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J. Serb. Chem. Soc. *73* (11) 1039–1049 (2008) UDC **Ambrosia artemisiifolia*:638.138:615.28–188
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Chemical composition and biological activity of the acetone extract of *Ambrosia artemisiifolia* L. pollen

SLAVICA SOLUJIĆ[1]*, SLOBODAN SUKDOLAK[1], NENAD VUKOVIĆ[1], NEDA NIĆIFOROVIĆ[1] and SNEŽANA STANIĆ[2]

[1]*Department of Chemistry, Faculty of Science, University of Kragujevac, P.O. Box 12, 34000 Kragujevac and* [2]*Department of Biology, Faculty of Science, University of Kragujevac, P.O. Box 12, 34000 Kragujevac, Serbia*

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Abstract: In this study, the chemical components, antimicrobial and genotoxic biological activities of the acetone extract of *Ambrosia artemisiifolia* L. pollen were examined. Two lactones were identified: ambrosin and artesovin. The antimicrobial activity of the acetone extract of *A. artemisiifolia* L. pollen was examined on ten different bacterial species using the disc diffusion method and the microdilution method in Mueller-Hinton broth dilution. The minimal inhibitory concentration of the acetone extract of *A. artemisiifolia* pollen varied between 1.25−6.50 mg mL^{-1}. The genotoxic effect of the acetone extract of *A. artemisiifolia* pollen on a eukaryotic model system *Drosophila melanogaster* was investigated using the SLRL test.

Keywords: *Ambrosia artemisiifolia* L.; pollen; extract; antimicrobial activity; genotoxicity.

INTRODUCTION

The *Ambrosia* (Asteraceae) genus is classified as belonging to the Heliantheae tribe. There are about 20 species of this genus in Europe and the ambrosia species is the most widespread one.[1] Ambrosia is conquering Europe with enormous speed due to the ability of its pollen to travel extremely fast, up to 300 km h^{-1}, if the wind is favourable.[2–5] Various measurements and testing have shown that the pollen concentrations in the European air have increased by up to 5 times in the last 10 years.

The *Ambrosia artemisiifolia* L. plant is an invasive, allergenic plant which produces large amounts of pollen.[6] The human immune system responds to the antigen present in the pollen through the so-called polinosis process.[7,8] During research of the *A. artemisiifolia* L. plant, the sesquiterpene lactones ambrosin, isa-

* Corresponding author. E-mail: ssolujic@kg.ac.yu
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belin, psilostachyn,[9,10] cumanin and peruvin,[11,12] as well as triterpenoids of the α- and β-amyrine type and derivatives of caffeic acid [6,13] were identified.

The sesquiterpene lactones are characterized by the presence of α,β-unsaturated γ-lactone moiety[14] and possess antibacterial, antifungal, antiprotozoal, anthelmintic, analgesic, schistosomicidal, genotoxic and mutagenic activities.[15–18]

In addition, various studies have suggested a presence of both genotoxic and mutagenic activities[19–21] of the sesquiterpene lactones. Genotoxic substances can perform deleterious actions on the genetic material of cells, thus affecting their integrity, and may, therefore, be potentially mutagenic or carcinogenic,[22–25] specifically those capable of causing genetic mutations and contributing to the development of tumours.[26–28] The sesquiterpene lactones go through a cellular metabolic transformation thereby acquiring the ability to damage DNA.[20,29–33] They operate by inhibiting certain enzymes responsible for maintaining the integrity of cells and, consequently, the integrity of an organism. The sesquiterpene lactones can react with the nucleophilic centres of intracellular macromolecules.[20] Such a reaction occurs with the thiol group of glutathione,[17] which is an important intracellular compound participating in the inactivation of chemical substances and it may be efficient in protecting cells and macromolecules such as DNA.[18,33]

Some of the above-mentioned examined lactones exhibit antimicrobial properties. Lactones exhibit bacteriostatic properties by inhibiting bacterial growth, or bactericidal, killing the bacteria. The critical attack site of anti-cell wall agents lies in the peptidoglycan layer. This layer is essential for the survival of bacteria in hypotonic environments. The loss or damage of this layer destroys the rigidity of the bacterial cell wall, resulting in death.[34–36]

This paper presents the results of the chemical isolation and identification of two lactones from the acetone extract of the pollen and some biological effects of this extract, *i.e.*, its antibacterial activity against some pathogenic bacteria and its genotoxic effect on a eukaryotic model system *Drosophila melanogaster* using the SLRL test.

