ANTAGONISTIC EFFECTS OF *SATUREJA HORTENSIS* ESSENTIAL OIL AGAINST AFB_1 ON HUMAN LYMPHOCYTES *IN VITRO*

S. CEKER [1, 2], G. AGAR [2], L. ALPSOY [3], G. NARDEMIR [1, 2],H.E. KIZIL [4]

[1] Agri Ibrahim Cecen University, Central Research and Application Laboratories, Agri, TR- 04100, Turkey
[2] Ataturk University, Faculty of Science, Department of Biology, Erzurum, TR-25240, Turkey
[3] Fatih University, Faculty of Science, Department of Biology, Istanbul, Turkey
[4] Bayburt University, Education Faculty, Bayburt, Turkey
E-mail: lalpsoy@fatih.edu.tr

Satureja hortensis L. (Lamiaceae) has been used as a folk remedy to treat various such as cramps, muscle pains, nausea, indigestion, diarrhea, and infectious diseases. In this study, the antagonistic effects of essential oil of S. hortensis (SHE) were studied against aflatoxin B_1 (AFB_1) in human lymphocytes in vitro. The analysis of the essential oil was performed by using Gas chromatography-mass spectrometry (GC-MS). Anti-genotoxic effects of the SHEs was evaluated using sister chromatid exchange (SCE), micronuclei (MN) tests against AFB_1. Also level of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities used to determine the anti-oxidative effects of the SHEs. This result showed AFB_1 (5 μM) increased the frequencies of SCE, MN and the level of MDA. AFB_1 at the same concentration decreased the activities of SOD and GPx. However, different concentrations of SHE with AFB_1 decreased the frequency of SCE and MN and level of MDA and also increased the activities of SOD and GPx significantly. Especially, the 1.0, 1.5, 2.0 μL dose of SHE are more effective than other doses. The results of this experiment have clearly shown that SHE has strong antioxidative and antigenotoxic effects, these biological activities of SHEs can be due to its component.

Keywords: *Satureja hortensis, aflatoxin B_1, essential oils of Satureja*

Introduction. *Satureja hortensis* L. (Lamiaceae) is an aromatic and medical plant. Leaves, flowers, and stems of *S. hortensis* are frequently used as tea or additives in commercial spice mixtures for many foods to offer aroma and flavor and called «sater, ank, or anug» by the people from different regions in Turkey [1]. *S. hortensis* has also been used as a folk remedy to treat various ailments such as cramps, muscle pains, nausea, indigestion, diarrhea, and infectious diseases. It is used in the traditional medicine in Iran for treating stomach and intestinal disorders [2].

Extracts of *S. hortensis* have been shown to have antimicrobial, antioxidant, antispasmodic and sedative properties [2]. In previous studies, the contents of essential oils and extracts of medicinal plants were demonstrated that they have antimicrobial, antioxidant, and other biological activities. The chemical composition of essential oils may be different due to the origin, the locality, the environmental conditions, and the stage of development of the collected plant material [3].

Although the biological activity of an essential oil is mainly attributed to its major components, the synergistic or antagonistic effect of a compound in a minor percentage in the mixture has to be considered. The essential oils from different *Satureja* species have been found to be different both qualitatively and quantitatively [4, 5]. The essential oil of cultivated summer *S. hortensis* has been found to be rich in α-terpinene and carvacrol [6]. In additional, the essential oil of *S. hortensis* which is widely distributed in the Anatolia region of Turkey, has been measured in the following percentage; thymol (29.0 %), carvacrol (26.5 %), α-terpinene (22.6 %), and *p*-cymene (9.3 %) [7].

Many edible plant species such as tea, fruits, juices, spices, and vegetables throughout the history of mankind have been attractive to scientists as natural sources of compounds that are safer than the synthetic ones. Many other plants have also been screened for their antioxidant capacities, and attempts led to the introduction of natural antioxidants such as those from rosemary and sage [8].

