Antimicrobial activity of Indian meal moth silk, *Plodia interpunctella*

Olgica Stefanović*, Filip Vukajlović, Tamara Mladenović, Dragana Predojević, Ljiljana Čomić and Snežana B. Pešić

University of Kragujevac, Faculty of Science, Institute of Biology and Ecology, Radoja Domanovića 12, 34000 Kragujevac, Republic of Serbia

In this study, inhibitory effects of crude silk produced by 5th larval instar of moth *Plodia interpunctella*, as well as ethanol, acetone, ethyl acetate, hexane and dichloromethane silk extracts were evaluated against 18 bacterial strains (ATCC strains/isolates) and 6 fungal ATCC strains *in vitro*. Among the tested extracts, the acetone silk extract had the highest activity against Gram-negative bacteria, while all the extracts showed moderate to high activity against Gram-positive bacteria and fungi. The dilution of 1/2, 1/4 of stock samples showed higher microbial activity. The crude silk was not bioactive. This is the first report on antimicrobial activity of different extracts of silk of *P. interpunctella* larvae. These results open new avenues for future research in using this major storage insect pest in biomedical applications.

Keywords: Antimicrobial activity, bacteria, fungi, insect pests and silken web.

MICROORGANISMS are well accommodated to different environmental stress conditions owing to numerous adaptive mechanisms. The number of resistant or less susceptible bacteria to antimicrobial agents is ever-growing, causing difficulties in the eradication of bacterial infections or control of foodborne pathogenic bacteria[1]. Besides, opportunistic infections caused by pathogen *Candida albicans* or mould infections caused by *Aspergillus* sp. are frequent[2]. Faced with these problems, the search for new antimicrobial agents has been intensified in the last decades and the naturally occurring compounds isolated from bacteria, fungi, lichens, and plants have been intensively studied, resulting in the identification of numerous bioactive compounds[3–6]. However, the chemical compounds produced by insects are still poorly understood and they certainly deserve further studies[7].

Silk is a natural, water-insoluble material constructed from fibres, with an important role in the survival and protection of its animal producers (not only spiders or silkworm)[8–10]. Larvae of different insect species, mostly from orders Hymenoptera, Trichoptera and Lepidoptera, possess the ability to spin silk fibres for building the cocoons during pupation[11,12]. Insect silks are under growing research interest due to special characteristics of constituent fibres, particularly their exceptional strength, elasticity and tenacity, as well as biodegradability[11,13]. Moreover, it is known that insects and their products are being used in folk medicine[14–16]. The silk of silkmoth *Bombyx mori* pupae is perhaps the most studied. The recent studies confirmed its antibacterial properties[17]. The silkworm pupae extract has a very complex chemical composition containing compounds such as insulin-like heterodimeric peptide – bombyxin, omega-3 fatty acids, different amino acids, vitamin B2, etc. and is useful in antioxidant, anti-ageing cosmetic preparations and supplements[18]. Besides, ethanol extract of silkworm pupae in one *in vivo* experiment on mice, injected with the alcohol, increased the activity of alcohol dehydrogenase and increase alcohol detoxification[18]. Modern scientific techniques provided more knowledge on insect silks production, their chemical characteristics, and properties, which led to the use of these materials in tissue engineering, producing of silk hydrogel for the treatment of breast cancer, manufacturing of silk biomaterials for the treatment of the infections, etc.[8].

Indian meal moth, *Plodia interpunctella* (Hübner, 1813) (Lepidoptera: Pyralidae), a notorious worldwide distributed pest in animal and human food storages, produces silk webs. Its larval stages inflict most damage – not only by feeding on different substrates, such as cereal grain, flour, dried fruits and vegetables, spices, tea, nuts, bird food, dry feed, chocolate, etc.[19–21], but also by contaminating the substrate with excrements and exuviae. Additionally, larvae continuously spin a silk net, both inside and on the top of the food surface[22].

While producing the silk by labial glands, *P. interpunctella* larvae incorporate their excreta which consists a variety of chemical compounds, which presents the larval defence system, i.e. possess potential antimicrobial activity and represent the possible source of new antimicrobial compounds for use in biomedicine. The preliminary research conducted on silken web antimicrobial activity in our laboratories gave some confirmative results[23]. Recently, Milutinović *et al.*[24] provide significant information about *P. interpunctella* 5th instar larval silk extracts (with DMSO and Trypsin) as a potential biomaterial usable as a substrate for growing normal human fibroblasts MRC-5. Moreover, tested extracts possess certain anticancer properties against tested HCT-116 colorectal carcinoma cells[24].

