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Protective Effects of *Origanum onites* and Its Components on Lead-Nitrate Induced Genotoxicity in Root Cells of *Allium cepa* L.

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Abstract: This study investigates the protective effects of components (Thymol; Thy, Carvacrol; Car, Linalool; Lin, and *α*-Pinene; AP) and essential oil of *Origanum onites* L. (*O. onites*-EO), against lead nitrate-induced cytotoxicity and genotoxicity in *Allium cepa* L. (*A. cepa*) root tip cells. These components obtained from *O. onites* were characterized by gas chromatography (GC). *A. cepa* bulbs were exposed to 6.25-12.5 mg/L concentrations of the *O. onites*-EO/components of *O. onites*-EO for analyses of induction of cytogenetic damage. Then, these bulbs were exposed to 10 mg/L concentrations of lead nitrate for analyses of the protective effects of *O. onites* and its components. Mitotic abnormalities were evaluated for genotoxicity, and mitotic index (MI) for cytotoxicity. As a result of this study, lead nitrate increased the total chromosomal abnormality amount in *A. cepa*, indicating genotoxicity. MI was decreased with lead nitrate. However, this effect was significantly improved by components of *O. onites*-EO. This effect was shown with the decrease in the number of chromosomal abnormalities and increase in MI rates in lead nitrate-induced root cells after exposure to the components of *O. onites*-EO. The protective effect of *O. onites*-EO components against the damage caused by lead nitrate in cells can be listed as *α*-Pinene > Thymol > Carvacrol > Linalool. Among all essential oil components tested, *α*-Pinene was determined to have the strongest protective effect. Furthermore, the protective effect of the essential oil, which contains all the components, could not be determined. It has been observed that the components of essential oil have different effects, and it can be said that these components suppress the effects of each other in the mixture where they are found together. In conclusion, this study shows that the components of *O. onites*-EO have a protective effect on lead nitrate-induced *A. cepa* root cells.

Keywords: Anti-genotoxicity; essential oil; heavy metal toxicity; lead-nitrate; *Origanum onites*. © 2024 ACG Publications. All rights reserved

1. Introduction

The contamination of the ecosystem with heavy metals is a major problem worldwide. Among the primary pollutants, heavy metals are the most harmful substances [1]. Due to their toxic effects on living organisms, heavy metals are a cause for significant concern worldwide [2]. Lead (Pb), one of the heavy metals, is a toxic metal widely used in various industrial processes, leading to intense environmental pollution and health issues in many parts of the world. The primary sources of lead

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include industrial processes, food, cigarette smoke, drinking water, and household items such as house paint, storage batteries, and toys [3,4]. According to a report published in 2020 by UNICEF and Pure Earth, approximately 800 million children worldwide have blood lead (Pb) levels of 5 µg/mL or higher, which is a level of health concern. There is no safe level of lead exposure for the human body, and lead can lead to systemic toxicity [4-6]. Lead, unlike other metals such as zinc (Zn), copper (Cu), and manganese (Mn), shows toxicity by disrupting various physiological processes in cells, without having any beneficial functions [7]. The main lead toxicity is the formation of oxidative stress and the deactivation of sulfhydryl groups of antioxidants [7]. Studies have shown that in a plant with high lead concentration, the production of reactive oxygen species (ROS) accelerates, resulting in cell membrane damage and photosynthetic processes. As a result of this toxicity, it has been determined that lead inhibits the overall growth of the plant [8]. Furthermore, it is known that lead causes similar excessive reactive oxygen species (ROS) production in human cells. It is established that heightened levels of ROS can result in extensive and irreparable cell damage by oxidizing DNA, RNA, carbohydrates, proteins, and lipids. As a result of this process, it has been determined that it causes cell death through apoptosis or necrosis [9]. These toxicity consequences can cause oxidative damage that accumulates throughout life and is associated with aging and age-related diseases such as cardiovascular diseases, cancer, neurodegenerative disorders, and other chronic diseases [10]. Living organisms use molecules known as antioxidants produced by antioxidant defense systems to counteract ROS generated. However, if antioxidant molecules are not produced in the cell to detoxify such reactive intermediates or repair the resulting damage and the ROS level continues to rise, oxidative stress occurs. Many researchers show that oxidative stress in living cells results from an imbalance between the production of free radicals and the production of antioxidants [7]. In this context, supplementation of herbal antioxidants is needed. Several plants possess a range of antioxidant phytochemicals and bioactive compounds, making them valuable for addressing diseases linked to oxidative stress. For this reason, the medicinal properties of herbal products cause an increase in the demand and use of herbal products in the world [11]. *Origanum onites* (*O. onites*) appears as an important alternative in the treatment of such diseases. *O. onites*-essential oil (*O. onites*-EO) has been shown to have antiviral, antioxidant, anticancer, and proapoptotic properties [12].