Aflatoxins are biologically active secondary metabolites produced by certain species of Aspergillus molds including *Aspergillus parasiticus, Aspergillus nominus* and *Aspergillus flavus* [9, 10]. Aflatoxins caused the cellular and DNA damage via two different ways. Firstly, aflatoxins especially AFB_1 ($C_{17}H_{12}O_6$) is metabolized to AFB_1-8,9-oxide by cytochrome P450 system and forms adduct primarily at N7 position of guanine and are responsible for its mutagenic and carcinogenic effects [11, 12]. Secondly, aflatoxins produce reactive oxygen

species (ROS) such as superoxide radical anion, hydrogen peroxide and lipid hydroperoxides. The hydroxyl radicals interact with DNA and produces mutations [13, 14].

In present study, it was aimed to determine whether *S. hortensis* essential oil (SHE) has any protective effect against AFB_1 mutagen agent by measuring sister chromatid exchange (SCE) and micro nucleus (MN) frequencies and activities superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA) level in human lymphocyte cell culture *in vitro*.

Materials and methods. *Plant material. S. hortensis* plants were collected during the flowering stage in July 2009, from the eastern part of Erzurum city of Turkey (ATA-9350). The identification of plant materials was confirmed by a plant taxonomist, Assoc. Prof. Dr. Yusuf KAYA, in the Department of Biology, Ataturk University, Erzurum, Turkey.

Isolation of the Essential Oil. The air-dried and ground aerial parts of plants collected were submitted to water distillation for 3 h using a Clevenger type apparatus (yield 1.98 %, v/w). The obtained essential oil was dried over anhydrous sodium sulfate and, after it was filtered, stored at 4 °C until tested and analyzed.

GC-MS Analysis Conditions. The analysis of the essential oil was performed using a Hewlett-Packard 5890 II GC, equipped with a HP-5 MS capillary column (30 m × 0.25 mm × 0.25 μm) and a HP 5972 mass selective detector. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium was the carrier gas at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively. The oven temperature was programmed for GC analysis. Diluted samples (1/100, v/v, in acetone) of 1.0 μL were injected manually and in the splitless mode. The components were identified based on the comparison of their relative retention time and mass spectra with those of commercial standards (for the main components), NBS75K library data of the GC-MS system, and literature data. The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on nonpolar phases reported in the literature [15].

Microscopic Evaluation. Blood samples were obtained by vein puncture from four (two men and two women) healthy non-smoking volunteer donors. This study was approved by the local ethics committe. Experiments were also conformed to the guidelines of the World Medical Assembly (Declaration of Helsinki). Lymphocyte cultures were set up by adding 0.5 mL of heparinised whole blood to RPMI-1640 chromosome medium supplemented with 15 % heat-inactivated fetal calf serum, 100 IU/mL streptomycin, 100 IU/mL penicillin and 1 % L-glutamine. Lymphocytes were stimulated to divide by 1 % phytohaemaglutinin. AFB_1 (in concentra-

Table 1. **Essential oil content of *S. hortensis***

Retention Index	Retention time	Components	%	Identificitation methods
983	11.84	β-Pinene	0.33	GC, MS, RI
1023	13.75	α-Terpinene	0.55	GC, MS, RI
1034	14.24	*p*-Cymene	3.14	GC, MS, RI
1067	15.72	γ-Terpinene	9.05	GC, MS, RI
1172	21.61	Borneol	0.64	GC, MS, RI
1178	21.99	Terpinene-4-ol	0.96	GC, MS, RI
1289	26.97	Thymol	0.10	GC, MS, RI
1296	27.43	Carvacrol	79.17	GC, MS, RI
1347	29.39	Thymol acetate	2.24	GC, MS, RI
1419	32.25	β-Caryophyllene	1.48	GC, MS, RI
1442	33.04	Aromadendrene	0.30	GC, MS, RI
1478	34.60	γ-Muurolene	0.25	MS, RI
1494	35.24	Viridiflorene	0.35	GC, MS, RI
1513	36.33	γ-Cadinene	0.51	MS, RI
1574	39.40	Spathulenol	0.92	MS, RI

tions of 5 μM), and essential oils of *S. hortensis* (SHE) were added to the cultures. The experiments were performed on seven groups as follows:

Culture 1: Control
Culture 2: 5 μM AFB_1
Culture 3: 5 μM AFB_1 + SHE 0.1 μL
Culture 4: 5 μM AFB_1 + SHE 0.5 μL
Culture 5: 5 μM AFB_1 + SHE 1.0 μL
Culture 6: 5 μM AFB_1 + SHE 1.5 μL
Culture 7: 5 μM AFB_1 + SHE 2.0 μL

