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Preliminary results on biological activity of a pollen extract of *Ambrosia artemisiifolia* L.

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This paper examines chemical components of the *Ambrosia artemisiifolia* L. plant acetone extract, and its two identified lactones: the ambrosin and dihydroambrosin lactones. In the examination, the *A. artemisiifolia* L. acetone extract was tested for its antimicrobial and genotoxic biological activities. A disc diffusion method was used to investigate and test out the antimicrobial activity of the plant extract by applying different quantities of the extract (5 mg and 10 mg per disk, respectively) to 10 different bacterial species. Extracts of five and 10 mg concentrations demonstrated inhibition zones ranging from 21.0 to 24.0 mm against eight investigated bacteria. The genotoxicity of the extract was tested on the eukaryotic model system *Drosophila melanogaster* using the Sex-Linked Recessive Lethal test (SLRL). The results presented here illustrate that tested lactones, more specifically ambrosin and dihydroambrosin lactones do induce recessive lethal mutations on the X-chromosome of *D. melanogaster* in II and III broods, based on which we may conclude that spermatides and spermatocytes stages fall into and represent a more sensitive stage of spermatogenesis.

Key words: *Ambrosia artemisiifolia*, extract, antimicrobial activity, genotoxicity.

INTRODUCTION

The *Ambrosia* (Asteraceae) genus is classified as a part of the Heliantheae tribe. The *Ambrosia artemisiifolia* L. plant is an invasive allergenic plant which produces large amounts of pollen (Wang *et al*., 2006). Human immune system responds to the antigen agent present in the plant through a process of so-called polinosis (Brunet *et al*., 1992a, b). The allergic reaction is thus produced and accompanied by increased production of IgE (Ruffin *et al*., 1990; Bond *et al*., 1991). People with an increased risk of allergic diseases or atopics were found to posses an inherited propensity for creation of IgE antibodies (Hedlin *et al*., 1989; Rafnar *et al*., 1991).

The studied plant, which originates from North America, can be found growing wild in many different areas (i.e. near roads, close by railway tracks, on banks of rivers and streams, in neglected gardens, etc). It is often rooted on corn, sugar beet and many other vegetable plants. Although there are about 20 species of this genus in Europe, the *Ambrosia* species is the most widespread one (Gajić, 1975). *Ambrosia* is conquering Europe with an enormous speed due to its pollen ability to travel extremely fast, as fast as 300 km h^{-1} if the wind is favourable (Jones, 1953; Comtois, 1998; Deen *et al*., 1998; Genton *et al*., 2005). Various measurements and testing showed that the pollen concentrations in the European air have increased 5 times in the last 10 years.

The sesquiterpene lactones ambrosin, isabelin, psilostachyn (Bloszyk *et al.*, 1992; Rugutt *et al.*, 2001), cumanin and peruvin (David *et al*., 1999; Parkhomenko *et al.*, 2005), triterpenoids of type α- and β-amyrine and derivatives of caffeic acid have all been identified in the *Ambrosia artemisiifolia* L. plant (Tamura *et al*., 2004; Wang *et al.*, 2006). The allergenic factor

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of *Ambrosia* is in the pollen it contains (Wan *et al*., 2002; Tao & He, 2005). Different ragweed proteins have been identified in pollen extracts, some of which have been found to possess enzymatic activities. At least 20 different enzymes have been identified in pollen extracts (Blanchard & Gardner, 1976). One of them is Ser protease with chymotrypsin-like specificity (Bagarozzi *et al.*, 1996). The second protease is a trypsin-like enzyme with a molecular weight of about 80 kD (Bagarozzi *et al.*, 1998). During pollen-initiated allergic reactions, pollen endopeptidase may be involved in the inactivation process of regulatory neuropeptides. Some of these proteins are antigenic and may have the potential of being allergenic in certain sensitized individuals (King *et al.*, 1964; Brunet *et al*., 1992). There are 52 antigens presently identified in aqueous extracts of ragweed pollen, out of which at least 22 act as allergens, as defined by their reactivity with the human IgE (Lowenstein & Marsh, 1983; Pilyavskaya *et al*., 1995).

