaceae (CRE) and a culture of *Escherichia coli* (Marian & Muste, 2023).

Plants of the *Echinops* genus are widespread all around the globe, and are typically used in medicine to mitigate symptoms of respiratory diseases, such as pain, inflammation, fever, sore throat, and cough (Hymete & Erko, 2005). Previous studies of chemical compositions of some *Echinops* plants found the presence of glycoside benzothiophene, flavone, alkaloids, polyacetylene thiophenes, and carbohydrates (Falah & Alizadeh Behbahani (2021). *Echinops setifer* has displayed an immune-modulating action, suggesting a high antimicrobial potential for control of infectious diseases. Thus, scientists recommend using it to control infectious diseases, such as COVID-19. Extract from *Echinops gracilis* O. Hoffm. demonstrated antibacterial and antioxidant properties against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*, the minimum inhibition concentrations (MIC) measuring 31.2, 15.6, and 31.2 μg/mL, respectively (Weyepe Lah & Dongo, 2021). Gaizirene & Gabriel (2023) reported that essential oils from *E. giganteus* killed the bacteria of *Staphylococcus aureus* and *Salmonella enteritidis*.

Abdul-Jalil (2020) observed the efficacy of alcohol extracts from the leaves and roots of *L. serriola* against *S. aureus* and *S. saprophyticus*, and Unver & Gurhan (2024) reported that essential oil of *L. serriola* displayed a strong inhibiting effect on the growth of the bacteria and fungi of the *Candida* genus, the MIC values ranging 0.47 to 1.87 μL/mL. Those effects were attributed to flavonoids and terpenoids present in the plant.

We studied the antimycotic action of different types of extracts from the aboveground and underground parts of *S. canadensis*. They exerted antibacterial activity against *Listeria monocytogenes* and *Staphylococcus aureus*, the phytopathogenic fungi *Monilinia fructicola*, *Botrytis cinerea*, *Aspergillus niger*, and *Penicillium expansum*, and also some phytopathogenic bacteria *Bacillus megaterium* and *Clavibacter michiganensis*, *Xanthomonas campestris*, *Pseudomonas fluorescens*, and *P. syringae* pv. *phaseolicola* (Elshafie & De Feo, 2019; Anžlovar & Dolenc Koce, 2020).

Janaćković & Marin (2022) observed strong antimicrobial action of essential oil from the aboveground part of *A. artemiisifolia* against two strains of *Xanthomonas campestris* and reference and natural isolates of *Ervinia amylovora*, the causative agents of black rot. Gram-positive bacteria were found to be more sensitive to the antimicrobial properties of essential oil compared to Gram-negative bacteria (Hamidović & Lalević, 2023). The results of the study by those authors revealed that the oil from *A. artemisiifolia* displayed antimicrobial action towards growth of the colonies of *Salmonella* spp. and *Bacillus subtilis*, but had an insignificant effect on growth of the colonies of *Escherichia coli*.

Aqueous alcohol extracts from the aboveground part of *Solidago graminifolia* exerted promising antioxidant and antimicrobial potentials, with powerful antibacterial activity against *Staphylococcus aureus* and strong antifungal effects against *Candida albicans* and *C. parapsilosis* (Toiu & Oniga, 2019). While having potent antimycotic and antioxidant properties, a study of essential oils of *Solidago virgaurea* L. found antibactericidal activity against Gram-positive microorganisms. Likewise, the authors considered the studied plants suitable for the production of bio-degradable packaging in the food industry (Malićanin & Danilović, 2024).

Babotă & Păltinean (2018) discovered that alcohol extracts from the flowers of *Helichrysum arenarium* (L.) Moench. and *Antennaria dioica* (L.) Gaertn. are valuable sources of chlorogenic acid and flavonoids. The ethanol extract from the aboveground part of *H. arenarium* exhibited antimicrobial activity of varying degrees (the growth inhibition zone was 8.8–20.4 mm wide) against *S. aureus*, *B. megaterium*, *C. glabrata*, *C. albicans*, and *Trichophyton* sp. (Eren & Güven, 2023).