For SCE demonstration, the cultures were incubated at 37 °C for 72 h, and 5-bromo-2-deoxyuridine at 8 μg/mL was added at the initiation of cultures. All cultures were maintained in the darkness. Next, 0.1 mg/mL of colcemide was added 3 h before harvesting to arrest the cells at metaphase. The cultures were centrifuged at 800 g for 10 min. Cells were harvested and treated for 30 min with hypotonic solution (0.075 M KCl) and fixed in a 1:3 mixture of acetic acid/methanol (vol/vol). Bromodeoxyuridine-incorporated metaphase chromosomes were stained with fluorescence plus Giemsa technique as described by Perry and Evans [16]. In SCE study, by selecting 20 satisfactory metaphases for each group, the results of SCE were recorded on the evaluation table. For each treatment condition, well-spread second division metaphases containing 42–46 chromosomes in each cell were scored, and the values obtained were calculated as SCEs per cell.

For MN analysis, cytochalasin B was added 44 h after PHA stimulation to a final concentration of 3 μg/ml. Twenty-eight hours later (after 72 h of culture) the cells were harvested by centrifugation (900 g × 10 min). The supernatant was removed, the cells were mixed thoroughly and 5 ml of cold hypotonic solution (0.05 M KCl) was added. The cells were subsequently incubated at 37 °C for 20 min and centrifuged again (900 g × 10 min). The pellet was mixed thoroughly and 5 ml fresh fixative (1 : 3 acetic acid : methanol) was added dropwise. This fixation procedure was repeated three times and the tube was centrifuged again. The cell pellet was then resuspended in 1 ml of fresh fixative, dropped on to a clean microscope slide, incubated at 37 °C or at room temperature overnight, and stained with Giemsa dye. Coded slides were scored blind by two independent individuals. Only binucleated cells were scored for MN analysis. For each subject, at least 2000 binucleated cells were analyzed for the presence of MN. For the MN scoring, the micronucleus criteria described by Countryman and Heddle were used: a diameter less than 1/3 of the main nucleus, non-refractivity, not touching, and with the same color as the nucleusor lighter [17].

Biochemical Analysis. The cell homogenates were prepared at a 1 : 10 (w : v) dilution in 10 mM potassium phosphate buffer, pH 7.4. Samples were centrifuged at 3000 rpm for 10 min at 4 °C, and the supernatants were collected and immediately assayed for enzyme activities. All samples were measured in six fold.

Measurement of SOD activity. Cu, Zn-SOD activity in the cell culture supernatant was detected by the method of Sun et al. [18]. Assay reagent in 2.45 mL [0.3 mM xanthine, 0.6 mM Na_2EDTA, 0.15 mM nitroblue tetrazolium (NBT), 0.4 M Na_2CO_3, 1 g/L bovine serum albumin] was combined with 100 μL of the sample. Xanthine oxidase (50 μL, 167 U/L) was added to initiate the reaction, and the reduction of NBT by superoxide anion radicals, which are produced by the xanthine-xanthine oxidase system, was determined by measuring the absorbance at 560 nm. Cu, Zn-SOD activity was expressed in units of SOD per mg protein, where 1 U is defined as that amount of enzyme causing half-maximal inhibition of NBT reduction.

Measurement of GPx activity. GPx activity in the cell culture supernatant was measured by the method of Paglia and Valentine [19]. Briefly, 50 μL of sample was combined with 100 μL of 8 mM NADPH, 100 μL of 150 mM reduced GSH, 20 μL of glutathione reductase (30 U/mL), 20 μL of 0.12 M sodium azide solution, and 2.65 mL of 50 mM potassium phosphate buffer (pH 7.0,

Table 2. **The frequencies of SCEs and MN in blood lymphocytes exposure to AFB_1 and SHE**

Groups	SCEs/Cell	MN/Cell
Control	6.60 ± 0.35	2.2 ± 0.002
AFB_1	8.20 ± 0.26 [a]	4.3 ± 0.002 [d]
AFB_1 + SHE 1	8.57 ± 0.23 [a]	5.7 ± 0.001 [d]
AFB_1 + SHE 2	8.40 ± 0.17 [a]	5.1 ± 0.001 [d]
AFB_1 + SHE 3	7.55 ± 0.87 [bc]	4.3 ± 0.002 [d]
AFB_1 + SHE 4	6.87 ± 0.14 [c]	2.6 ± 0.001
AFB_1 + SHE 5	7.23 ± 0.12 [c]	3.0 ± 0.001

Note. For SCE [a] $p < 0.001$ compared with control group, [b] $p < 0.005$ compared with contol group, [c] $p < 0.005$ compared with AFB_1 group. For MN [d] $p < 0.05$.

