



Antibacterial activity of etanolic extracts of insects and spiders

V. V. Zazharskyi*, V. V. Brygadyrenko**, N. M. Zazharska*, I. V. Borovyk*,
O. M. Kulishenko*, P. O. Davydenko*, N. M. Tishkina*, I. A. Biben*

*Dnipro State Agrarian and Economic University, Dnipro, Ukraine

**Oles Honchar Dnipro National University, Dnipro, Ukraine

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Dnipro State Agrarian
and Economic University,
Sergiy Efremov st., 25,
Dnipro, 49000, Ukraine.
E-mail:
zazharskiyv@gmail.com

Oles Honchar Dnipro
National University,
Nauky av., 72, Dnipro,
49010, Ukraine.
E-mail: brigad@ua.fm

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Despite the fact that preparations made from insects and spiders can be used against microorganisms that are pathogenic to humans and animals, their potential in this relation has not been explored fully. We studied the effects of ethanolic extracts of 12 insects belonging to the families Tettigoniidae, Oecanthidae, Acrididae, Forficulidae, Pyrrhocoridae, Pentatomidae, Carabidae, Coccinellidae, Erebididae, and Vespidae and four spiders of the families Araneidae and Lycosidae. Growth inhibition zones that exceeded six millimeters were produced by the following ethanolic extracts: the firebug *Pyrrhocoris apterus* against five species of microorganisms (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Bacillus subtilis*, and *Candida albicans*), caterpillars of *Euproctis chrysochroa* against four species (*Proteus mirabilis*, *Shigella flexneri*, *Enterococcus faecalis*, and *Clostridium perfringens*), imagoes of *Chorthippus dorsatus* against four species (*Escherichia coli*, *Proteus mirabilis*, *Shigella flexneri*, and *Enterococcus faecalis*), and the spider *Lycosa singoriensis* against four species (*Escherichia coli*, *Proteus mirabilis*, *Clostridium perfringens*, and *Candida albicans*). During our study of antibacterial and antifungal activities, these insects and spiders turned out to be the most promising for further studies on in vivo antibacterial activity. At the same time, the least encouraging results were produced by *Calathus fuscipes*, *Harmonia axyridis*, *Coccinella septempunctata*, *Forficula auricularia*, and *Araneus marmoreus*. The ethanolic extracts of the insects *Pyrrhocoris apterus*, *Chorthippus dorsatus*, *Tettigonia viridissima*, and *Oecanthus pellucens*, and also the spider *Lycosa singoriensis* had the highest inhibitory activity against *E. coli*. A moderate inhibition of the growth of *E. coli* was demonstrated by the extracts derived from *Forficula auricularia*, *Palomena prasina*, and *Araneus diadematus*. A significant activity toward the bacterium *Klebsiella pneumoniae* was displayed by the extracts of *P. apterus* and *T. viridissima*, and a moderate activity was demonstrated by the *Harpalus rufipes* extract. *Proteus mirabilis* was observed to be highly sensitive to the extracts of *Eu. chrysochroa*, *P. gallicus*, *Ch. dorsatus*, *P. apterus*, *T. viridissima*, and *Oe. pellucens* and the extract of *L. singoriensis*. The bacterium *P. mirabilis* was moderately sensitive to two extracts from the ground beetles, *H. rufipes* and *C. fuscipes*. The most intense inhibition of the growth of the bacterium *Shigella flexneri* was produced by the extracts from the insects *Eu. chrysochroa*, *Ch. dorsatus*, *C. septempunctata*, and also the spider *Argiope bruennichi*. No ethanolic extracts of the insects and spiders inhibited the growth of *Pseudomonas aeruginosa* and *Listeria monocytogenes*. A high antimicrobial activity against *E. faecalis* was exhibited by the extracts of the insects *P. gallicus*, *Eu. chrysochroa*, *Ch. dorsatus*, *P. prasina*, and *Oe. pellucens*, and also the ethanolic extracts of the spiders *A. bruennichi* and *A. diadematus*. The bacterium *Staphylococcus aureus* was low-sensitive to the alcohol extracts of three insects and one spider. Only the alcohol extract of *P. apterus* effectively inhibited the growth of the colonies of *Bacillus subtilis*. A notable inhibitory action toward the bacterium *Clostridium perfringens* was displayed by the extracts of *Eu. chrysochroa*, *P. prasina*, *A. diadematus*, and *L. singoriensis*. Of all the tested samples of alcohol extracts of insects and spiders, a fungicidal activity against *Candida albicans* was exerted by *Eu. chrysochroa*, *P. prasina*, *P. apterus*, *A. marmoreus*, *A. diadematus*, and *L. singoriensis*.