The aims of this study were to extract different bioactive compounds of silk of *P. interpunctella* larvae in solvents of different polarity (ethanol, acetone, ethyl acetate, hexane and dichloromethane) and evaluate the antimicrobial effect of these silk extracts as well as the crude silk against 18 bacterial strains (ATCC strains/isolates) and 6 fungal ATCC strains *in vitro*. In this study, the antimicrobial potential of extracts of *P. interpunctella* silk was further tested.

A total of 50 *P. interpunctella* 5th instar larvae were placed in each of 50 dry, sterile, glass Petri dishes (10 cm

*For correspondence. (e-mail: olgica.stefanovic@pmf.kg.ac.rs)

in diameter) for silk production. During the 48 h, larvae freely produced the silken web attaching it to the inner surface of the Petri dishes in laboratory conditions, at 24 ± 3°C, relative humidity 60 ± 10% and 14 : 10 (L : D) photoperiod. After that, larvae were removed from the Petri dishes and substituted by new 50 larvae which continue the silk production. Before the transfer of fresh larvae into Petri dishes, all visible impurities were removed from the silk by fine brush. This procedure was repeated five times to collect high-quality silk for further experiments. Used larvae originated from Central Serbia *P. interpunctella* population which were grown for ~50 generations on standard laboratory diet for *P. interpunctella* in the Laboratory of General and Applied Entomology at Faculty of Science, University of Kragujevac, in thermostat chamber, at 28 ± 1°C, relative humidity 60 ± 10% and 14 : 10 (L : D) photoperiod[25]. The collected silk was extracted and experimentally analysed in Microbiological Laboratory, Faculty of Science, University of Kragujevac, Serbia.

The silk was extracted by maceration. In 100 ml of the each of solvents (ethanol, acetone, ethyl acetate, hexane and dichloromethane), 1 g of shredded silk was added. After 72 h of extraction, the samples (silk + solvent) were concentrated to solvent-free (dry) product, i.e. solvents were removed by vacuum-evaporation at 40°C. The stock samples for experiments were prepared by suspending 150 mg of extracted silk in 3 ml of 10% DMSO. 10% DMSO was prepared into liquid nutritive media. The control set confirmed that 10% of DMSO had no negative impact on the growth of microorganisms. Besides, in experiments, because of serial two-fold dilutions of samples, the 10% DMSO was additionally diluted (≤5% DMSO).

Table 1. Test microorganisms

Bacteria	Reference strains
Staphylococcus aureus	ATCC 25923
Bacillus subtilis	ATCC 6633
Escherichia coli	ATCC 25922
Strains isolated from human material	
Staphylococcus aureus LM48	Pyogenic infection
Staphylococcus aureus LM65	Skin
Enterococcus spp. LM41	Urine
Escherichia coli LM56	Wound
Escherichia coli LM31	Urine
Proteus mirabilis LM46	Pyogenic infection
Klebsiella pneumoniae LM50	Urine
Pseudomonas aeruginosa LM58	Wound
Strains isolated from food	
Escherichia coli O157 LM1	Cheese
Klebsiella oxytoca LM14	Cheese
Proteus spp. LM74	Raw meat
Salmonella enterica LM24	Egg
Salmonella typhimurium LM25	Egg
Strains of environmental origin	
Bacillus subtilis LM54	Air sample
Micrococcus luteus LM55	Air sample
Fungi	
Candida albicans	ATCC 10231
Aspergillus niger	ATCC 16888
Aspergillus flavus	ATCC 9170
Aspergillus fumigatus	ATCC 204305
Penicillium italicum	ATCC 10454
Penicillium chrysogenum	ATCC 24791

Antimicrobial activity was evaluated against 18 bacterial strains (ATCC strains and isolates) and 6 fungal ATCC strains (Table 1). The bacterial isolates were obtained from the Institute of Public Health, Kragujevac, Serbia. The bacterial ATCC strains and isolates were kept in a 20% glycerol/medium stock at –80°C and fungal strains in paraffin oil/medium stock at 4°C for further use. The bacterial strains were subcultured on nutrient agar (Torlak, Serbia) and fungal strains on Sabouraud dextrose agar (Torlak, Serbia), twice, before the experiments.