5 mM EDTA) and the tubes incubated for 30 min at 37 °C. The reaction was initiated with the addition of 100 μL of 2 mM H_2O_2 solution, mixed rapidly by inversion, and the conversion of NADPH to NADP was measured spectrophotometrically for 5 min at 340 nm. The enzyme activity was expressed as units per g protein using an extinction coefficient for NADPH at 340 nm of $6.22 \cdot 10^{-6}$.

Measurement of MDA level. MDA levels in the cell culture supernatant were determined spectrophotometrically according to the method described by Ohkawa et al. [20]. A mixture of 8.1 % sodium dodecyl sulphate, 20 % acetic acid, 0.9 % thiobarbituric acid was added to 0.2 mL of sample, and distilled water was added to the mixture to bring the total volume up to 4 mL. This mixture was incubated at 95 °C for 1 h. After incubation, the tubes were left to cool under cold water and 1 mL distilled water with 5 mL n-butanol/pyridine (15 : 1, v/v) was added, followed by mixing up. The samples were centrifuged at 4000 × g for 10 min. The supernatants were removed, and absorbances were measured with respect to a blank at 532 nm. Tetraethoxypropane (1,1,3,3-tetraethoxypropane) was used as the standard. Lipid peroxide levels were expressed as mol/l MDA. Protein concentrations in the cell culture supernatant were determined by Bradford method [21]. All photometrical measurements were performed with a DU 530 spectrophotometer (Beckman Instruments, Fullerton, California, USA) in a quartz cuvette.

Statistical analysis. The statistical analysis of MN frequencies was performed by use of the χ^2 test. For statistical analysis of biochemical parameters and analysis of SCE values Mann–Whitney U-test was used. A value of P less than 0.05 was accepted as statistically significant. Results were expressed as mean ± SD. For these procedures, SPSS 15.0 version for Windows (SPSS Inc, Chicago, Illinois, USA) was used.

Results. GC-MS analysis of the crude oil isolated from dried aerial parts of *S. hortensis* resulted in the identification of 15 compounds. Carvacrol (79.17 %), gamma-terpinene (9.05 %), terpinene-4-ol (4.55 %), *p*-cymene (3.14 %), thymol acetate (2.24 %) and β-caryophyllene (1.48 %) were the main components (87.4 %) of the oil based on GC-MS analysis (Table 1).

AFB_1 caused significant formations of SCE and MN on peripheral lymphocytes as seen in Table 2. Such an increase was found to be statistically significant ($p < 0.05$). Whereas, SCE and MN frequencies decreased by different concentrations (1.0, 1.5, 2.0 μL) of *S. hortensis* simultaneous treatments with AFB_1. Such a decrease was found to be statistically significant ($p < 0.05$). Other doses of *S. hortensis* were not caused a decrease in SCE and MN frequencies. The 1.5–2 μL dose of SHE were more effective than other doses.

The results of the effect of essential oil on antioxidant enzymes in human blood cells was determined by measuring GPx, SOD and MDA activities as shown in Table 3. AFB_1 caused a decrease in the activities of SOD, GPx and an increase in MDA level. These results were found to be statistically significant ($p < 0.05$).

However, these effects of AFB_1 decreased cotreatment with higher concentrations of essential