EXPERIMENTAL

The melting points (m.p.) were recorded on a Kofler hot stage apparatus and are uncorrected. Microanalysis for carbon and hydrogen was performed using a Carlo Erba 1106 microanalyser. The IR spectra were run on Perkin-Elmer Grating Spectrophotometers, models 137 and 337. The NMR spectra were recorded on a Varian Gemini 200 spectrometer (^{1}H at 200 MHz and ^{13}C at 50 MHz) in $CDCl_3$, using TMS as the internal standard. The extract was monitored and separated by thin-layer chromatography (TLC) on MN-silica gel P/UV_{254} with $CaSO_4$.

Extraction of Ambrosia artemisiifolia *pollen*

Ambrosia artemisiifolia L. plants were collected from the region of Kragujevac, in central Serbia. The biomass was freeze-dried on the same day as it was collected. A voucher specimen of the plant (BEOU No. 16171) was deposited in the Herbarium of the Department of Biology of the Faculty of Science, University of Belgrade, Serbia. The pollen was separated from the plant leaves.

The pollen was first broken into small pieces using a cylindered crusher. Then, pollen pieces (60 g) were extracted with acetone (500 mL) using a Soxhlet apparatus.[37] The extract was filtered through a paper filter (Whatman, No. 1) and evaporated on a rotary evaporator. The crude acetone extract (1645 mg) of the pollen was washed with petroleum ether (70−90 °C) at room temperature during a 24 h period. The secondary petroleum ether extract (l430 mg) contained palmitic acid wax. The residue was dissolved in 50 mL of a 0.20 % solution of lead acetate in a 1:1 mixture of ethanol and water. After stirring over night, the solution was filtered, evaporated to a quarter of its volume, extracted with chloroform, dried over anhydrous sodium sulphate, evaporated and dissolved in acetone. The primary acetone extract pollen (820 mg) was stored in a dark glass bottle for further processing. The components were separated by preparative chromatography on MN-silica gel P/UV_{254} with $CaSO_4$ and a 6:4 mixture of benzene and ethyl acetate. The silica gel layer was extracted with ethyl acetate. The extracts were filtered and evaporated and the residues analysed spectroscopically. Two lactones were identified. The first was ambrosin (**1**), light white crystals with a m.p. 146–148 °C (ethanol) and R_f = 0.36 (benzene:ethyl acetate = 6:4). The second lactone was dihydroambrosin (**2**), white crystals with a m.p. 165–167 °C (diethyl ether) and R_f = 0.50 (benzene:ethyl acetate = 6:4).

Test microorganism

The bacterial strains used in these experiments were *Bacillus mycoides* (IPH), *Pseudomonas fluorescens* (B28), *Erwinia carotovora* (B31), *Enterobacter cloacae* (B22), *Klebsiella pneumoniae* (B26), *Agrobacterium tumefaciens* (B11), *Azotobacter chroococcum* (B14), *Staphylococcus aureus* (IHP), *Proteus* sp. (IHB) and *Pseudomonas aeruginosa* (IPH).

All of the tested bacteria cultures were obtained from the Institute for Health Protection (IPH) in Kragujevac and the Faculty of Agriculture, University of Belgrade, Serbia. The identity of the bacterial strains was confirmed in Laboratory for Microbiology at the Department of Biology, Faculty of Science, University of Kragujevac, Kragujevac, Serbia.