El-Kalamouni & Talou (2017) reported that *B. cereus* was the most sensitive bacterium to the oil extract of *A. millefolium* in the utilized concentrations (5 and 10 μg/mL). The smallest growth inhibition zones were produced for the cultures of *S. typhimurium* and *S. agona*, whereas *S. epidermidis*, *S. enteritides*, and *E. coli* were resistant to all the concentrations of the preparation. The extracts from *A. millefolium* demonstrated antifungal activity and inhibiting effects against the development of *Rhizopus stolonifer* (65.7%), *Verticillium dahliae* (56.3%), *Colletotrichum gloeosporioides* (60.9%), *Botrytis cinerea* (50.8%) and *Aspergillus niger* (40.7%).

The nematocidal activities of plants of the Asteraceae family have been described in the scientific literature to a lesser degree. However, there are data regarding noticeable nematocidal properties of plants of the genus Artemisia. Nikitin et al. (2023) studied the nematocidal activity of ethanol extracts from five different species of *Artemisia* L. towards the free-living soil nematode *Caenorhabditis elegans*. The authors have confirmed that the death of nematodes was observed within the concentration range of 31.3–1,000.0 μg/mL.

Liu et al. (2019) assessed the nematocidal properties of ethyl acetate, ethanol, and aqueous extracts from different organs (flowers, leaves, stems, and roots) of common wormwood *A. absinthium* to support the potential agricultural applications of this plant, including its use as an industrial crop. According to results of their studies, the highest concentration of total thiophene and nematocidal activity were recorded in the ethyl acetate extract from the roots of this plant. Our studies of the effects of ethanolic extracts from *Artemisia* plants also indicated absence of notable nematocidal properties against nematode larvae parasitizing ruminants. Perhaps, the difference in the results obtained by different authors is associated with the the object of study and its development stage, as well as concentrations of the extracts. Therefore, according to our studies, the 0.1% concentration of ethanolic extract was ineffective. Our previous studies revealed low nematocidal activity of aqueous extract of plants of this genus against the invasive larvae of *S. papillosus*. However, the concentration of this extract equaled 3% (Boyko & Brygadyrenko, 2016).

Thus, according to our results, the ethanolic extracts inhibited the growth of colonies of many species of microorganisms of the families Enterobacteriaceae, Enterococcaceae, Listeriaceae, Staphylococcaceae, Bacillaceae, and Clostridiaceae, and also fungi of the family Saccharomycetacea. It is somewhat concerning that the strains of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *E. faecalis*, and *B. subtilis* which we studied were resistant to ampicillin (the growth inhibition zone equaled 0.0–2.0 mm).

Conclusion

For the first time, a study was carried out on the complex inhibiting actions of 12 species of plants of the Asteraceae family towards 10 strains of bacteria, one strain of fungi, and larvae of three species of nematodes. The alcoholic extracts from the aboveground parts of *A. austriaca*, *L. serriola*, *A. artemisiifolia*, *S. virgaurea*, roots of *A. vulgaris*, *E. ritro*, *L. serriola*, *S. canadensis*, *A. artemisiifolia*, *S. autumnalis*, and leaves of *E. ritro* had no noticeable effects on the multiresistant strains of *E. coli*,

P. mirabilis, *S. marcescens*, *L. ivanovi*, *P. aeruginosa*, *L. monocytogenes*, *S. aureus*, *C. perfringens*, and *C. albicans*. We observed intensive inhibitory effects of ethanol-based extracts against various multiresitant strains of bacteria or fungi, in particular, the aboveground parts of *S. canadensis*, *C. xanthiifolia*: *S. canadensis*, *C. xanthiifolia* were effective against 5; *J. arachnoidea*, *S. autumnalis*, *H. arenarium* exhibited activity against 4; fruits of *E. ritro* exerted inhibiting effects on 4; flowers of *E. ritro* were active against 4; galls of *A. vulgaris* demonstrated activity towards 3; the aboveground part of *A. millefolium* was effective against 3, and roots of *J. arachnoidea* and *S. virgaurea* inhibited 3 of the 11 studied strains of bacteria or fungi.

We observed no noticeable nematocidal effects of ethanol extracts of the studied species of plants against ruminant parasites such as *M. capillaris*, *S. papillosus*, and *H. contortus*. After 24 hour exposure to 0.1% solutions of those extracts, 95% of the nematode larvae remained vital. We belive it is possible to recommend ethanol extracts from *S. canadensis*, *C. xanthiifolia*, *J. arachnoidea*, and *H. arenarium* or individual compounds present in those plants for further research of combating polyresistant strains of the abovementioned microorganisms.

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