Keywords: antibacterial activity; fungistatic activity; *Tettigonia viridissima*; *Oecanthus pellucens*; *Chorthippus dorsatus*; *Forficula auricularia*; *Pyrrhocoris apterus*; *Palomena prasina*; *Harpalus rufipes*; *Calathus fuscipes*; *Harmonia axyridis*; *Coccinella septempunctata*; *Euproctis chrysochroa*; *Polistes gallicus*; *Araneus diadematus*; *Araneus marmoreus*; *Argiope bruennichi*; *Lycosa singoriensis*; polyresistant strain.

Introduction

As of now, biodiversity is notably declining (Melnichuk et al., 2024; Szyszko-Podgórska et al., 2024). More and more often, studies in different regions of Europe register high homogenization of biocoenoses due to the loss of autochthonous species, decrease in the biomass of local populations, and the domination of species that are able to withstand anthropogenic impacts (Méndez-Rojas et al., 2021; Zazharskyi et al., 2025). As a consequence, sharp and large-scale declines in the numbers of insects are reported (Homburg et al., 2019; Wagner, 2020). This multifactorial phenomenon includes different biotopes and is associated with diverse plant cover, properties of soils, and peculiarities of the land use. The content of nutrients for plants in soil, floristic diversity, species composition, and types of land use are interrelated (Fründ et al., 2010; Kolchuk et al., 2024).

Individuals of most invertebrate species are constantly in contact with bacteria and yeasts that cover the soil surface, foliage, and animal remains and feces. Being several centimeters or millimeters long,

invertebrates are much more suitable as a nutritional source for microorganisms because they do not have a constant elevated body temperature, unlike people and warm-blooded animals. Arthropods cannot afford not to have antibacterial or antifungal resistance because their intersegmental membranes can be easily mechanically damaged, with hemolymph leaking outside and contacting with hundreds of microorganisms. The closer invertebrates live to the surface, the stronger the antibacterial and antifungal protection they must have. If an insect or a spider spends most of the time in grass stands or tree crowns, ultraviolet sunrays often disinfect their living environments, killing bacteria and fungi. By contrast, when an arthropod hides most of the time in foliage or under rocks, its living environment is hundreds or thousands times more saturated with bacteria and fungi. Over dozens of millions of years of evolution alongside microorganisms, various groups of arthropods must have obtained different mechanisms of antimicrobial resistance.

For example, *Harpalus rufipes* (Degeer, 1774) and *Calathus fuscipes* (Goeze, 1777) are some of the commonest beetles in agroce-

noses, pastures, and urbanized ecosystems. By consuming dozens of species of invertebrates, including weakened, ill, and dead individuals, those ground beetles ingest a high number of pathogenic bacteria and fungi. How do they succeed in opposing pathogens? Unfortunately, this issue currently lacks substantial studies. There is a scarcity of reports on species of beetles, in particular plant-eating weevils that can increase the likelihood of survival of microbacteria pathogenic to humans and invertebrates in the environment by retaining them in their intestines (Zazharskyi et al., 2023). Ye et al. (2023) determined that the LCTX-F2 peptide, found in the poison of the spider *Lycosa singoriensis* (Laxmann, 1770) is the first known procoagulant component that enhances blood coagulation by activating the coagulation factors. All of this indicates the fragmented pattern of information pertaining to the antibacterial resistance of arthropods, and also a necessity of evaluating such a resistance in order to find alternative antibacterial and antifungal drugs for treatment of humans and vertebrates.

The objective of the study was to evaluate the antibacterial activities of 16 ethanolic extracts of insects and spiders in relation to 11 species of microorganisms. Until now, species of invertebrates have been poorly studied for antibacterial effects, although they may have a great potential in modern human and veterinary medicine.

Material and methods

A total of 16 species of insects and spiders (Table 1) was collected in the territory of the Botanical Garden of Oles Honchar Dnipro National University (Boyko et al., 2020). They were weighed, coated

with 70% ethanol, fragmented, and stored for 14 days at room temperature in 70% ethanol, and then filtered. Per 10 g of 70% ethanol, 2 g of insects and spiders were taken. Then, to assess the antibacterial properties, 0.1 mL of filtered alcohol extracts was transferred to 6 mm-diameter paper disks. The disks were dried in sterile conditions at a temperature of 10 °C in a microbiological safety locker HR1200-IIA2-D (China).

The antibacterial activity of the tinctures was assessed using the disk diffusion method in agar. Of the daily inoculations of microorganisms, we prepared weighed amounts according to McFarland turbidity standard of bacterial suspension, which equals 0.5 units (McF) 1.5×10^8 CFU (colony-forming units). This parameter was determined using a DEN-1 densitometer (Latvija, Table 2). The obtained weighed amount was transferred to Mueller-Hinton agar (Himedia, India, 2023) with further cultivation in a TCO-80/1 thermostat (Factory of New Technologies and Marketing Equipment, Ukraine, 2015) for 24 h at a temperature of 37 °C. Over the inoculations we placed disks (n = 8) saturated with corresponding alcohol extracts of 16 insects and spiders (Table 1).