Antibacterial activity of the pollen acetone extract

The antibacterial activity of the acetone extract of the pollen was examined using the nutrient agar broth disc diffusion method and the microdilution method in Mueller-Hinton broth.[38,39] The disc diffusion method was performed using a 24 h culture which was reseeded on the nutrient broth at a temperature of 37 °C. The concentrations of the cultures were adjusted to 5.6×10^6 CFU mL^{-1} with sterile water. One mL of suspension was added over the plates containing nutrient agar broth in order to achieve a uniform microbial growth on both the control and test plates. The acetone extract of pollen was dissolved in 96 % ethanol (100 mg mL^{-1}) and sterilized. Under aseptic conditions, empty sterilized discs (Whatman No. 5, 14 mm diameter) were impregnated with 250, 100 and 50 μL of different concentrations (10 mg/disc and 5.0 mg/disc) of the respective extract, and placed on the agar surface. The plates were left for 30 min at room temperature to allow diffusion of the extract and then incubated at 37 °C.

After the incubation period (48 h), the zone of inhibition was measured and is presented in mm. A paper disc of the solvent (ethanol) was used as the control and Sinacilin® was used as a standard antibiotic for comparison. Each test was performed in triplicate and repeated three times.

The minimal inhibitory concentration (*MIC*) of the acetone extract was determined by the microdilution two-fold serial technique. A series of two-fold dilutions of the extract, ranging from 0.10 to 10 mg mL^{-1}, was prepared in Mueller-Hinton broth with the addition 0.10 mL of a suspension of the bacterial spores (5.4×10^6 CFU mL^{-1}). The results were determined after

24 h and the *MIC* values were determined as the lowest concentration of the extract which inhibited visible growth of each organism. Sinacilin (1.0 mg mL^{-1}) was chosen as the control drug.

Genotoxic activity of the pollen acetone extract

The sex-linked recessive lethal test for mutagenicity (or SLRL test) was performed with laboratory stocks of *Drosophila melanogaster* (obtained from the Umea Stock Centre, Sweden). The examined "Canton-S" types were individuals with a normal phenotype ("wild" type), while "Basc" line flies are characterized with individuals homozygous for a balancer X-chromosome, which carries two genetic markers: Bar (B) which produces a narrow shaped eye under homo- and hemizygous conditions and a kidney shaped eye when heterozygous in females. An eye restricted to a narrow vertical bar of 80 facets appears in males and 70 facets in homozygous females. Heterozygous females have an intermediate number of facets (360) compared to the homozygous females (70) and wild type (780).[40]

The character can be regarded as partially dominant; white-apricot (wa) – changes the red eye color into a light orange and is expressed only in homozygous females and hemizygous males (while in heterozygotes females are not expressed); scute (sc) – a recessive mutation that reduces the number of thoracic bristles. This mutation is linked with a long inversion on the X-chromosome, which is necessary for the suppression of crossing-over, which could change the existing gene combinations on the treated chromosome.[41-43]

Three-day old Canton-S males (N = 30) were starved in empty bottles for 5 h prior to treatment and then transferred and fed in bottles with a filter paper soaked with a 5.0 % solution of the acetone extract of *A. artemisiifolia* for 24 h. After another 24 h of recovery on standard medium, each male was mated individually to three Basc females in 30 bottles, which made brood I. After two days, the males were transferred to new vials with three Basc line virgins (brood II) and after three days, the males were transferred again into fresh vials with three Basc virgins (brood III). These males stayed with the females for three days and were then removed. The females were left for five days to lay eggs, and then they were removed. The solvent 1.0 % sucrose served as the negative control.

After F_1 emerged, brother–sister mating was allowed for several days and 10 females from each vial were placed individually into new vials. Each vial would give the progeny of one treated X-chromosome.

In F_2, the phenotypes were scored according to eye colour and shape. The absence of wild type males indicated the presence of a recessive lethal induced by the test substance.

The stocks were maintained and all experiments were performed under optimal conditions (t = 25 °C, relative humidity = 60 %, 12/12 h light/dark regime) on a standard nutritive medium for *Drosophila* (corn flour, yeast, agar, sugar and nipagin to prevent mould and infection).