Investigations of the natural origin chemical agent antimicrobial and genotoxic effects have provided us with strong reasons for engaging in a more comprehensive research of this heterogeneous group of compounds. Natural products, like essential oils (e.g. sage), alkaloids (pyrrolizidines from Compositae, Leguminosae), glukosides (cycasin from the genus *Cycas*), natural colours, riboflavin, aflatoxin, metabolism products of the fungus *Aspergillus flavus*, showed mutagenicity in experiments with *Salmonella typhimurium* and *Drosophila melanogaster* (Williams *et al.*, 1980; Haveland-Smith, 1981; Morgan & Hoffman, 1983; Simić *et al.*, 2000). Therefore, the objective of the present paper was to investigate the biological effects of the pollen acetone extract of the *Ambrosia artemisiifolia* L. plant through the application of different methods.

MATERIALS AND METHODS

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

Pollen extraction

Pollen was broken into small pieces by using a cylindered crasher and pollen pieces (30 g) were extracted with acetone using a Soxhlet apparatus. The mixture was filtered through a filter paper (Whatman, No. 1) and evaporated in a rotary evaporator. A crude extract of pollen (0.54 g) was stored in a dark glass bottle for further processing. The acetone extract of pollen was purified by thin layer chromatography on a MN-silica gel (on the plate 20×20 cm, a mining size of 0.25 mm) P/UV_{254} with $CaSO_4$. Elution was performed with 250 ml of benzene and ethyl acetate mixture at the ratio of 6:4. The two components were identified with Rf 0.36 and 0.50. The components were then separated by preparative chromatography on a MN-silica gel (on the plate 20×40 cm, a mining size of 1.75 mm) P/UV_{254} with $CaSO_4$ using benzene and ethyl acetate mixture at the ratio of 6:4. The layer of silica gel was extracted with ethyl acetate. The extract was filtered and evaporated. The crude extract was analysed with IC, H^1-NMR and MS.

Melting points (m.p.) were recorded on a Kofler-hot stage apparatus. Microanalysis of carbon and hydrogen was carried out with a Carlo Erba 1106 microanalyser. The IR spectra were run on a Perkin-Elmer Grating Spectrophotometers Model 137 and Model 337, ν in cm^{-1}. UV-VIS spectra were recorded on a Varian Super Scan-3 spectrophotometer. The NMR spectra were recorded on a Varian Gemini 200 spectrometer (^1H at 200 MHz and ^{13}C at 50 MHz) in a $CDCl_3$ solvent using TMS as the internal standard. Chemical shifts (δ) are given in ppm. The following abbreviations were used: s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet. Mass spectroscopic analyses were carried out with a Finnigen MAT 8230 and GC/MSD Agilant 6980/5973MSD.

Microorganisms

The bacteria used were: *Bacillus mycoides* (IHP), *Pseudomonas fluorescens* (FSB28), *Erwinia carotovora* (FSB31), *Enterobacter cloaceae* (FSB 22), *Klebsiella pneumoniae* (FSB26), *Agrobacterium tumefaciens* (FSB11), *Azotobacter chrococcum* (FSB14), *Staphylococcus aureus* (IHP), *Proteus* sp. (IHP) and *Pseudomonas aeruginosa* (IHP).

All tested bacterial cultures were obtained from the Institute of Health Protection (IHP) and the Faculty of Agriculture, University of Belgrade, Serbia. The laboratory of Microbiology at the Department of Biology, Faculty of Science, University of Kragujevac, Serbia, confirmed the identification of all tested micro organisms (FSB No).

Antibacterial activity of the pollen extract

The antibacterial activity of the acetone pollen extract was investigated by the disc diffusion method on nutrient agar broth. The method was performed using a 24 hrs culture that was reseeded on nutrient broth at 37°C. The cultures were adjusted to 5.6×10^6 CFU ml^{-1} with sterile water. One millilitre of suspension was added over the plates containing nutrient agar broth to get a uniform microbial growth on both control and test plates. The extract of pollen was dissolved with 96% ethanol (100 mg ml^{-1}) and then sterilized. Under aseptic conditions, empty sterilized discs (Whatman no. 5, T14 mm) were impregnated with 250 µl, 100 µl and 50 µl, of different respective extract concentrations (25 mg/disc, 10 mg/disc, 5 mg/disc,) 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 the 37°C. After the incubation period (48 hrs), the zone of inhibition was measured and expressed in mm (Schalkowsky & Hunt, 1997). The paper disc of the solvent (acetone) was used as a control. Sinacilin antibiotic was constituted as a standard antibiotic for comparison. Each test was performed in triplicate.