As the positive control, we used disks with 10 µg of ampicillin trihydrate (Himedia Laboratories Pvt. Limited, Mumbai, Maharashtra, India), a broad-spectrum semisynthetic 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 of microorganisms (Antibiotic Zone Scale-C, model PW297, India) and the TpsDig2 software (F. James Rohlf, USA, 2016). The data in tables are represented as $x \pm SD$ (mean \pm standard deviation).

Table 1

Sixteen species of insects and spiders that we used to prepare ethanolic extracts and the most important data on their antibacterial activity

Class	Order	Family	Species	Most important literature sources about medical properties	
Insecta	Orthoptera	Tettigoniidae	<i>Tettigonia viridissima</i> (Linnaeus, 1758)	Sonchieu & Mireille (2019); Radev & Petkova (2024)	
		Oecanthidae	<i>Oecanthus pellucens</i> (Scopoli, 1763)	Labadessa & Todisco (2016); Meßer & Klady (2023)	
		Acrididae	<i>Chorthippus dorsatus</i> (Zetterstedt, 1821)	Winter & Schielzeth (2021)	
	Dermaptera	Forficulidae	<i>Forficula auricularia</i> Linnaeus, 1758	Staerkle & Kölliker (2008); Cantrall (1972)	
		Pyrrhocoridae	<i>Pyrrhocoris apterus</i> (Linnaeus, 1758)	Käfer & Stabenheiner (2023)	
	Hemiptera	Pentatomidae	<i>Palomena prasina</i> (Linnaeus, 1761)	Ozdemir & Ozer (2022); Hamidi & Tavella (2023)	
		Carabidae		<i>Harpalus rufipes</i> (Degeer, 1774)	Brygadyrenko & Reshetniak (2014a, 2014b); Cavaliere & Giglio (2019); Avtaeva et al. (2021); Szyszko-Podgórska & Niznikowski (2024)
	Coleoptera			<i>Calathus fuscipes</i> (Goeze, 1777)	Pozsgai & Littlewood (2021); Ilko & Peterkova (2023)
		Coccinellidae		<i>Harmonia axyridis</i> (Pallas, 1773)	Berkvens & De Clercq (2007); Liu & Francis (2025)
				<i>Coccinella septempunctata</i> (Linnaeus, 1758)	Yadav & Agrawal (2022); Yang & Ding (2024)
Lepidoptera	Erebidae	<i>Euproctis chryorrhoea</i> (Linnaeus, 1758)	İlçin (2022)		
Arachnida	Araneae	Vespidae	<i>Polistes gallicus</i> Linnaeus, 1761 (гніздо)	Perez-Bote & Rodriguez Riano (2020); Stabenheiner & Turillazzi (2022); Kovac & Stabenheiner (2023)	
				<i>Araneus diadematus</i> (Clerck, 1758)	Hanichenko & Lapyga (2020); Nyffeler & Bonte (2020); Fischer & Uhl (2021)
		Araneidae		<i>Araneus marmoreus</i> (Clerck, 1758)	Sarkar & Uniyal (2023); Wang & Zhang (2025)
				<i>Argiope bruennichi</i> (Scopoli, 1772)	Dobrzycka-Krahel (2024); Noguchi (2025)
			Lycosidae	<i>Lycosa singoriensis</i> (Laxmann, 1770)	Li & Rong (2020); Ye & Liu (2023)

Table 2

Taxonomic composition of 11 species of microorganisms we studied

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

Results

The tested ethanolic extracts of arthropods exerted an inhibitory activity against certain strains of microorganisms (Table 3–5). The extracts of the insects *Pyrrhocoris apterus*, *Chorthippus dorsatus*,

Tettigonia viridissima, and *Oecanthus pellucens* and also the tarantula *Lycosa singoriensis* displayed the highest inhibitory activity toward *E. coli* (11.5, 11.7, 12.6, 10.6, and 11.6 mm, hereinafter the average radius of the growth inhibition zone is given in mm). A moderate ability to inhibit the growth of the bacterium *E. coli* was exhibited by

the extracts of *Forficula auricularia* (8.8), *Palomena prasina* (8.6), and *Araneus diadematus* (8.5). At the same time, we found a resistance of the studied strain of *E. coli* to the ethanolic extracts of the insects *Harpalus rufipes*, *Calathus fuscipes*, *Euproctis chrysorrhoea*, nests of the paper wasp *Polistes gallicus*, and the ladybugs *Harmonia axyridis* and *Coccinella septempunctata*. Ampicillin, used as the positive control, demonstrated no efficacy against *E. coli*, indicating resistance of the strain we studied to this antibiotic (Table 3).