The inoculums were prepared in sterile physiological saline by the direct colony suspension method[26,27]. The density of the inoculums was determined using a McFarland densitometer (BioSan, Latvia). For standardization of bacterial inoculums and the inoculum of yeast, 0.5 McFarland standard was used which indicated the density of a bacterial suspension of $1–2 \times 10^8$ colony forming units (CFU)/ml and yeast suspension of $1–5 \times 10^6$ cells/ml. The initial inoculums were further diluted in sterile saline to give a bacterial count of 1×10^6 CFU/ml and 1×10^4 cell/ml of yeast. The spore suspensions of filamentous fungi were prepared by collecting spores from the slant agar directly in sterile physiological saline and then diluted at the ratio of 1 : 1000.

Antimicrobial activity of silk extracts was tested using the microdilution method[26,27]. The stock samples were prepared by suspending 150 mg of extracted silk in 3 ml of 10% DMSO. In the 96-well microtiter plates (Sarstedt, Germany), a series of double dilutions of the stock samples in the range from 2^{-1} to 2^{-6} (1/2 to 1/64) were prepared in Mueller–Hinton broth (Torlak, Serbia) for bacteria and Sabouraud dextrose broth (Torlak, Serbia) for fungi. The total volume in the wells was 100 µl. 10 µl of each bacterial and fungal inoculum was inoculated into appropriated wells and incubated at 37°C/24 h for bacteria and yeast and at 28°C/72 h for filamentous fungi. After the incubation, the bacterial growth was detected spectrophotometrically, measuring the absorbance of an inoculated medium at a wavelength of 630 nm using a microtiter plate reader (RT-2100C, Rayto, China). The control wells containing medium alone and medium + extract were included in the analysis to serve as blanks. The growth of filamentous fungi and yeast was visually observed.