The total number of treated X-chromosomes is equal to the sum of the lethal and non-lethal cultures and the frequency of sex-linked recessive lethal was calculated from the ratio of the number of lethal to the total number of treated X-chromosomes. The significance of the difference in the percentage of lethals was tested using the test for big independent samples (testing of difference between proportions).[44,45]

RESULTS AND DISCUSSION

Many different kinds of metabolites, including sesquiterpene lactones, phenolics, coumarins and flavonoids have been identified from *A. artemisiifolia* L.[9,11,13,15] In the above-ground parts of *A. artemisiifolia* L. species, many struc-

turally different sesquiterpene lactones were identified, among them coronopilin, dihydropartenolide, psilostachyin, cumanin, peruvin, artemisiifolin, isabelin, ambrosin and cumamin.[11]

The following lactones were identified in the methanol extract of common ragweed, *A. artemisiifolia*: the sesquiterpene lactones psilostachyins A, B and C, paulitin and isopaulitin. Psylostachyins A and C block cells in mitosis, which act as novel checkpoint inhibitors of G2/DNA damage.[46] It was suggested that these compounds can easily bind covalently to target proteins.[47]

On the basis the present experimental results, it was concluded that the ambrosia pollen acetone extract contained two lactones, ambrosin and artesovin in the ratio of 3:1 (Fig. 1). The structures of the sesquiterpene lactones ambrosin and artesovin were assigned on the basis of UV–Vis, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data.

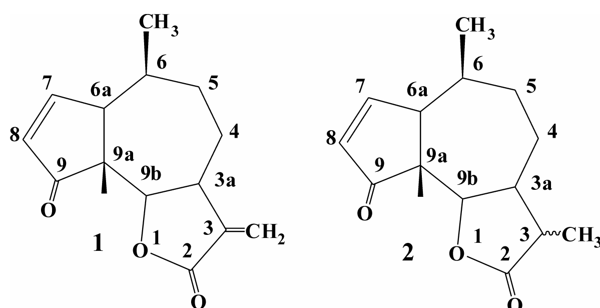

Fig. 1. Chemical structure of ambrosin (**1**) and artesovin (**2**).

*Ambrosin (6,9a-dimethyl-3-methylene-3,3a,4,5,6,6a-hexahydroazuleno[4,5-*b*]-furan-2,9(9a*H*,9b*H*)-dione) (**1**)*. Light white crystals; m.p. 146−148 °C (ethanol); R_f = 0.36 (6:4 benzene:ethyl acetate); IR (KBr, cm^{-1}): 1755 (γ-lactone), 1710, 1660 (enone (lactone)), 1605 (enone (cyclpentenone)), 1420, 1378, 1140; ^{1}H-NMR (200 MHz, $CDCl_3$, δ, ppm): 1.01 (3H, *d*, CH_3–C_6, ^3J = 6.8 Hz), 1.12−1.48 (4H, *m*, C_4, C_5), 1.33 (3H, *s*, CH_3–C_{9a}), 1.71 (1H, *m*, C_6), 2.39 (1H, *t*, C_{6a}, $^3J_{6a,6}$ = 5.9 Hz, $^3J_{6a,7}$ = 5.8 Hz), 2.69 (1H, *bq*, C_{3a}, $^3J_{3a,9b}$ = 6.7 Hz, $^3J_{3a,4}$ = 6.2 Hz, $^3J_{3a,4}$ = 6.1 Hz), 4.21 (1H, *d*, C_{9b}, $^3J_{3a,9b}$ = 6.7 Hz), 5.65 (1H, *d*, CH_2 methylene, J = 1.3 Hz), 6.20 (1H, *d*, C_8, $^3J_{7,8}$ = 6.3 Hz), 6.25 (1H, *d*, CH_2 methylene, J = 1.3 Hz), 7.02 (1H, *dd*, C_7, $^3J_{7,8}$ = 6.3 Hz, $^3J_{6a,7}$ = 5.8 Hz); ^{13}C-NMR (50 MHz, $CDCl_3$, δ, ppm): 169.97 (C_2), 138.7 (C_3), 43.43 (C_{3a}), 24.8 (C_4), 31.15 (C_5), 33.9 (C_6), 49.56 (C_{6a}), 169.87 (C_7), 128.1(C_8), 211.8 (C_9), 53.47 (C_{9a}), 79.65 (C_{9b}), 18.79 (CH_3 at C_6), 17.6 (CH_3 at C_{9a}), 120.05 (CH_2 methylene); MS (*m/z*): 93, 125, 145, 166, 189, 204, 231, M$^+$ 246.1256; UV–Vis (EtOH) (λ_{max}, nm (ε, L mol^{-1} cm^{-1})): 217 (17100) (α,β-unsaturated ketone and α,β-unsaturated γ-lactone) and 343 (35) (carbonyl).