Two ethanolic extracts exerted a significant antibacterial activity against the epizootic strain of *K. pneumoniae*, exceeding that of ampicillin: *P. apterus* (12.5) and *T. viridissima* (10.6). The extract of *H. rufipes* (6.7) exhibited a moderate ability to inhibit the growth of the colonies of *K. pneumoniae*, indicating its partial antibacterial activity. The stain of *K. pneumoniae* was completely insensitive to the ethanolic extracts of other six species of studied insects: *C. fuscipes*, *Eu. chrysorrhoea*, *P. prasina*, *P. gallicus*, *H. axyridis*, and *C. septempunctata*. The growth of the colonies was not inhibited, evidencing the absence of antimicrobial activity of hemolymph of these insects. Meanwhile, in control group, ampicillin also did not inhibit the growth of the bacterial colonies of *K. pneumoniae*, indicating the antibiotic resistance of the tested strain to beta-lactams.

Table 3

Antibacterial effect of ethanolic extracts of insects and spiders on *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Shigella flexneri* ($x \pm SD$, $n = 8$)

Species	<i>E. coli</i>		<i>K. pneumoniae</i>		<i>P. mirabilis</i>		<i>Sh. flexneri</i>	
	Test	control	test	control	test	control	test	control
<i>Harpalus rufipes</i>	0 ± 0***	4.32 ± 0.13	6.67 ± 1.33**	4.12 ± 0.28	8.64 ± 1.28***	18.31 ± 3.44	0 ± 0***	17.22 ± 3.26
<i>Calathus fuscipes</i>	0 ± 0***	4.42 ± 0.24	0 ± 0***	4.25 ± 0.21	8.67 ± 1.31***	18.26 ± 2.83	0 ± 0***	17.42 ± 3.04
<i>Euproctis chrysorrhoea</i>	0 ± 0***	4.42 ± 0.14	0 ± 0***	4.07 ± 0.33	10.62 ± 1.34***	19.02 ± 3.51	10.68 ± 1.32***	17.16 ± 2.81
<i>Palomena prasina</i>	8.62 ± 1.31***	4.28 ± 0.21	0 ± 0***	4.22 ± 0.14	0 ± 0***	18.17 ± 2.46	0 ± 0***	17.20 ± 2.18
<i>Polistes gallicus</i> (nest)	0 ± 0***	5.34 ± 0.32	0 ± 0***	4.63 ± 0.25	10.74 ± 1.42***	18.23 ± 2.91	0 ± 0***	17.61 ± 3.03
<i>Chorhippus dorsatus</i>	11.66 ± 1.41***	5.16 ± 0.24	4.74 ± 0.68	4.20 ± 0.26	10.74 ± 1.93***	18.07 ± 3.12	8.35 ± 1.41***	17.03 ± 1.82
<i>Harmonia axyridis</i>	0 ± 0***	4.09 ± 0.34	0 ± 0***	4.13 ± 0.27	0 ± 0***	18.02 ± 2.33	0 ± 0***	16.44 ± 3.15
<i>Coccinella septempunctata</i>	0 ± 0***	5.54 ± 0.20	0 ± 0***	4.26 ± 0.32	0 ± 0***	17.54 ± 2.61	8.46 ± 1.32***	17.02 ± 3.24
<i>Forficula auricularia</i>	8.76 ± 1.42***	5.08 ± 0.16	2.74 ± 0.33**	3.69 ± 0.24	0 ± 0***	18.26 ± 3.42	0 ± 0***	17.13 ± 3.21
<i>Pyrrhocoris apterus</i>	11.54 ± 1.38***	5.24 ± 0.21	12.46 ± 1.42***	4.08 ± 0.31	11.36 ± 1.62***	17.51 ± 3.25	0 ± 0***	16.27 ± 3.34
<i>Tettigonia viridissima</i>	12.62 ± 1.34***	5.23 ± 0.19	10.61 ± 1.17***	4.12 ± 0.34	12.65 ± 1.33***	17.44 ± 3.28	0 ± 0***	17.32 ± 2.24
<i>Oecanthus pellucens</i>	10.61 ± 1.32***	4.27 ± 0.23	2.63 ± 0.52***	4.26 ± 0.22	10.68 ± 1.32***	18.13 ± 2.84	0 ± 0***	18.15 ± 3.22
<i>Araneus marmoreus</i>	0 ± 0***	4.32 ± 0.17	0 ± 0***	4.17 ± 0.21	0 ± 0***	17.32 ± 3.06	0 ± 0***	17.23 ± 2.91
<i>Argiope bruennichi</i>	0 ± 0***	5.24 ± 0.31	2.42 ± 0.21***	4.63 ± 0.28	0 ± 0***	18.08 ± 3.12	10.24 ± 1.63***	18.02 ± 3.13
<i>Araneus diadematus</i>	8.48 ± 1.73***	4.26 ± 0.22	2.46 ± 0.32***	4.23 ± 0.34	0 ± 0***	17.51 ± 3.03	0 ± 0***	17.44 ± 3.20
<i>Lycosa singoriensis</i>	11.62 ± 1.70***	5.03 ± 0.18	0 ± 0***	4.81 ± 0.26	11.72 ± 1.44***	18.23 ± 2.61	0 ± 0***	18.13 ± 2.82

Note: disks with 10.0 µg of ampicillin were used for all bacteria as positive control; * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared with the control.