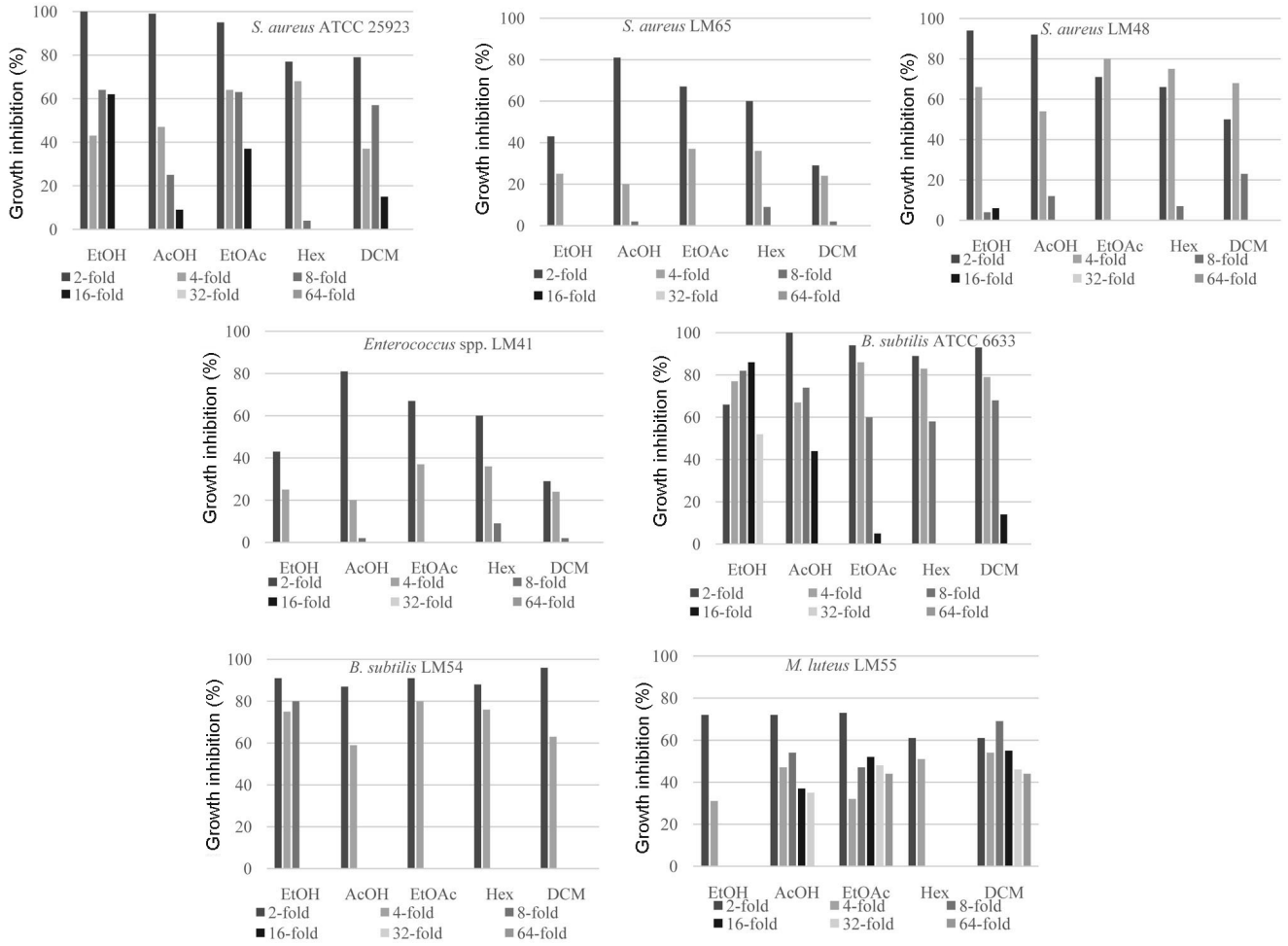

Figure 1. Effect of ethanol (EtOH), acetone (AcOH), ethyl acetate (EtOAc), hexane (Hex) and dichlormethane (DCM) silk extracts on growth of Gram-positive bacteria.

Antibacterial activity was defined as the percentage of growth inhibition and was calculated according to the equation[28]

$$\% \text{ of growth inhibition} = \frac{A_c - A_s}{A_c} \times 100,$$

where A_c is the absorbance of the growth control (bacteria + medium) and A_s is the absorbance of a sample.

Antibacterial activity of crude silk was assayed by mixing 20 mg of silk and 2 ml of liquid nutrient medium, Mueller–Hinton broth (Torlak, Serbia). The medium was inoculated with 100 µl of a given bacterium (1×10^6 CFU/ml). After the incubation at 37°C/24 h, the absorbance of the tested samples was monitored at a wavelength of 630 nm. The inhibitory effect of crude silk on bacterial growth was determined by comparing the sample absorbance data with the absorbance data of growth control.

The results of the antibacterial activity of ethanol, acetone, ethyl acetate, hexane and dichloromethane extracts of silk of *P. interpunctella* larvae against a panel of Gram-negative and Gram-positive bacterial strains are shown in Figures 1 and 2. Serial two-fold dilutions (2^{-1} to 2^{-6}) of a stock sample (150 mg of extracted silk suspended in 3 ml of 10% DMSO) were tested. The activity depended on the type of extract, the tested dilution of stock sample, and bacterial strains. It was expected that using the solvents of different polarities, will result in the isolation of different groups of silk compounds, which will influence the activity of the extract. Among the tested silk extracts, acetone extract exhibited the highest activity, followed by the ethyl acetate, hexane, dichloromethane, and ethanol extract against Gram-negative bacteria. On the other side, the tested silk extracts showed significant activity against Gram-positive bacteria. In general, Gram-positive bacteria were more susceptible to the tested extracts than Gram-negative bacteria.

Strains of *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, and *Micrococcus luteus* LM55 showed the highest sensitivity to the extracts. The growth