O. onites, a member of the Lamiaceae family, is used as a medicinal plant in the treatment of some diseases thanks to its bioactive molecules. The genus *Origanum* is represented by 41 species and 52 taxa in the world, and 23 species and 32 taxa in Türkiye [13]. It is known that the endemic rate of *Origanum* taxa in Türkiye is more than 44%, and this high rate shows that Türkiye is the gene center of *Origanum*. Today, *Origanum* is used as herbal medicine and is very important in world trade [14]. This is why *Origanum* attracts the attention of researchers [15,16,17]. Furthermore, the common feature of all *Origanum* species is that they contain high amounts of essential oil, and the main component of the essential oil is carvacrol and/or thymol [14]. Essential oil, which is a fragrant oily liquid obtained from different parts of the plant, has many properties such as antifungal, and antimicrobial [18], antioxidant [19], insecticide [20], hepatoprotective [21], cytotoxic activity [22] and anticancer [23,24]. It seems that the phytochemicals of *Origanum* have various biological activities [12,24]. These activities are carried out thanks to the phytochemicals contained in the essential oil of *O. onites*. The essential oil contains mainly carvacrol and thymol, as well as *α*-terpinene, *p*-cymene, linalool, *β*-bisabolene, *α*-thujene, *α*-pinene and other components [12].

In this study, the protective effect of *O. onites*-EO and some major elements of *O. onites*-EO against cytotoxicity and genotoxicity in *Allium cepa* (*A. cepa*) root tip cells induced by lead nitrate was investigated. In this way, both the direct activity of the molecules and their synergistic effects in the essential oil were tested. Comparison of the protective effects of these molecules was carried out with the *A. cepa* assay. *A. cepa* assay is frequently used to monitor the toxicogenetic activity of bioactive compounds [25,26]. The rapid proliferation of *A. cepa* cells and the low number of chromosomes enable better analysis of structural and numerical changes. Therefore, it is a method used in studies of genotoxicity and protective effects of bioactive compounds [26,27].

2. Materials and Methods

2.1. Plant Material

Aerial parts of *Origanum onites* were collected from Pelitli village of Ivrindi, Balıkesir, on July 05, 2023. The species was identified by Prof. Dr. Tuncay Dirmenci, from Balıkesir University. It was identified by Dr. Tuncay Dirmenci (Balıkesir University, Necatibey Education Faculty, Department of Biology Education). A voucher specimen (TD 5341) was deposited at the Herbarium of Department of Biology Education at Balıkesir University.

2.2. Chemicals

Lead nitrate (LN) ($Pb(NO_3)_2$, 99.5 %) and Giemsa (G-5637) were purchased from Merck®. Glacial acetic acid (Meck 100063,) and ethanol (Sigma Aldrich 24105-M, 96%) and hydrochloric acid (Tekkim, TK.400155, 30 %) were used. Carvacrol was isolated from the *O. onites* and its purity was determined as 97 % by GC-FID. Thymol (98.5 %), linalool (97 %) and α-pinene (99 %) were obtained from Sigma-Aldrich.