Genotoxicity

The Sex-Linked Recessive Lethal test for mutagenicity (SLRL test) was performed with laboratory stocks of *Drosophila melanogaster* (obtained from the Umea Stock Centre, Sweden). *Canton-S* line flies had a normal phenotype (*wild* type), while *Basc* line flies were characterized by individuals homozygous phenotype for an X-chromosome balancer carrying two genetic markers: *Bar (B)* which produces a narrow eye shape in homo- and hemizygous conditions and a kidney shaped eye when heterozygous in females. Eye restricted to a narrow vertical bar of 80± facets appears in males and 70± facets in homozygous females. The heterozygous female has an intermediate number of facets (360±) between homozygous females (70±) and a wild-type (780±). The character can be regarded as partially dominant; *white-apricot* (w^a) – alters the red eye colour into light orange and is expressed only in homozygous females and hemizygous males; *scute (sc)* – recessive mutation that reduces the number of thoracic bristles. This mutation is linked with the long inversion on the X-chromosome, necessary for suppression of crossover that could potentially change the existing gene combinations on the treated chromosome (Lee *et al.*, 1983).

Three-days-old *Canton-S* males (N = 30) were starved in empty bottles for 5 hrs prior to the treatment, and then transferred and fed in the bottles containing filter paper soaked with a 5% solution of the acetone extract for 24 hrs. After another 24 hrs of recovery on a standard medium, each male was mated individually to three *Basc* females, in 30 bottles, creating brood I. Two days later, males were transferred to a new set of vials containing three virgins of *Basc* line (thus creating brood II). After three days, males were transferred again to the fresh vials containing three *Basc* virgins (brood III). These males stayed with females for three days and were removed afterwards. Females were left alone for five days to lay eggs, and then removed. The solvent 1% Sucrose served as the negative control (Lewis & Bacher, 1968).

After F_1 emerged, brother-sister mating was allowed for several days, and 10 females from each vial were placed individually into the 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 the *wild* type males indicated the presence of recessive lethal agent induced by the test substance.

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

The total number of treated X-chromosomes is equal to the sum of lethal and non-lethal cultures, and the frequency of sex-linked recessive lethal cultures was calculated according to the ratio between the number of lethal cultures to the total number of treated X-chromosomes. A significance of the percentage difference regarding lethal cultures was performed through a testing for big independent samples (testing the difference between proportions – Petz, 1985).

RESULTS AND DISCUSSION

Through examination many different kinds of metabolites including sesquiterpenic lactones, phenolics, coumarins and flavonoids have been identified from the *A. artemisiifolia* L. (Bloszyk *et al*., 1992; Milosavljević *et al*., 1999; Tamura *et al*., 2004; Parkhomenko *et al*., 2006). In the above-ground parts of *A. artemisiifolia* L. species many structurally different sesquiterpenic lactones were identified – some of them being co-

ronopilin, dyhidropartenolide, psilostachyin, cumanin, peruvin, artemisiifolin, isabelin, ambrosin, and cumamin (Parkhomenko *et al*., 2005).

The α,β-unsaturated lactones are the subject of interest in phytochemistry and medicine, due to their biological properties as cytotoxic, antitumor, antimicrobial agents and allergens (Kebede, 1994; Parkhomenko *et al.*, 2005). Previous literature gears us toward the fact that the existing pollen proteins are responsible for their allergenic activity (Wan *et al*., 2002), although there is no proof whether those lactones actually contribute to such reaction along with existing proteins as their carriers (Warshaw & Zug, 1996; Moller *et al*., 2002).

The following lactones were identified from the methanol extract of common ragweed *A. artemisiifolia*: the sesquiterpene lactones psilostachyins A, B and C, paulitin and isopaulitin. Psilostachyins A and C block cells in mitosis and act as a novel checkpoint inhibitors of the G/2/DNA damage (Sturgeon *et al*., 2005), are suggesting that these compounds can easily bind covalently to the target proteins (Hwang *et al*., 1996; Borchardt *et al*., 2001).

At this point we performed a selective pollen extraction using acetone. Only two lactones, ambrosin and dihydroambrosin, were identified in the acetone extract (in the ratio of 3:1) of ambrosia pollen (Figs 1 and 2). The presence of these lactones is in accordance with the presence of the ambrosic acid (Milosavljević *et al*., 1999), acting as a precursor in acid-catalysed intra molecular cyclization to form ambrosin, and ambrosin-like lactones (Herz *et al*., 1977). Besi-

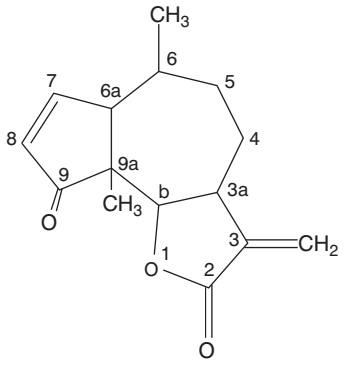

FIG. 1. $C_{15}H_{18}O_3$, MW 246.30, 6,9a-dimethyl-3-methylene-3,3a,4,5,6,6a-hexahydroazuleno[4,5-b]furan-2,9 (9aH,9bH)-dione (ambrosin).