Table 3. **The effects of AFB_1 and SHE on MDA, SOD and GPx enzymes activities**

Groups	MDA (nmol/mL)	SOD (U/mg protein)	GPx (U/gr protein)
Control	96.00 ± 2.86	0.82 ± 0.17	12.62 ± 0.91
AFB_1	149.33 ± 2.13 [a]	0.33 ± 0.07 [b]	7.23 ± 0.68 [b]
AFB_1 + SHE 1	150.40 ± 3.40 [a]	0.52 ± 0.05 [b]	7.50 ± 0.79 [b]
AFB_1 + SHE 2	121.60 ± 1.43 [b]	0.73 ± 0.041 [d]	10.69 ± 0.85 [cd]
AFB_1 + SHE 3	98.760 ± 1.43 [ce]	0.81 ± 0.04 [f]	12.29 ± 0.29 [cd]
AFB_1 + SHE 4	108.80 ± 3.50 [cf]	0.82 ± 0.03 [f]	12.62 ± 0.91 [cd]
AFB_1 + SHE 5	107.20 ± 0.12 [df]	0.81 ± 0.01 [f]	12.62 ± 0.91 [cd]

Note. [a] $p < 0.001$ compare with control group, [b] $p < 0.05$ compare with control group, [c] $p < 0.001$ compare with AFB_1-treatment group, [d] $p < 0.05$ compare with AFB_1-treatment group, [e] $p < 0.001$ compare with AFB_1 + SHE 1, [f] $p < 0.005$ compare with AFB_1 + SHE 1.

oil. In summary, significant increases in the activities of SOD, GPx and decreases in level of MDA were observed compared with treatment of AFB_1 alone.

Discussion. GC-MS analysis of the crude oil isolated from dried aerial parts of *S. hortensis* resulted in the identification of 15 compounds. In the previous studies, the essential oils from different *Satureja* species have been found to differ qualitatively and quantitatively [5, 7, 22]. The essential oil of the cultivated *S. hortensis* plant has been observed to be rich in thymol, carvacrol, and terpinene chemotype. In addition that, other studies by Ghannadi have been reported that thymol/carvacrol (1:1 59.7 %), α-terpinene (12.8 %), and *p*-cymene (9.3 %) are major constituents of the oil isolated from the seeds of *S. hortensis* [23]. *S. hortensis* essential oil has also been reported as a source of gamma terpinen, thymol, carvacrol and other phenols [6, 7, 22, 24–26]. In this study it has been observed that SHE has relatively higher amounts of carvacrol (79.12 %), terpinene – chemotype (14.05 %), *p*-cymene (3.14 %) and thymol (2.24 %). As emphasized before in some researches, essential oil content can be affected by the influence of several local, climatic, and seasonal factors. For example, severe water stress was reported to alter carvacrol/α-terpinene contents [7, 22, 27].

The antigenotoxic activities of SHE against AFB_1 in the present study were assessed by evaluating MN and SCE frequencies as shown in Table 2. SHE was found to be effective against genotoxic effects of AFB_1. The observed antigenotoxic activity of SHE can attribute to the essential oils compositions. Antigenotoxic activity of the essential oils is apparently related to their high carvacrol, terpinene, particularly *p-a* cymene and thymol. In a previous study, it has been reported that carvacrol has antimutagenic activity on SCE induced mitomycin C in human lymphocyte cultures [28]. In addition, Soltani et al. [29], has showed that *S. hortensis* has protective properties on DNA lesions in rat lymphocytes exposed to oxidative stress and this protective effect have been linked to compounds such as thymol, carvacrol, and γ-terpinene. The observed antigenotoxic activities of the essential oil are thought to be related to the high content of these components in the oil especially, carvacrol.

Also, our results showed that AFB_1 caused a decrease in SOD and GPx activities and an increase in level of MDA. But, these effects of AFB_1 on enzymes decreased cotreatment with *S. hortensis* essential oils. Previous researches have reported *S. hortensis* essential oils have antioxidant capacity and the antioxidant capacity have been linked to compounds such as thymol, carvacrol, and α-terpinene, γ-terpinene, *p*-cymene [7, 30–32]. The antioxidant capacity of *S. hortensis* could be depended on carvacrol which is the major component in the essential oil of *S. hortensis.* The antimutagenic effect of carvacrol may be due to its antioxidant property as reported by some researches [28, 29]. In summary, anti-genotoxic effect of SHE may be attributed to the antioxidant activity of the mentioned compounds. The essential oils of *S. hortensis* have antigenotoxic and the antioxidant activities therefore; they can be used as a natural preservative ingredient in the food or pharmaceutical industry.

The authors are thankful to the Fatih University, Research Project Foundation (Contract no: P50031301_B (2923) for financial support of this study.