The strains of *P. aeruginosa* and *E. faecalis*, which belong to the families Pseudomonadaceae and Enterococcaceae, were fully resistant to ampicillin (Table 4). No inhibition of the growth of *P. aeruginosa* was observed when subject to the ethanolic extracts of the insects and spiders we tested. A high antimicrobial activity toward *E. faecalis* was displayed by the extracts of *P. gallicus* (8.4), *Eu. chrysorrhoea* (8.7), *Ch. dorsatus* (10.5), *P. prasina* (10.6), and *Oe. pellucens* (10.6), and also the extracts of the spiders *A. bruennichi* (8.2) and *A. diadematus* (8.6). None of the studied samples of insect and spider extracts that we studied inhibited *Listeria monocytogenes* (Table 4).

The ethanolic extracts of insects and spiders had no pronounced antimicrobial activity against *Staphylococcus aureus* (Table 5). This bacterium was low-sensitive to three alcohol extracts of insects (*Eu. chrysorrhoea*, *Ch. dorsatus*, and *T. viridissima*) and one extract of spider (*A. bruennichi*).

Only the alcohol extract of *P. apterus* (14.4) was effective at inhibiting the growth of the colonies of *Bacillus subtilis*. At the same time, the strain of *B. subtilis* demonstrated complete resistance to ampicillin, which indicates mechanisms of resistance to this beta-lactam antibiotic (Table 5).

A significant inhibitory activity against the bacterium *Clostridium perfringens* was exhibited by four alcohol extracts, in particular the insects *Eu. chrysorrhoea* (8.6) and *P. prasina* (8.6) and the spiders *A. diadematus* (10.5) and *L. singoriensis* (30.7). At the same time, the strain of *C. perfringens* was entirely unaffected by the ethanolic extracts of the insects *H. rufipes*, *C. fuscipes*, *P. gallicus*, *Ch. dorsatus*,

Proteus mirabilis was highly sensitive to seven ethanolic extracts, of the insects *Eu. chrysorrhoea*, *P. gallicus*, *Ch. dorsatus*, *P. apterus*, *T. viridissima*, and *Oe. pellucens* and the tarantula *L. singoriensis*. The growth inhibition zones ranged 10.6 to 12.7 mm. The bacterium *Proteus mirabilis* was observed to have a moderate sensitivity to two insect extracts, derived from *H. rufipes* and *C. fuscipes* (8.6 and 8.7 mm, respectively). At the same time, the extracts of the insects *P. prasina*, *H. axyridis*, *C. septempunctata*, and *F. auricularia* and spiders *A. diadematus*, *A. bruennichi*, and *A. diadematus* demonstrated no antimicrobial activity against the polyresistant strain of *P. mirabilis* (Table 3).

Some ethanolic extracts of insects and spiders exhibited a notable antibacterial activity toward *Shigella flexneri*. The strongest inhibition of the growth of the bacterium was produced by the extracts of the insects *Eu. chrysorrhoea* (10.7 mm), *Ch. dorsatus* (8.4 mm), *C. septempunctata* (8.5 mm), and also the spider *A. bruennichi* (10.2 mm). At the same time, the strain of *Sh. flexneri* was resistant to a number of extracts: from the insects *H. rufipes*, *C. fuscipes*, *P. prasina*, *P. gallicus*, *H. axyridis*, *F. auricularia*, *P. apterus*, *T. viridissima*, and *Oe. pellucens* and the spiders *A. diadematus*, *A. diadematus*, and *L. singoriensis* (Table 3).

H. axyridis, *C. septempunctata*, *P. apterus*, *T. viridissima*, and *Oe. pellucens* and the spiders *A. diadematus* and *A. bruennichi* (Table 5).

Of all the tested samples of ethanolic extracts of insects and spiders, a fungicidal activity against *Candida albicans* (the growth inhibition zone of 4 mm; Table 5) was exerted by six extracts: three insects (*Eu. chrysorrhoea*, 4.0; *P. prasina*, 4.1; and *P. apterus*, 6.3) and three spiders (*A. marmoreus*, 6.0; *A. diadematus*, 6.1; and *L. singoriensis*, 8.2). At the same time, the polyresistant strain of *C. albicans* was insensitive to the extracts of the insects *H. rufipes*, *C. fuscipes*, *P. gallicus*, *Ch. dorsatus*, *H. axyridis*, *C. septempunctata*, *F. auricularia*, *T. viridissima*, and *Oe. pellucens* and the spider *A. bruennichi*.