Light white crystals, m.p. 146-148°C (ethanol), $R_f = 0.36$ (benzene : ethyl acetate = 6:4) **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); **IR** (KBr, cm^{-1}): 1755 γ-lacton, 1710, 1605 enone (cyclpentenone), 1660 enone (lactone), 1420, 1378, 1140; **^{1}H NMR** (200 MHz, $CDCl_3$, δ, ppm): 1.01 (3H, d, $^3J = 6.8$ Hz, CH_3-C6), 1.33 (3H, s, CH_3-C9a,), 2.39 (1H, t. $^3J_{6a,6} = 5.9$ Hz, $^3J_{6a\ 7} = 5.8$ Hz, C6a), 2.69 (1H, bq, $^3J_{3a,9b} = 6.7$ Hz, $^3J_{3a\ 4} = 6.2$ Hz, $^3J_{3a\ 4} = 6.1$Hz, C3a), 4.21 (1H, d, $^3J_{3a,9b} = 6.7$ Hz, C9b), 5.65 (2H,d, J = 1,3 Hz, CH_2 methylene), 6.25 (2H, d, J = 1,3 Hz, CH_2 methylene), 7.02 (1H, dd, $^3J_{7,8} = 6.3$Hz, $^3J_{6a\ 7} = 5.8$ Hz, C7), 6,20 (1H, d, $^3J_{7,8} = 6.3$Hz, C8), 1.71 (1H, m, C6), 1.12-1.48 (4H, m, C4,C5);
^{13}C NMR (50 MHz, $CDCl_3$, δ, ppm): 169.97 (C2), 138.7 (C3), 43.43 (C3a), 24.8 (C4), 31.15 (C5), 33.9 (C6), 49.56 (C6a), 169.87 (C7), 128.1(C8), 211.8 (C9), 53.47 (C9a), 79.65 (C9b), 18.79 (CH_3 at C6), 17.6 (CH_3 at C9a), 120.05 (CH_2 methylene);
MS (m/z): 93, 125, 145, 166, 189, 204, 231, M^+ 246.1256.

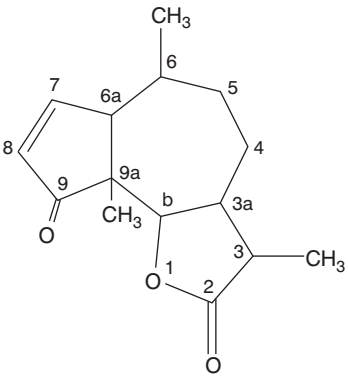

FIG. 2. $C_{15}H_{20}O_3$, MW 248.30, 3,6, 9a-dimethyl-3,3a,4,5,6,6a-hexahydroazuleno[4,5-b]furan,9(9aH,9bH)-dione (dihydro 3-ambrosin).

White crystals, m.p. 165-167°C (diethyl ether), $R_f = 0.50$ (benzene : ethyl acetate = 6:4); **UV-VIS** (EtOH) (λ_{max}, nm, ε (L mol^{-1} cm^{-1}): 212 (15100) α,β unsaturated ketone) and 347 (20) (carbonyl); **IR** (KBr, cm^{-1}): 1775 γ-lacton, 1707, 1615 enone (cyclpentenone), 1420, 1370,1140;
^{1}H NMR (200 MHz, $CDCl_3$, δ, ppm): 1.01 (3H, d, $^3J = 6.7$Hz, CH_3-C6), 1.21 (3H, d, $^3J = 7.7$Hz, CH_3-C6), 1.34 (3H, s, CH_3-C9a), 2.47 (1H, t, $^3J_{6a,6} = 7.9$ Hz, $^3J_{6a\ 7} = 5.5$ Hz, C6a), 2.61 (1H, dq, $^3J_{3,\ Me} = 7.7$ Hz, $^3J_{3a\ 3} = 9.5$ Hz, C3), 2.81 (1H, m, $^3J_{3a,9b} = 10.4$ Hz, $^3J_{3a\ 3} = 9.5$ Hz, C3a), 4.61 (1H,d, $^3J_{3a,9b} = 10.4$ Hz, C9b), 7.1(1H, dd, $^3J_{7,8} = 5.9$Hz, $^3J_{6a\ 7} = 5.5$ Hz, C7), 6,07 (1H, d, $^3J_{7,8} = 5.9$ Hz, C8), 1.68 (1H, m, C6), 1.12-1.48 (4H, m, C4,C5);
^{13}C NMR (50 MHz, $CDCl_3$, δ, ppm): 178.3 (C2), 41.4 (C3), 45.4 (C3a), 26.7 (C4), 30.1 (C5), 35.2 (C6), 51.6 (C6a), 161.7 (C7), 129.2(C8), 209.8 (C9), 54.7(C9a), 85.6 (C9b), 19.9 (CH_3 at C6), 20.6 (CH_3 at C9a), 12.9 (CH_3 at C3);
MS (m/z): 125, 145, 166, 189, 203, 218, 233, M^+ 248.1412.