*Artesovin (3,6,9a-trimethyl-3,3a,4,5,6,6a-hexahydroazuleno[4,5-*b*]furan--2,9(9a*H*,9b*H*)- dione) (**2**)*. White crystals; m.p. 165–167 °C (diethyl ether), R_f = 0.50 (benzene:ethyl acetate = 6:4); IR (KBr, cm^{-1}): 1775 (γ-lactone), 1707, 1615 (eno-

ne (cyclpentenone)), 1420, 1370, 1140; ^{1}H-NMR (200 MHz, $CDCl_3$, δ, ppm): 1.01 (3H, *d*, CH_3–C_6, ^3J = 6.7 Hz), 1.12–1.48 (4H, *m*, C_4,C_5), 1.21 (3H, *d*, CH_3–C_6, ^3J = 7.7 Hz), 1.34 (3H, *s*, CH_3–C9a), 1.68 (1H, *m*, C_6), 2.47 (1H, *t*, C_{6a}, $^3J_{6a,6}$ = 7.9 Hz, $^3J_{6a,7}$ = 5.5 Hz), 2.61 (1H, *dq*, C_3, $^3J_{3,Me}$ = 7.7 Hz, $^3J_{3a,3}$ = 9.5 Hz), 2.81 (1H, *m*, C_{3a}, $^3J_{3a,9b}$ = 10.4 Hz, $^3J_{3a,3}$ = 9.5 Hz), 4.61 (1H, *d*, C_{9b}, $^3J_{3a,9b}$ = = 10.4 Hz), 6.07 (1H, *d*, C_8, $^3J_{7,8}$ = 5.9 Hz), 7.1 (1H, *dd*, C_7, $^3J_{7,8}$ = 5.9 Hz, $^3J_{6a,7}$ = 5.5 Hz); ^{13}C-NMR (50 MHz, $CDCl_3$, δ, ppm): 178.3 (C_2), 41.4 (C_3), 45.4 (C_{3a}), 26.7 (C_4), 30.1 (C_5), 35.2 (C_6), 51.6 (C_{6a}), 161.7 (C_7), 129.2(C_8), 209.8 (C_9), 54.7(C_{9a}), 85.6 (C_{9b}), 19.9 (CH_3 at C_6), 20.6 (CH_3 at C_{9a}), 12.9 (CH_3 at C_3); MS (*m/z*): 125, 145, 166, 189, 203, 218, 233, M^+ 248.1412; UV–Vis (EtOH) (λ_{max}, nm (ε, L mol^{-1} cm^{-1})): 212 (15100) (α,β-unsaturated ketone) and 347 (20) (carbonyl).

The presence of these lactones is in accordance with the presence of ambrosic acid,[15] which acts as a precursor in intra molecular cyclization to form ambrosin and ambrosin-like lactones.[48]

The bacterial strains used in the present study demonstrated the commercial potential of the pollen as an active allelochemical[36] and antimicrobial mixture for some human and phytopatogenic bacteria.[6]

The results of the antibacterial activity (disc diffusion and microdilution methods) of the primary acetone extract of the pollen of *A. artemisiifolia* L. on some bacteria are presented in Table I.

TABLE I. Antibacterial activity of the acetone extract of *A. artemisiifolia* pollen (*Pseudomonas fluorescens*, 0.063 mg ml^{-1}, *Bacillus mycoides*, 0.039 mg ml^{-1})