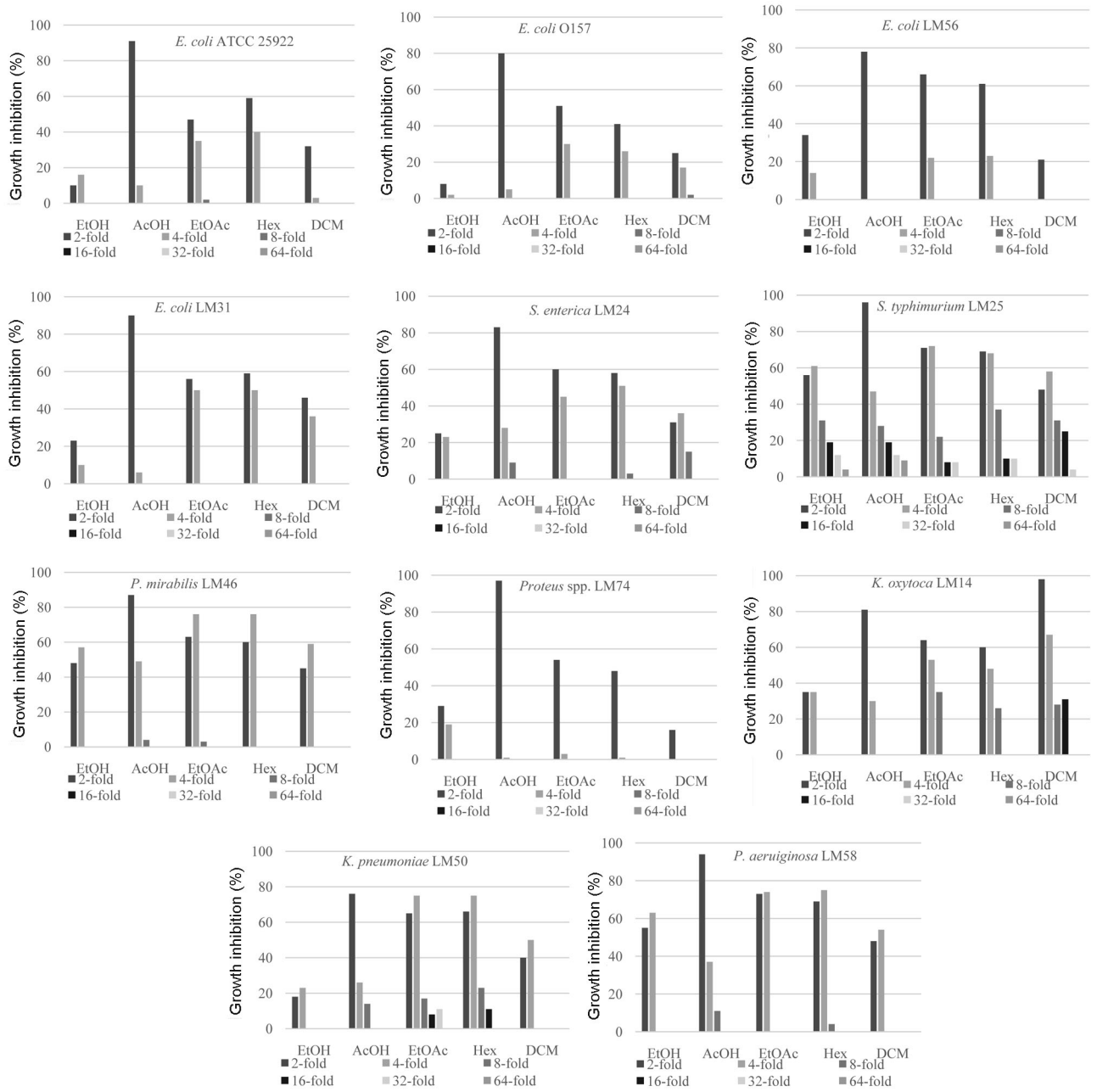

Figure 2. Effect of ethanol (EtOH), acetone (AcOH), ethyl acetate (EtOAc), hexane (Hex) and dichlormethane (DCM) silk extracts on growth of Gram-negative bacteria.

of *S. aureus* ATCC 25923, at 2^{-1} dilution, was 100% inhibited with ethanol extract, 99% with acetone extract, and 95% with ethyl acetate extract (Figure 1). Concerning the mentioned strain, *S. aureus* LM48 and *S. aureus* LM65 showed lower sensitivity to the tested extracts. The highest percentage of growth inhibition was noticed at 2^{-1} dilution of all tested extracts and it was in a range of 50–94% for *S. aureus* LM48 and from 29% to 81% for *S. aureus* LM65 (Figure 1). Growth of *B. subtilis* ATCC 6633 and *B. subtilis* LM54 was inhibited by all tested extracts (Figure 1). Regarding *M. luteus* LM55, acetone, ethyl acetate and dichloromethane extracts were active at all tested dilutions. Against *Enterococcus* spp. LM41, ethanol, acetone, ethyl acetate and dichloromethane extracts acted at a range of 2^{-1}–2^{-3} dilutions and the percentage of inhibition was up to 89%. The greatest activity was exhibited by the acetone extract (Figure 1).