Discussion

Over recent years, the resistance of pathogenic strains of microorganisms to antibiotics has been one of the most serious problems in the sphere of public health (EFSA, 2024). Excessive and uncontrolled use of antibiotics in agriculture and medicine promoted the emergence and spread of resistant bacterial strains, which significantly complicates the treatment of infectious diseases. Such pathogens become less sensitive to traditional antibiotics, in particular fluorine quinolones and macrolides (Liu et al., 2019; Jehanne et al., 2021). In this context, insects and spiders are considered a promising source of new antibacterial compounds, which can be an innovative and ecologically sustainable solution for increasing the safety of food products and overcoming the problem of antimicrobial resistance (Saadoun et al., 2022). In particular, fats obtained from the larvae of *Hermetia illucens*

(Linnaeus, 1758) (Diptera, Stratiomyidae) by lyophilization defatting with the use of supercritical fluid extraction (SFE) demonstrated a

strong antibacterial effect against *Campylobacter* spp.

Table 4

Antibacterial effects of the ethanol extracts of insects and spiders

on *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Listeria monocytogenes* ($\bar{x} \pm SD$, n = 8)

Species	<i>P. aeruginosa</i>		<i>E. faecalis</i>		<i>L. monocytogenes</i>	
	test	control	test	control	test	control
<i>H. rufipes</i>	0 ± 0***	1.54 ± 0.27	0 ± 0***	1.74 ± 0.22	0 ± 0***	24.16 ± 3.24
<i>C. fuscipes</i>	0 ± 0***	1.33 ± 0.31	0 ± 0***	1.31 ± 0.28	0 ± 0***	25.22 ± 3.26
<i>Eu. chrysorrhoea</i>	0 ± 0***	1.52 ± 0.44	8.66 ± 1.23***	0 ± 0	0 ± 0***	24.34 ± 3.14
<i>P. prasina</i>	1.11 ± 0.20**	1.65 ± 0.23	10.62 ± 1.31***	1.34 ± 0.24	0 ± 0***	23.41 ± 3.23
<i>P. gallicus</i> (nest)	0 ± 0***	1.28 ± 0.22	8.43 ± 1.27***	1.42 ± 0.31	0 ± 0***	21.36 ± 3.12
<i>Ch. dorsatus</i>	1.16 ± 0.24*	1.74 ± 0.21	10.46 ± 1.22***	1.53 ± 0.26	0 ± 0***	23.64 ± 3.23
<i>H. axyridis</i>	0 ± 0***	1.54 ± 0.36	0 ± 0***	1.31 ± 0.14	0 ± 0***	22.28 ± 3.41
<i>C. septempunctata</i>	0 ± 0***	1.63 ± 0.25	0 ± 0	0 ± 0	0 ± 0***	24.32 ± 2.84
<i>F. auricularia</i>	0 ± 0***	1.33 ± 0.31	0 ± 0***	1.52 ± 0.23	0 ± 0***	23.24 ± 3.12
<i>P. apterus</i>	0 ± 0***	1.54 ± 0.27	0 ± 0***	1.34 ± 0.27	2.58 ± 0.23***	23.21 ± 3.25
<i>T. viridissima</i>	0 ± 0***	1.72 ± 0.30	0 ± 0***	1.53 ± 0.31	0 ± 0***	22.06 ± 3.24
<i>Oe. pellucens</i>	1.34 ± 0.23*	1.81 ± 0.22	10.64 ± 1.32***	1.42 ± 0.25	0 ± 0***	21.10 ± 3.22
<i>A. marmoreus</i>	0 ± 0***	1.43 ± 0.24	8.62 ± 1.27***	1.54 ± 0.18	0 ± 0***	22.17 ± 3.12
<i>A. bruennichi</i>	1.23 ± 0.22*	1.62 ± 0.31	8.23 ± 1.45***	1.33 ± 0.42	0 ± 0***	23.44 ± 2.83
<i>A. diadematus</i>	0 ± 0***	1.84 ± 0.28	0 ± 0***	1.24 ± 0.23	0 ± 0***	23.21 ± 3.16
<i>L. singoriensis</i>	0 ± 0***	1.66 ± 0.32	0 ± 0***	1.46 ± 0.41	2.34 ± 0.21***	21.32 ± 2.64

Note: see Table 3.