2.3. Isolation of Essential Oil

The essential oil of *Origanum onites* was obtained by hydrodistillation for 3 h in a Clevenger-type apparatus. Essential oil was isolated using 100 g of finely chopped plants. The essential oil was stored at + 4ºC. The study was conducted according to previously described method in the literature [28].

2. 4. Analysis of Essential Oil

2.4.1. GC MS Analysis

The essential oils were diluted with CH_2Cl_2 (1:10) and analyzed using a Trace 1600 GC series gas chromatograph (Thermo Scientific, Inc., Waltham, MA, USA) and a Thermo TSQ 9610 mass spectrometer (Thermo Scientific, Waltham, MA, USA). The separation was carried out in a DB-5 capillary column. Helium was used as a carrier gas at a flow rate of 0.8 mL/min. The oven temperature was kept at 60 °C for 5 min, programmed to 280 °C at a rate of 4 °C/min and kept constant at 240 °C for 5 min. The split ratio was adjusted to 50:1, and the injector temperature was maintained at 250°C. Mass spectra were recorded at 70 eV over a mass range from *m/z* 35 to 650 [28].

2.4.2. GC Analysis

The GC analysis was carried out using a Thermo Scientific Trace GC 1600. The FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms [29,30].

2.5. Test Material and Experimental Design

Allium cepa (*A. cepa*) (2n=16) bulbs used as the test material were obtained from a commercial market. The bulbs were used for the modified *A. cepa* assay to evaluate the genotoxic and anti-genotoxic effect of the *O. onites*-EO/Components of *O. onites*-EO [31]. For genotoxic and anti-genotoxic tests, *A. cepa* bulbs were placed in 50 mL sterile tubes filled with dH_2O and the bulbs were germinated at room temperature for 24 h. For the genotoxicity test, five germinated *A. cepa* bulbs per concentration were transferred into a tube with two different concentrations (12.5-6.25 mg/L) of the *O. onites*-

EO/Components of *O. onites*-EO for 24 h. The distilled water and lead nitrate (10 mg/L) were used as negative control and positive control, respectively. After 24 h exposure to *O. onites*-EO/Components of *O. onites*-EO, the meristematic cell region of the root tip cells was excised and prepared for analysis with the help of routine preparation techniques [32]. The microscopic analysis of cells was performed. For the anti-genotoxicity test, five germinated *A. cepa* bulbs per concentration were transferred into a tube with two different concentrations (12.5-6.25 mg/L) of the *O. onites*-EO/Components of *O. onites*-EO for 24 h. After 24 h exposure to *O. onites*-EO/Components of *O. onites*-EO, the bulbs were transferred into a tube with lead nitrate (10 mg/L) for 24 h. To briefly summarize, in this study, to investigate the protective effect of *O. onites*-EO/components of *O. onites*-EO, the *A. cepa* bulbs were first exposed to the phytochemicals of *O. onites* essential oil and then were exposed to lead nitrate. At the end of the period of exposure, the *A. cepa* bulbs were washed in distilled water and prepared for analysis with the help of routine preparation techniques [32]. The microscopic analysis of cells was performed. Distilled water was used as the negative control and lead nitrate (10 mg/L) was used as the positive control.

2.6. Genotoxic and Antigenotoxic Analysis

For cytological examinations, preparation methods such as fixation, staining, and squashing were used with minor modifications following the procedure described by Darlington and La Cour (1979) [28]. At the end of the 24-hour treatment period, *A. cepa* bulb roots were cut to a length of 1-2 cm and placed in a mixture of alcohol and glacial acetic acid (3:1) for fixation overnight at +4°C. The roots were kept in 70% alcohol until microscopic examination. After removing them from alcohol, the roots were washed with distilled water and hydrolyzed in 1N HCl at 60°C for 15 min. Squash preparations were made using acetocarmine stain. Additionally, 3000 cells were counted to calculate the mitotic index (MI). The mitotic index represents the ratio of cells undergoing mitotic division to the total number of cells in a cell population. The mitotic index and chromosomal aberrations were computed following the established procedure detailed by Bakare et al. [33]. The mitotic index was obtained as follows:

Mitotic Index (%) = Number of Dividing Cells / Total Number of Cells x 100

The percentage of aberrant cells was obtained as follows:

Aberrant cells (%) = Number of Aberrant Cells / Total Number of Cells x 100

2.7. Statistical Analysis

The data were analyzed using SPSS 20 for Windows (SPSS Inc., Chicago, IL, USA) software, and the results were expressed as mean ± standard error. The significance of differences between treatment groups and the negative control was evaluated using the Kruskal-Wallis test, which is a non-parametric test. A p-value of less than 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Chemical Composition of the O. onites-EO

The essential oil yield of *Origanum onites* was found to be 3.2 % (v/w). Table 1 presents the outcomes of the components found in the essential oils of *O. onites*. The relative percentage of carvacrol in *O. onites* essential oil was determined as 83.21%. It has been once more determined that carvacrol is the highest among the phytochemicals contained in the essential oil of *O. onites*. The relative percentages of thymol, linalool, and *α*-pinene, which are the other phytochemicals used on genetoxicity

assay in this study, were found to be 2.10%, 3.04%, and 0.17%, respectively (Table 1) as described in the literature [12]. Essential oil components used for bioactivity assays are shown as bold in Table 1.

Table 1. Essential oil composition of *O. onites*

Compound	RRI	Range of RRI[a]	Relative amounts (%)	Identification method
α-Tuhujen	935	920-940[b]	0.15	RRI, MS
***α*-Pinene**	937	924-941[b]	0.17	RRI, MS, ST
Camphene	953	933-954[b]	0.10	RRI, MS
1-octen-3ol	970	964-979[q,r]	0.01	RRI, MS
Sabinene	973	944-980[b]	0.01	RRI, MS
β-Pinene	986	964-985[b]	0.01	RRI, MS, ST
3-octonal	993	980-996[i,t]	0.01	RRI, MS
Mycrene	995	981-993[b]	0.52	RRI, MS
Δ3-Carene	1006	1010-1020[b]	0.01	RRI, MS
α-phellandrene	1008	995-1011[b]	0.09	
α-Terpinene	1018	1010-1020[f]	0.69	RRI, MS, ST
p-Cymene	1026	1014-1027[f]	2.38	RRI, MS
β-Phellandrene	1030	1026-1032[b]	0.01	RRI, MS, ST
Limone	1032	1028-1038[b]	0.19	RRI, MS, ST
Eucalyptol	1035	1030-1035[f]	0.03	RRI, MS, ST
γ-Terpinene	1062	1042-1064[b]	2.77	RRI, MS, ST
Linolool oxide	1076	1061-1090[d]	0.02	RRI, MS, ST
α-Terpinolene	1089	1086-1089[f]	0.14	RRI, MS, ST
Linalool	1097	1098-1101[d]	3.04	RRI, MS, ST
Sabinene hydrate	1098	1084-1115[d]	0.03	RRI, MS
Isoborneol	1160	1156-1196[o,p]	1.02	RRI, MS
Borneol	1165	1152-1177[b]	0.02	RRI, MS
Terpinen-4-ol	1175	1154-1189[g]	0.51	RRI, MS
p-Cymene-8-ol	1190	1163-1207[b]	0.02	RRI, MS
α-Terpineol	1193	1178-1203[c]	0.19	RRI, MS
Carvacrol methyl ether	1240	1233-1246[j]	0.06	RRI, MS
Carvone	1244	1231-1250[k,l]	0.19	RRI, MS
Thymol	1288	1288-1298[b]	2.10	RRI, MS, ST
Carvacrol	1298	1298-1390[b]	83.21	RRI, MS, ST
Carvacrol acetate	1371	1341-1372[b]	0.15	RRI, MS
Trans-caryophyllene	1420	1396-1444[g]	0.38	RRI, MS, ST
Aromodendren	1448	1430-1450[e]	0.03	
Humulene	1458	1452-1470[e]	0.01	RRI, MS
Bisabolene	1507	1495-1509[c]	0.01	RRI, MS
δ-Cadinene	1528	1515-1547[d]	0.01	RRI, MS
Spathulamol	1583	1566-1601[a,f]	0.16	RRI, MS, ST
Caryophyllene oxide	1586	1578-1613[b]	0.32	RRI, MS, ST
Sclareol	2215	2212-220[h]	0.02	RRI, MS, ST
Total			98.79	