Among the Gram-negative bacteria, the most sensitive were strains of *S. typhimurium* LM25, *K. oxytoca* LM14, and *K. pneumoniae* LM50. The growth of *S. typhimurium* LM25 was, in the highest percentage (96), inhibited by the acetone extract. The other extracts also exhibited significant activity at different concentrations (Figure 2). On the other hand, only 2^{-1} dilution of tested silk extracts

Table 2. Antifungal activity of silk extracts

Species	Ethanol extract	Acetone extract	Ethyl acetate extract	Hexane extract	Dichlormethane extract
Candida albicans ATCC10231	1/2	1/2	1/2	1/2	1/2
Aspergillus niger ATCC 16404	1/2	1/2	1/2	1/2	1/2
Aspergillus flavus ATCC 9170	1/4	1/4	1/4	1/4	1/4
Aspergillus fumigatus ATCC 204305	1/4	1/4	1/4	1/4	1/4
Penicillium italicum ATCC 10454	1/4	1/8	1/4	1/4	1/4
Penicillium chrysogenum ATCC 24791	1/8	1/8	1/8	1/8	1/8

Data present dilutions of tested extract which inhibit the growth.

showed significant activity against *S. enterica* LM24 (Figure 2). *K. oxytoca* LM14 was sensitive to almost all extracts in all tested dilutions. The tested silk extracts exhibited an inhibitory effect in the range of 26–98% (Figure 2). The growth of *K. pneumoniae* LM50 was inhibited by the acetone silk extract (76% of inhibition) (Figure 2). Double and four-fold dilutions of the silk extracts prevented the growth of *P. aeruginosa* LM58, *Proteus* spp. LM74, *P. mirabilis* LM46, four strains of *E. coli* and *E. coli* ATCC 25922 up to 97%. For the other tested dilutions (2^{-3}–2^{-6}), low per cent of growth inhibition (0–11%) was noticed (Figure 2).

The crude silk did not show inhibitory effects on bacterial growth (data not shown).

The results of the antifungal activity of ethanol, acetone, ethyl acetate, hexane and dichloromethane extracts of silk of *P. interpunctella* larvae against 6 tested fungal strains are shown in Table 2. The extracts were active in 2^{-1}–2^{-3} dilution range. The most sensitive species were *P. chrysogenum* ATCC 24791 and *P. italicum* ATCC 10454, moderate sensitivity was shown by *A. flavus* ATCC 9170 and *A. fumigatus* ATCC 204305, while *C. albicans* ATCC 10231 and *A. niger* ATCC 16404 were the most tolerant.

In this study, the antimicrobial (antibacterial and antifungal) activity of *P. interpunctella* silk extracts and crude silk were evaluated. This species is well known as a pest of stored food. Because of its huge economic importance, several research efforts about this insect pest are focused on better understanding its biology and ecology, with the aim to find the possible solution for control, elimination, and reduction of major economic damages caused by it[19–21]. It is known that the larvae form a cocoon of silk which has a protective role during the pupation[22]. In the silk web, the larvae incorporate different chemical compounds that could express different biological activities, including antimicrobial one. According to literature, this is the first report on testing of antimicrobial activity of ethanol, acetone, ethyl acetate, hexane and dichloromethane silk extracts as well as crude silk against a panel of bacterial and fungal strains. This research is a continuation of our earlier, pioneer research conducted a few years ago[23], where *P. interpunctella* acetone silk extract was tested against 20 microorganisms (10 bacterial strains and 10 fungal strains) resulting in different bioactivity. The best results were observed against bacteria of the genus *Bacillus*, followed by *Staphylococcus aureus* ATCC 25923 and a clinical isolate of *S. aureus*, while among fungi, the best results were observed against *Rhodotorula* sp. and *Penicillium chrysogenum*[23].

The inhibitory effect of silk extracts on different human pathogenic bacteria and fungi, as well as contaminants of food, is confirmed. The extracts of silk in five solvents showed different intensity of activity. The acetone extract was the most active, which means that certain bioactive compounds of silk, soluble in acetone, possess antimicrobial properties. The results showed that Gram-positive bacteria were more sensitive than Gram-negative bacteria (compare Figures 1 and 2). This is probably because of the complex structure of the cell wall of Gram-negative bacteria which consists of peptidogly can layer and lipopolysaccharide–phospholipid layer – an outer membrane which can prevent diffusion of chemical compounds[29]. Significantly, the silk extracts exhibited activity against foodborne bacteria – *S. typhimurium* LM25, *S. enterica* LM24, and *K. oxytoca* LM14, as well as against some pathogenic strains *K. pneumoniae* LM50, *P. aeruginosa* LM58, *P. mirabilis* LM46 and *S. aureus* LM48.