Table 5

Antibacterial effects of the ethanolic extracts of insects and spiders

on *Staphylococcus aureus*, *Bacillus subtilis*, *Clostridium perfringens*, and *Candida albicans* ($\bar{x} \pm SD$, n = 8)

Species	<i>S. aureus</i>		<i>B. subtilis</i>		<i>C. perfringens</i>		<i>C. albicans</i>	
	test	control	test	control	test	control	test	control
<i>H. rufipes</i>	0 ± 0***	31.12 ± 3.63	1.14 ± 0.23	1.28 ± 0.31	0 ± 0***	15.24 ± 1.43	0 ± 0***	13.32 ± 1.54
<i>C. fuscipes</i>	0 ± 0***	31.26 ± 3.52	0 ± 0***	1.26 ± 0.32	0 ± 0***	15.31 ± 1.52	0 ± 0***	14.06 ± 2.13
<i>Eu. chrysorrhoea</i>	1.28 ± 0.14***	32.34 ± 3.43	1.03 ± 0.12	1.33 ± 0.24	8.62 ± 1.27***	14.03 ± 1.84	4.02 ± 0.31***	13.14 ± 1.66
<i>P. prasina</i>	0 ± 0***	31.23 ± 3.22	0 ± 0***	1.27 ± 0.42	8.64 ± 1.32***	14.25 ± 1.61	4.13 ± 0.22***	14.22 ± 1.41
<i>P. gallicus</i> (nest)	0 ± 0***	32.62 ± 3.16	0 ± 0***	1.34 ± 0.33	0 ± 0***	14.23 ± 1.26	0 ± 0***	13.38 ± 2.23
<i>Ch. dorsatus</i>	1.42 ± 0.24***	32.35 ± 3.31	0 ± 0***	1.44 ± 0.22	0 ± 0***	15.24 ± 1.73	0 ± 0***	13.21 ± 2.74
<i>H. axyridis</i>	0 ± 0***	32.54 ± 3.27	0 ± 0***	1.31 ± 0.23	0 ± 0***	15.42 ± 1.44	0 ± 0***	13.63 ± 2.02
<i>C. septempunctata</i>	0 ± 0***	31.13 ± 3.02	0 ± 0***	1.40 ± 0.26	0 ± 0***	15.36 ± 1.32	0 ± 0***	14.12 ± 2.35
<i>F. auricularia</i>	0 ± 0***	32.34 ± 3.25	0 ± 0***	1.24 ± 0.32	2.53 ± 0.24***	15.23 ± 1.22	0 ± 0***	13.36 ± 1.27
<i>P. apterus</i>	0 ± 0***	31.19 ± 3.32	14.42 ± 1.34***	1.21 ± 0.34	0 ± 0***	15.12 ± 1.34	6.33 ± 1.42***	13.34 ± 1.53
<i>T. viridissima</i>	1.26 ± 0.17***	31.03 ± 3.36	0 ± 0***	1.54 ± 0.31	0 ± 0***	15.24 ± 1.32	0 ± 0***	14.21 ± 2.56
<i>Oe. pellucens</i>	0 ± 0***	31.21 ± 3.03	0 ± 0***	1.62 ± 0.17	0 ± 0***	15.13 ± 1.23	0 ± 0***	13.28 ± 1.72
<i>A. marmoreus</i>	0 ± 0***	32.44 ± 3.25	0 ± 0***	1.42 ± 0.22	0 ± 0***	14.22 ± 1.18	6.02 ± 1.13***	13.34 ± 1.41
<i>A. bruennichi</i>	2.14 ± 0.12***	31.30 ± 3.14	0 ± 0***	1.23 ± 0.12	0 ± 0***	14.34 ± 2.65	0 ± 0***	13.06 ± 2.23
<i>A. diadematus</i>	0 ± 0***	32.42 ± 3.31	0 ± 0***	1.46 ± 0.24	10.46 ± 1.72***	15.31 ± 2.24	6.14 ± 1.22***	13.32 ± 2.48
<i>L. singoriensis</i>	0 ± 0***	31.27 ± 3.22	0 ± 0***	1.32 ± 0.23	30.72 ± 1.44***	14.42 ± 2.32	8.23 ± 0.74***	13.73 ± 2.12

Note: see Table 3.

The study by Silvan et al. (2025) revealed that those fats inhibited the growth of *C. jejuni* and displayed a bactericidal activity toward the strains of *E. coli*. The authors highlight that lauric and linoleic acids provided a complete inhibition of the growth of *C. jejuni* and *E. coli*, whereas myristic, palmitic, and oleic acids exerted no significant antimicrobial effect against those pathogens.