Microorganism	Zones of inhibition, mm[a,b,c]					*MIC*[e] mg mL^{-1}
	Concentration of extract				Standard[d]	
	5 mg	10 mg	5 mg	10 mg	10 mg	
	24 h		48 h		24 h	
B. mycoides	18.0±1	23.0±0.5	14.5±0.5	17.0±1	22.0±0.5	3.50
P. fluorescens	17.0±1	21.0±1	0	17.0±1	13.2±0.7	1.25
A. tumefaciens	19.0±0.5	24.0±1	18.0±0.5	23.0±0.5	15.0±0.5	2.50
E. carotovora	17.0±0.5	23.0±0.5	0	21.0±0.5	14.1±0.5	2.25
E. cloacae	19.0±1	26.0±0.5	0	24±1	10.6±0.5	2.50
A. chroococcum	18.0±1	23.0±0.3	0	22±1	10.6±0.5	2.50
K. pneumoniae	20.0±0.5	22.0±0.5	17±1	18±1	12.5±0.5	6.50
S. aureus	21.0±0.3	23.0±0.5	22.0±0.5	23±0.5	12.5±0.5	4.75
Proteus sp.	0	0	0	0	36.8±0.7	0
P. aeruginosa	0	0	0	0	0	0

[a]Mean value±*SD*, *N* = 3; [b]"0" absence of antimicrobial activity; [c]solvent control acetone was negative; [d]sinacilin, 10 mg/disc; [e]*MIC* standard: sinacilin, 1.0 mg mL^{-1}

These results show that the acetone extract has a high antibacterial activity against all ten investigated bacteria. All the cultures had in common the fact that the investigated concentrations had an inhibitory effect on the development of a

large number of bacteria. The 10 mg concentration exhibited a high degree of inhibition over the 24 h development period. The inhibition level varied from 21.0 to 26.0 mm. The extract demonstrated the highest inhibition of the growth of *Enterobacter cloacae*, with an inhibition zone of 26.0 mm, while the inhibition zone with the other bacteria ranged from 21.0 to 24.0 mm.[37]

In the 48 h-cultures, a slight decrease in the inhibition was registered, except for the *Staphylococcus aureus* culture.[49] There were two significant differences with this bacterium. The first one is that the inhibition ability of the extract was very similar for the samples of 5 and 10 mg of extract per disc and it varied from 21.0 to 23.0 mm. The second difference is that the level of inhibition remained the same as that after 24 h of development.[50,51] For most of the examined bacteria, after a time period of 48 h with 10 mg of extract, a slight decrease of the effect was observed, while no bacteriostatic effect was registered after 48 h with 5 mg of extract in the case of the *Pseudomonas fluorescens*, *Erwinia carotovora*, *E. cloacae* and *Azotobacter chroococcum* cultures. In general, this class of bacteria was more resistant.

Such a resistance could be due to the permeability barrier provided by the cell wall or the membrane accumulation mechanism.[34,35] Compared to the standard ability of sinacilin, with 10 mg per disc, all of the examined bacteria experienced an inhibitory growth in the presence of the acetone extract of 5−12 mm, *i.e.*, the bacterial growth was 1−1.5 times slower than the one in the presence of the antibiotic.

The examined concentration of the acetone extract did not demonstrate an inhibition effect on the growth of *Proteus* sp. and *Pseudomonas aeruginosa*. The absence of susceptibility of these bacteria to the pollen extract was not entirely unexpected, and it is based both on their anatomical and biochemical characteristics.[34]

At a concentration of 5 mg extract per disc during a 24-hour incubation, the extract was bacteriostatic towards eight of the ten examined bacteria, which can be the result of the inhibition process of the synthesized cell wall. At a concentration of 10 mg extract, the effect was bactericidal and is contingent to the inhibition of the bacterial metabolism, with the most prominent inhibition recorded on the ribosome protein synthesis.[34,35] The minimal inhibitory concentration of the acetone extract of *A. artemisiifolia* pollen varied between 1.25−6.50 mg mL^{-1}.

Previous literature geared us towards the fact that the existing pollen proteins are responsible for their allergenic activity,[52] although there is no proof whether the lactones present actually contribute to such a reaction using the existing proteins as their carriers.[53]

Using a short test for the detection of mutagenicity in *Drosophila melanogaster in vivo* conditions, it was found that the examined plant extract had mutagenic properties.[54] The results are given in Table II.