The silk extracts inhibited the growth of the tested fungi. The most sensitive were species of the genus *Penicillium* (*P. italicum* and *P. chrysogenum*) – common contaminants of the food, which is similar to results obtained by Vasić *et al.*[23]. In that study, the acetone extract of *P. interpunctella* silk have been tested and it was found that from 10 fungal strains, five strains did not react. *Aspergillus flavus*, *A. niger* ATCC 16404, *Candida albicans* ATCC 10231, *C. albicans* (clinical isolate) and *Saccharomyces boulardii*, did not react, whereas *Rhodotorula* sp. and *Penicillium chrysogenum* expressed very high sensitivity, while *P. italicum*, *Trichoderma viride* and *Botrytis cinerea* expressed moderate sensitivity.

The literature search resulted in not finding similar researches on antimicrobial effects of the *P. interpunctella* silk extracts, making it difficult to discuss the results.

The results obtained in this study open a new approach for the control of pathogenic and foodborne microorganisms and potential application of insect metabolites as antimicrobial agents. The next steps will be oriented to

the chemical determination of active substances and additional biological experiments with *P. interpunctella* silk, so that after collecting as much knowledge as possible, the silk produced by these food pests can be used in the industry of biomaterials, or as protective medicinal material for humans.

The chemical compounds produced by insects are considered to be a promising treasure for new bioactive compounds whose investigation should be of great scientific and practical interest. Different extracts were prepared in order to isolate different silk compounds. The intensities of the antimicrobial activity of the compounds extracted from this silk were varying. Among the 18 tested bacterial and fungal strains, half of them, both pathogenic and foodborne were the most sensitive. The acetone extract was the most promising for further research.

Conflict of interest: The authors declare that they have no conflict of interest.