In Thailand, Boonsai (2012) tested the antibacterial activity of propolis from hives of *Apis mellifera* Linnaeus, 1758 (Hymenoptera, Apidae). The crude methanol extract of propolis (CME) demonstrated a better antibacterial activity, with a minimal inhibition concentration of 5 mg/mL, and was effective at inhibiting *S. aureus* and *E. coli*. The studies by Benli & Yigit (2008) revealed that an acetone extract of the web of *Eratigena atrica* (C. L. Koch, 1843) (Araneae, Agelenidae) had a high antibacterial activity against the *B. subtilis* (maximum inhibition zone of 15.0 mm) and *E. coli* (12.0 mm), and the poison of *Agelena labyrinthica* (Clerck, 1757) (Araneae, Agelenidae) was effective against *B. subtilis*.

In 1981, Steiner and Boman first identified the antibacterial peptides cecropins in the cecropia moth *Hyalophora cecropia* (Linnaeus, 1758) (Lepidoptera, Saturniidae). In the following years, similar peptides with antibacterial activity were found in a broad spectrum of representatives of fauna: ranging bacteria and insects of different orders to amphibians, humans, and other mammals. Gomesin became the first peptide isolated from spider that displayed antimicrobial activity.

It is a high-cation peptide, composed of 18 amino-acid residues, in particular four cysteines, which form two disulfide bonds (Mandard & Vovelle, 2002). Tian & Geng (2024) reported that cecropin A (Bmcecropin A) from *Bombyx mori* (Linnaeus, 1758) (Lepidoptera, Bombycidae) exerted antibacterial, antiviral, antifilamentous fungal, and inhibitory effects against tumor cells. Moreover, spider toxins have a significant potential against pests, which can be effectively used in agriculture (King & Maggio, 2002).

Langenegger & Kuhn-Nentwig (2019) studied the antimicrobial activity of the poison of the spider *Cupiennius salei* Keyserling, 1877 (Araneae, Trechaleidae). They isolated several antimicrobial peptides from it, synthesized their analogues and observed the peptides to demonstrate a lytic activity in relation to human erythrocytes and an insecticidal action toward *Drosophila melanogaster* Meigen, 1830 (Diptera, Drosophilidae). In another study, Okada & Possani (2019) purified five peptides called oxioylipines from the poison of the spider *Oxyopes kitabensis* (Araneae, Oxyopidae) and determined that they have antimicrobial, hemolytic, and insecticidal activities.

Our previous studies revealed that secondary metabolites, synthesized by plants, insects, and spiders, are capable of inhibiting the growth and development of pathogenic microorganisms (Zazharskyi et al., 2019, 2020). Some of the analyzed species demonstrated a potential for further pharmaceutical use (Zazharskyi et al., 2024b). Insects and spiders contain compounds with antibacterial properties and

present a large interest as an alternative to antibiotics or disinfecting agents for the control of antibiotic-resistant bacteria (Zazharsky et al., 2024a). The study we conducted confirmed that ethanolic extracts of different species of insects and spiders exert pronounced antimicrobial activity toward a large spectrum of microorganisms, in particular representatives of the families Enterobacteriaceae, Enterococcaceae, Listeriaceae, Staphylococcaceae, Bacillaceae, and Clostridiaceae, and also fungi of the family Saccharomycetaceae. The results we obtained indicate that such extracts have good prospects of being alternatives in antimicrobial therapy, and also can be used in the development of nutraceuticals.

At the same time, some strains, particularly *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Bacillus subtilis* were highly resistant to ampicillin. This manifested in the absence or just minimal degree of inhibition of the growth of their colonies (diameters of growth inhibition zones measured 0.0–2.0 mm), which suggests a low efficacy of this antibiotic against polyresistant isolates of these bacteria.

Conclusion

For the first time, there were studied the inhibitory effects of ethanolic extracts of 12 insects, belonging to the families Tettigoniidae, Oecanthidae, Acrididae, Forficulidae, Pyrrhocoridae, Pentatomidae, Carabidae, Coccinellidae, Erebididae, and Vespidae, and four spiders, belonging to the families Araneidae and Lycosidae, against 10 strains of bacteria and one strain of fungi. Only one alcohol extract, from *H. axyridis*, exerted no notable antimicrobial activity toward the polyresistant strains of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *Sh. flexneri*, *P. aeruginosa*, *E. faecalis*, *L. monocytogenes*, *S. aureus*, *B. subtilis*, *C. perfringens*, and *C. albicans*. The ethanolic extracts of most invertebrates manifested antimicrobial actions against the polyresistant strains of bacteria and fungi. In particular *P. prasina*, *T. viridissima*, *Oe. pellucens*, and *A. diadematus* were effective against three; *Eu. chrysorrhoea*, *Ch. dorsatus* and *L. singoriensis* exhibited the activity against four, and *P. apterus* effectively inhibited the growth of five of 11 studied strains of bacteria and fungi. The ethanolic extracts of the insects *Eu. chrysorrhoea*, *Ch. dorsatus*, *P. apterus* and the spider *L. singoriensis* are promising for further research oriented at combating polyresistant microorganisms.

The authors declare no conflict of interests.

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