des the lactones, the TLC chromatography analysis of acetone extract, as a non-polar phase identified some lipoid compounds in traces, while the individual components of a mixture were not examined (Koessler, 2007).

Bacterial strains used in our experiment demonstrate the commercial potential of pollen as active allelochemical (Kebede, 1994) and as an antimicrobial mixture for some human and phytopathogenic bacteria (Wang *et al*., 2006).

The results representing antibacterial activity (disc diffusion method, Benson 1990) of the acetone extract of ambrosia pollen are presented in Table 1.

These results showed that the acetone extract has the highest antibacterial activity against all ten investigated bacteria. All cultures have one thing in common; the investigated concentrations have an inhibitory effect on the development of a larger number of bacteria, while the 10 mg concentration exhibited a higher degree of inhibition over the 24 hours development period. The inhibition level varied from 21.0 to 26.0 mm and the extract demonstrated the highest inhibition ability with *Enterobacter cloacae* from 26.0 mm, while with other bacteria inhibition ranged from 21.0 to 24.0 mm (vanden Berghe & Vlietinck, 1991).

In the 48 hour-cultures, a slight decline of inhibition was noticed, except for the culture of *Staphylococcus aureus* (Chalchat *et al*., 2004*).* There are two significant differences with this bacterium. The first one is that inhibition ability is very similar in the both samples of 5 mg and 10 mg of extract per disc, and it varies from 21.0 to 23.0 mm. The second difference refers to the fact that the level of inhibition remains after 24 hrs of development (Akande & Hayashi, 1998; Chalchat *et al*., 2004). For the most of the examined cases of bacteria, after the time period of 48 hrs, and under the concentration of 10 mg, a slight weakening of the bacteriostatic effect was noticed, while the absence of susceptibility occurs under the concentration of 5 mg in the case of *Pseudomonas fluorescens*, *Erwinia carotovora*, *Enterobacter cloacae* and *Azotobacter chrococcum* cultures. In general, this class of bacteria is more resistant. Such resistance could be due to the permeability barrier provided by the cell wall or the membrane accumulation mechanism (Adwan & Hasan, 1998; Zhi *et al*., 2008).

Compared to a standard ability of sinacilin, at a concentration of 10 mg per disk, all bacteria have an inhibitory development in the presence of the acetone extract for 5-12 mm, i.e., bacterial development is 1-1.5 times slower than the one in the presence of antibiotics.

The examined concentration of acetone extract does not demonstrate an inhibition effect on the growth of *Proteus* and *Pseudomonas aeruginosa*. The absence of susceptibility of this bacteria to the plant extracts was not entirely unexpected, and it is based both on its anatomical and morphological characteristics (Gale *et al*., 1981; Murray, 1991; Adwan & Abu-Hasan, 1998; Srinivasan *et al*,. 2001; Asghari *et al*., 2006; Zhi *et al*., 2008).

TABLE 1. Antibacterial activity of acetone extract of pollen of *Ambrosia artemisiifolia* (standard: sinacilin 10 mg/disc). Data are average (± sd) diameters (in mm) of inhibition zones from three independent observations