АНТАГОНИСТИЧЕСКОЕ ДЕЙСТВИЕ ЭФИРНОГО МАСЛА *SATUREJA HORTENSIS* НА AFB_1 В ЛИМФОЦИТАХ ЧЕЛОВЕКА *IN VITRO*

S. Ceker, G. Agar, L. Alpsoy, G. Nardemir, H.E. Kizil

Satureja hortensis L. (Lamiaceae) используется как народное средство для лечения разных заболеваний, таких как спазмы, мышечные боли, тошнота, нарушения пищеварения, диарея и инфекционные заболевания. В настоящей работе на примере лимфоцитов человека in vitro изучали антагонистическое действие эфирного масла *S. hortensis* (SHE) на афлатоксины B_1 (AFB_1). Анализ эфирных масел проводился с помощью газовой хроматографии – масс-спектрометрии (GC-MS). Антигенотоксический эффект SHE был оценен на основании изучения обмена сестринских хроматид (SCE) и микроядерного теста против AFB_1. Кроме того, для оценки антиоксидативных эффектов SHE использовали уровень активностей малоновых диальдегидов (MDA), супероксиддисмутазы (SOD) и глютатионпероксидазы (GPx). Показано, что AFB_1 (5 μM) увеличивает частоту SCE, MN и уровень MDA. AFB_1 в той же концентрации снижает активность SOD и GPx. Однако различные концентрации SHE с AFB_1 снижали частоту SCE, MN и уровень MDA, а также существенно увеличивали активность SOD и GPx.

Концентрации SHE 1.0, 1.5, 2.0 μL были наиболее эффективными. Результаты работы четко показали, что SHE имеет сильный антиоксидативный и антигенотоксический эффект. Такая биологическая активность определяется химическим составом SHE.