1. World Health Organization (WHO), Antimicrobial resistance, 2018; https://www.who.int/antimicrobial-resistance/en/
2. Badiee, P. and Hashemiyadeh, Z., Opportunistic invasive fungal infection: diagnosis and clinical management. *Indian J. Med. Res*., 2014, **139**, 195–204.
3. Ullah, H. and Ali, S., Classification of anti-bacterial agents and their functions. In *Antibacterial Agents* (ed. Kumavath, R. N.), IntechOpen, London, 2017, pp. 1–16.
4. Kosanić, M. and Ranković, B., Lichen secondary metabolites as potential antibiotic agents. In *Lichen Secondary Metabolites Bioactive Properties and Pharmaceutical Potential* (ed. Ranković, B.), Springer International Cham Heidelberg, New York, Dordrecht, London, 2015, pp. 81–104.
5. Stefanović, O., Radojević, I., Vasić, S. and Čomić, Lj., Antibacterial activity of naturally occurring compounds from selected plants. In *Antimicrobial Agents* (ed. Bobbarala, V.), IntechOpen, Croatia, 2012, pp. 1–24.
6. Venturini, M. E., Rivera, C. S., Gonzalez, C. and Blanco, D., Antimicrobial activity of extracts of edible wild and cultivated mushrooms against foodborne bacterial strains. *J. Food Protect*., 2008, **71**, 1701–1706.
7. Rahnamaeian, M. *et al.*, Insect antimicrobial peptides show potentiating functional interactions against Gram-negative bacteria. *Proc. Biol. Sci*., 2015, **282**, 1–10.
8. Korayem, A. M. *et al.*, A Drosophila salivary gland mucin is also expressed in immune tissues: evidence for a function in coagulation and the entrapment of bacteria. *Insect Biochem. Mol. Biol*., 2004, **34**, 1297–1304.
9. Korayem, A. *et al.*, Evidence for an immune function of lepidopteran silk proteins. *Biochem. Biophys. Res. Commun*., 2007, **352**, 317–322.
10. Shaik, A. H. and Sehnal, F., Hemolin expression in the silk glands of *Galleria mellonella* in response to bacterial challenge and prior to cell disintegration. *J. Insect Physiol*., 2009, **55**, 781–787.
11. Sutherland, D. T., Young, J. H., Weisman, S., Hayashi, C. Y. and Merritt, D. J., Insect silk: one name, many materials. *Annu. Rev. Entomol*., 2010, **55**, 171–188.
12. Fedič, R., Žurovec, M. and Sehnal, F., The silk of Lepidoptera. *J. Insect Biotechnol. Sericol.*, 2002, **71**, 1–15.
13. Fedič, R., Žurovec, M. and Sehnal, F., Correlation between fibroin amino acid sequence and physical silk properties. *J. Biol. Chem*., 2003, **278**, 35255–35264.
14. Dossey, A. T., Insects and their chemical weaponry: new potential for drug discovery. *Nat. Prod. Rep*., 2010, **27**, 1737–1757.
15. Cherniack, P. E., Bugs as drugs, Part 1: Insects. The 'new' alternative medicine for the 21st century? *Altern. Med. Rev*., 2010, **15**, 124–135.
16. Ratcliffe, N., Azambuja, P. and Brasileiro, M. C., Recent advances in developing insect natural products as potential modern day medicines. *Evid-Based Compl. Alt. Med*., 2014, p. 21; Article ID 904958.
17. Nwekwo, W. C., Antimicrobial activity of silk fibroin microparticles. Ph D thesis, Graduate School of Applied Sciences, Near East University, Nicosia, 2015.
18. Kwon, M. G. *et al.*, Isolation and analysis of natural compounds from silkworm pupae and effects of its extracts on alcohol detoxification. *Entomol. Res*., 2012, **42**, 55–62.
19. Predojević, D. Z., Vukajlović, F. N., Tanasković, S. T., Gvozdenac, S. M. and Pešić, S. B., Influence of maize kernel state and type on life history of *Plodia interpunctella* (Lepidoptera: Pyralidae). *J. Stored Prod. Res*., 2017, **72**, 121–127.
20. Gvozdenac, S. M. *et al.*, Life history of *Plodia interpunctella* Hübner on sunflower seeds: Effects of seed qualitative traits and the initial seed damage. *J. Stored Prod. Res*., 2018, **79**, 89–97.
21. Vukajlović, F. N. *et al.*, Life history of *Plodia interpunctella* (Lepidoptera: Pyralidae) on dried fruits and nuts: effects of macronutrients and secondary metabolites on immature stages. *J. Stored Prod. Res*., 2019, **83**, 243–253.
22. Mohandass, S., Arthur, F. H., Zhu, K. Y. and Throne, J. E., Biology and management of *Plodia interpunctella* (Lepidoptera: Pyralidae) in stored products. *J. Stored Prod. Res*., 2007, **43**, 302–311.
23. Vasić, S., Vukajlović, F., Radojević, I., Stefanović, O., Pešić, S. and Čomić, Lj., Antimicrobial effects of a silken web produced by the larvae of *Plodia interpunctella*. International conference on antimicrobial research, Book of abstracts, Madrid, Spain, 2014.
24. Milutinović, M., Čurović, D., Nikodijević, D., Vukajlović, F., Predojević, D., Marković, S. and Pešić, S., The silk of *Plodia interpunctella* as a potential biomaterial and its cytotoxic effect on cancer cells. *Anim. Biotechnol.*, 2019; doi:10.1080/10495398.2019.1575848.
25. Silhacek, D. L. and Miller, G. L., Growth and development of the Indian meal moth, *Plodia interpunctella* (Lepidoptera: Phycitidae) under laboratory mass-rearing conditions. *Ann. Entomol. Soc. Am.*, 1972, **65**, 1084–1087.
26. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Ninth Edition. CLSI document M07-A9, Wayne, Pennsylvania, USA, 2012.
27. National Committee for Clinical Laboratory Standards (NCCLS). Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard – Second Edition. NCCLS document M27-A2. NCCLS, Wayne, Pennsylvania, USA, 2002.
28. Wang, J., Liu, H., Zhao, J., Gao, H., Zhou, L., Liu, Z., Chen, Y. and Sui, P., Antimicrobial and antioxidant activities of the root bark essential oil of *Periploca sepium* and its main component 2-hydroxy-4-methoxybenzaldehyde. *Molecules*, 2010, **15**, 5807–5817.
29. Nikaido, H., Molecular basis of bacterial outer membrane permeability revisited. *Microbiol. Mol. Biol. Rev*., 2003, **67**, 593–656.

ACKNOWLEDGEMENT. This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant Numbers 41010 and 173038.

Received 29 September 2019; revised accepted 3 January 2020

doi: 10.18520/cs/v118/i10/1609-1614