Microorganism	Zones of inhibition (mm)[a]				
	extract				standard
	5 mg	10 mg	5 mg	10 mg	10 mg
	24 h		48 h		24 h
Bacillus mycoides	18.0 ± 1.0	23.0 ± 0.5	14.5 ± 0.5	17.0 ± 1.0	22.0 ± 0.5
Pseudomonas fluorescens	17.0 ± 1.0	21.0 ± 1.0	0[a]	17.0 ± 1.0	13.2 ± 0.7
Agrobacterium tumefaciens	19.0 ± 0.5	24.0 ± 1.0	18.0 ± 0.5	23.0 ± 0.5	15.0 ± 0.5
Erwinia carotovora	17.0 ± 0.5	23.0 ± 0.5	0[a]	21.0 ± 0.5	14.1 ± 0.5
Enterobacter cloacea	19.0 ± 1.0	26.0 ± 0.5	0[a]	24.0 ± 1.0	10.6 ± 0.5
Azotobacter chrococcum	18.0 ± 1.0	23.0 ± 0.3	0[a]	22.0 ± 1.0	10.6 ± 0.5
Klebsiella pneumoniae	20.0 ± 0.5	22.0 ± 0.5	17.0 ± 1.0	18.0 ± 1.0	12.5 ± 0.5
Staphylococcus aureus	21.0 ± 0.3	23.0 ± 0.5	22.0 ± 0.5	23.0 ± 0.5	12.5 ± 0.5
Proteus sp.	0[a]	0[a]	0[a]	0[a]	36.8 ± 0.7
Pseudomonas aeruginosa	0[a]	0[a]	0 [a]	0[a]	0[a]

[a] Solvent controls of acetone were negative

TABLE 2. Frequencies of SLRL mutations after treatment of *Drosophila melanogaster* males with acetonic extract of pollen of *Ambrosia artemisiifolia*

	Sucrose (negative control)	*Ambrosia* (test group)	$t_{s/a}$
I brood ∑	300	244	1.65
N_o of lethals	5	10	$p > 0.05$
% of lethals	1.67	4.10	
II brood ∑	269	204	4.40
N_o of lethals	5	26	$p < 0.001^{***}$
% of lethals	1.86	12.74	
III brood ∑	252	236	2.97
N_o of lethals	6	20	$p < 0.01^{**}$
% of lethals	2.38	8.47	
I+II+III ∑	821	684	5.41
N_o of lethals	16	56	$p < 0.001^{***}$
% of lethals	1.95	8.19	

The extract of 5 mg concentration, during the 24-hour period acts as bacteriostatic towards eight out of ten examined bacteria, while the recorded effect is a result of the inhibition process of the synthesized cell wall.

The extract of 10 mg concentration is bactericidal and is contingent upon the inhibition of the bacterial metabolism, with the most prominent inhibition recorded on the ribosome protein synthesis (Pyatkin & Krivoshein, 1980; Coenye *et al.*, 2007; Umesha *et al.*, 2008).

Using short tests for the detection of mutagenicity in *Drosophila melanogaster in vivo* conditions, we were able to determine a mutagenic effect of the investigated plant. The results are presented in Table 2.

The concentration of 5% has induced sex-linked recessive lethal mutations on the X-chromosome of *Drosophila melanogaster* males, which were treated acutely with the extract (broods II and III). The frequency of germinative mutations induced by these pollen components is significantly higher than the frequency of mutations induced by sucrose (negative control). The obtained results showed that the spermatid cell line (brood II) is particularly sensitive to the extract.

Our results showed that the tested extract induces recessive, lethal X-linked mutations in postmeiotic germinative cell lines-spermatids and premeiotic line-spermatocytes, while the spermatozoids are more resistant to the genotoxic effects of the investigated agent.

The statistically significant difference in the increase of frequency of the sex-related lethal in the tested group of males of *Drosophila melanogaster* (compared to the negative control) indicated that a certain chemical component in the *Ambrosia* pollen, induces mutations in male germinative cells of this eukaryotic species. Statistically significant differences in II and III brood, shown in Table 2, confirm the same sensitivity of the germinative cells of premeiotic (diploid) and postmeiotic stages (haploidic spermatids).

The experimentally proven genotoxicity of the *Ambrosia* pollen extract needs further investigation i.e., determination of the chemical structure of the pollen agent that is capable of inducing hereditary genetic changes in this *in vivo* system.

On the basis of experimental work it was concluded that two lactones, ambrosin and dihydroambrosin, were identified in the acetone extract (in the ratio of 3:1) of *Ambrosia* pollen.

A 5 mg extract concentration is bacteriostatic for the most of the examined bacteria during the 24-hour development period, while the 10 mg extract concentration is bactericidal for eight out of ten examined bacteria.

The lactones mixture (3:1) of ambrosin and dihydroambrosin, induces recessive lethal mutations on X-chromosome of *Drosophila melanogaster* in II and III broods, and it was concluded that spermatides and spermatocytes are more sensitive stages of spermatogenesis than others.

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