REFERENCES

1. *Baytop T.* Turkce Bitki Adları Sozlugu (A Dictionary of Vernacular Names of Wild). – Ankara : Turk Dil Kurumu Yayınları, 1994. – 508 p.
2. *Hajhashemi V., Sadraei H., Ghannadi A.R., Mohseni M.* Antispasmodic and anti-diarrhoeal effect of *Satureja hortensis* L. essential oil // J. Ethnopharm. – 2003. – **71**, № 1/2. – P. 187–192.
3. *Deans S.G., Svoboda K.P.* Antibacterial activity of summer savory (Satureja-Hortensis L) essential oil and its constituents // J. Hort. Sci. – 1989. – **64**, № 2. – P. 205–210.
4. *Tumen G., Baser K.H.C.* Essential oil of *Satureja spicigera* (C. Koch) Boiss. from Turkey // J. Essent. Oil Res. – 1996. – **8**. – P. 57–58.
5. *Tumen G., Kirimer N., Ermin N., Baser K.H.C.* The essential oils of two new Satureja species from Turkey: *Satureja pilosa* and *S. icarica* // J. Essent. Oil Res. – 1998. – **10**. – P. 524–526.
6. *Gora J., Lis A., Lewandowski A.* Chemical composition of the essential oil of cultivated summer savory (*Satureja hortensis* L. cv. Saturn) // J. Essent. Oil Res. – 1996. – **8**. – P. 427–428.
7. *Gulluce M., Sokmen M., Daferera D. et al.* In vitro antibacterial, antifungal, and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of *Satureja hortensis* L. // J. Agricult. Food Chem. – 2003. – **51**, № 14. – P. 3958–3965.
8. *Schuler P.* Natural antioxidants exploited commercially / Food Antioxidants / Ed. F.B. Hudson. – London : Elsevier, 1990. – P. 99–170.
9. *Alpsoy L., Yildirim A., Agar G.* The antioxidant effects of vitamin A, C, and E on aflatoxin B1-induced oxidative stress in human lymphocytes // Toxicol. Ind. Health. – 2009. – **25**, № 2. – P. 121–127.
10. *Bedard L.L., Massey T.E.* Aflatoxin B1-induced DNA damage and its repair // Cancer Lett. – 2006. – **241**, № 2. – P. 174–183.
11. *Denissenko M.F., Cahill J., Koudriakova T.B. et al.* Quantitation and mapping of aflatoxin B1-induced DNA damage in genomic DNA using aflatoxin B1-8,9-epoxide and microsomal activation systems // Mutat Res. – 1999. – **425**, № 2. – P. 205–211.
12. *Wang J.S., Groopman J.D.* DNA damage by mycotoxins // Mutat. Res. – 1999. – **424**, № 1/2. – P. 167–181.
13. *Alpsoy L., Yalvac M.E.* Key roles of vitamins A, C, and E in aflatoxin B1-induced oxidative stress // Vitam. Horm. – 2011. – **86**. – P. 287–305.
14. *Halliwell B., Gutteridge J.M.C.* Oxidative stress: adaptation damage, repair and death. – New York : Oxford : Univ. press, 1999.
15. *Adams R.P.* Identification of essential oils components by gas chromatography / Mass Spectroscopy. – Illinois, USA, 2004. – 804 p.
16. *Perry P., Evans H.J.* Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange // Nature. – 1975. – **258**, № 5531. – P. 121–125.
17. *Countryman P.I., Heddle J.A.* The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes // Mutat Res. – 1976. – **41**, № 2/3. – P. 321–332.
18. *Sun Y., Oberley L.W., Li Y.* A simple method for clinical assay of superoxide dismutase // Clin. Chem. – 1988. – **34**, № 3. – P. 497–500.
19. *Paglia D.E., Valentine W.N.* Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase // J. Lab. Clin. Med. – 1967. – **70**, № 1. – P. 158–169.
20. *Ohkawa H., Ohishi N., Yagi K.* Assay for lipid peroxides in animal-tissues by thiobarbituric acid reaction // Anal. Biochem. – 1979. – **95**, № 2. – P. 351–358.
21. *Bradford M.M.* A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding // Anal. Biochem. – 1976. – **72**. – P. 248–254.
22. *Milos M., Radonic A., Bezic N., Dunkic V.* Localities and seasonal variations in the chemical composition of essential oils of *Satureja montana* L. and *S. cuneifolia* Ten // Flav. Frag. J. – 2001. – **16**, № 3. – P. 157–160.
23. *Ghannadi A.* Composition of the essential oil of *Satureja hortensis* L. seeds from Iran // J. Essent. Oil Res. – 2002. – **14**, № 1. – P. 35–36.
24. *Madsen H.L., Andersen L., Christiansen L. et al.* Antioxidative activity of summer savory (*Satureja hortensis* L.) and rosemary (*Rosmarinus officinalis* L.) in minced, cooked pork meat // Lebensm.-Unters. Forsch. – 1996. – **203**, № 4. – P. 333–338.
25. *Muller-Riebau F., Berger B.M., Yegen O., Cakir C.* Seasonal variations in the chemical compositions of essential oils of selected aromatic plants growing wild in Turkey // J. Agricul. Food Chem. – 1997. – **45**. – P. 4821–4285.
26. *Zargari A.* Medicinal Plants. – Tehran : Univ. Publ., 1990.
27. *Baher Z.F., Mirza M., Ghorbanli M., Rezaii M.B.* The influence of water stress on plant height, herbal and essential oil yield and composition in *Satureja*

ja hortensis L. // Flav. Fragr. J. – 2002. – **17**. – P. 275–277.

28. *Ipek M., Ipek A., Simon P.W.* Comparison of AFLPs, RAPD markers, and isozymes for diversity assessment of garlic and detection of putative duplicates in germplasm collections // J. Amer. Soc. Hort. Sci. – 2003. – **128**, № 2. – P. 246–252.
29. *Soltani F., Mosaffa F., Iranshahi M. et al.* Auraptene from ferula szowitsiana protects human peripheral lymphocytes against oxidative stress // Phytother. Res. – 2010. – **24**, № 1. – P. 85–89.
30. *Daferera D.J., Ziogas B.N., Polissiou M.G.* GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on *Penicillium digitatum* // J. Agricult. Food Chem. – 2000. – **48**, № 6. – P. 2576–2581.
31. *Daferera D.J., Ziogas B.N., Polissiou M.G.* The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, Fusarium sp. and *Clavibacter michiganensis* subsp *michiganensis* // Crop. Protect. – 2003. – **22**, № 1. – P. 39–44.
32. *Didry N., Dubreuil L., Pinkas M.* Antimicrobial activity of thymol, carvacrol and cinnamaldehyde alone or in combination // Pharmazie. – 1993. – **48**, № 4. – P. 301–304.

Received 26.11.12