TABLE II. Frequencies of SLRL mutations after treatment of *D. melanogaster* males with the acetone extract of *A. artemisiifolia* L. pollen

	Sucrose (negative control)	*Ambrosia* (test group)	$t_{s/a}$
I brood ∑	300	244	1.65
No. of lethals	5	10	$p > 0.05$
% of lethals	1.67	4.10	
II brood ∑	269	204	4.40
No. of lethals	5	26	$p < 0.001$[a]
% of lethals	1.86	12.74	
III brood ∑	252	236	2.97
No. of lethals	6	20	$p < 0.01$
% of lethals	2.38	8.47	
I+II+III ∑	821	684	5.41
No. of lethals	16	56	$p < 0.001$
% of lethals	1.95	8.19	

[a]Statistically significant difference

At the 5 % concentration level, it induced sex-linked recessive lethal mutations on the X-chromosome of *Drosophila melanogaster* males, which were treated acutely with this extract (broods II and III). The frequency of the germinative mutations induced by the pollen components was significantly higher than the frequency of mutations induced by sucrose (negative control). The obtained results show that the spermatic cell line (brood II) was especially sensitive to the influence of the examined extract.

The results of the experiments showed that the tested extract induced recessive, lethal X-linked mutations in postmeiotic germinative cell lines – spermatids and premeiotic line – spermatocytes, while the spermatozoids were more resistant to the genotoxic effects of the examined agent.

Since the established statistically significant difference in the increase in the frequency of sex-related lethals in the tested group of *D. melanogaster* males, compared to the negative control, represents a positive result, it was concluded that a chemical component in the ambrosia pollen induced the mutations in male germinative cells of this eukaryotic species. The statistically significant differentces in the II and III broods, shown in Table II, confirm the same sensitivity of germinative cells of the premeiotic (diploid) and postmeiotic stage (haploidic spermatids).

The experimentally proven genotoxicity of the ambrosia pollen extract demands further examination, *i.e.*, determination of the chemical structure of the pollen agent which is capable of inducing hereditary genetic changes in this *in vivo* system.

CONCLUSIONS

Two lactones, ambrosin and artesovin (in the ratio of 3:1, respecttively), were identified in the acetone extract of *Ambrosia artemisiifolia* pollen.

At a 5.0 mg concentration, the acetone extract of ambrosia pollen was bacteriostatic for most of the examined bacteria during a 24-h development period, while at the 10 mg concentration, the acetone extract of ambrosia pollen was bactericidal for eight out of the ten examined bacteria. The *MIC* of the acetone extract of *A. artemisiifolia* pollen varied between 1.25−6.50 mg mL^{-1}.

The lactones mixture (3:1) of ambrosin and artesovin induced recessive lethal mutations on the X-chromosome of *Drosophilia melanogaster* in the II and III broods. As a result, it was concluded that spermatides and spermatocytes were the more sensitive stages of spermatogenesis than the others were.

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ИЗВОД

ХЕМИЈСКИ САСТАВ И БИОЛОШКА АКТИВНОСТ АЦЕТОНСКОГ ЕКСТРАКТА ПОЛЕНА *Ambrosia artemisiifolia* L.

СЛАВИЦА СОЛУЈИЋ[1], СЛОБОДАН СУКДОЛАК[1], НЕНАД ВУКОВИЋ[1], НЕДА НИЋИФОРОВИЋ[1] и СНЕЖАНА СТАНИЋ[2]

[1]*Инсшишуш за хемију и* [2]*Инсшишуш за биологију, Природно–машемашички фкулшеш, й йр. 12, 34000 Крагујевац*

У овом раду испитан је хемијски састав ацетонског екстракта полена *Ambrosia artemisiifolia* и његова антимикробна и генотоксична биолошка активност. Идентификована су два лактона: амброзин и артезовин. Антимикробна активност ацетонског екстракта полена *A. artemisiifolia* одређена је применом дифузионе и микродилуциона метода у Mueller-Hinton храњљивом агару. Различите количине екстракта су нанете на дискове (5 и 10 mg) и испитане на 10 различитих бактерија. Минимална инхибиторна концентрација је у опсегу од 1,25–6,50 mg mL^{-1}. Генотоксични ефекат ацетонског екстракта полена *A. artemisiifolia* испитан је применом SLRL теста на еукариотском модел систему *Drosophilia melanogaster.*

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