RRI, relative retention indices calculated against n-alkanes. Identification methods: RI, identification based on the retention times (tR) of genuine compounds on DB5 column; MS, identified on the basis of computer matching of the mass spectra with those of the Wiley and NIST and Doruk Analitik and ACG libraries and comparison with literature data. ST, co-injection with standard compounds. GC analyses were replicated three times (mean RSD% value is 0.05)
[a]Literature RRI values for the compounds. the following letters shows the related references. b:[34]; c:[35]; d:[36]; e: [37]; f:[38]; g:[39]; h:[40];i:[41]; j:[42]; k:[43]; l:[44]; m:[45]; n:[46]; o:[47]; p:[48]; q: [49]; r:[50]; t:[51].

3.2.Cytological Examinations

It was determined that the mitotic index (MI) value of the group treated with lead nitrate (positive control) decreased 4.18 times compared to the negative control group and this decrease was statistically significant ($P \leq 0.001$). The decrease in MI level in cells is associated with the inhibition of DNA synthesis or the blocking of the G2 phase in the cell cycle [52]. In this context, it can be said that

lead nitrate (10 mg/L) seriously stops cell division and has high toxicity. In this study, it was observed that the tested dosages of essential oil, carvacrol, thymol, linalool, and *α*-pinene (12.5 mg/L and 6.25 mg/L) increased the MI values. It was observed that the MI values calculated by cytological examinations because of the treatment of these molecules were quite high compared to the negative control. It can be said that these dosages do not cause cytotoxicity and promote cell division. It has been observed that high dosages of *O. onites*-EO/components of *O. onites*-EO, except thymol, have higher MI values. It is seen that a high concentration (12.5 mg/L) of thymol reduces the MI level by 2.7 times compared to its low concentration (6.25 mg/L). There is no toxicity compared to negative control (Table 2). It was observed that chromosomal abnormalities rates (0.10%-0.40%) were quite low in *A. cepa* bulb root cells exposed to *O. onites*-EO/Components of *O. onites*-EOs (Table 2 and Figure 1).

Table 2. The effects of different concentrations of *O. onites*-EO/components of *O. onites*-EO and Lead nitrate on genotoxicity/antigenotoxicity parameters in *A. cepa* root tip cells.

Treatment (mg/L)	**Irreg. Meta.**	**Stickness**	**C-Mitosis**	**MN**	**Vag. Chr.**	**T. Ab. C. (%)**	**MI (%)**
NC	4.00 ± 1.58	7.75 ± 0.85	0.00 ± 0.00	0.00 ± 0.00	2.75 ± 0.47	1.45 ± 0.25^{b3}	10.65 ± 0.49
LN10	15.50 ± 2.06^{a3}	18.00 ± 1.73^{a3}	9.75 ± 2.28^{a3}	0.50 ± 0.50	3.50 ± 2.06	4.75 ± 0.48^{a3}	2.55 ± 0.82^{a3}
EO12.5	9.50 ± 3.69	11.00 ± 5.37	0.50 ± 0.29	0.00 ± 0.00	3.50 ± 1.94	0.20 ± 0.00	24.50 ± 8.37^{b2}
EO6.25	5.25 ± 3.09	5.00 ± 2.92	0.25 ± 0.25	0.00 ± 0.00	1.25 ± 0.95	0.35 ± 0.00	11.75 ± 693^{a1}
Car12.5	9.00 ± 2.97	16.25 ± 3.75	1.25 ± 0.95	0.00 ± 0.00	2.25 ± 1.65	0.10 ± 0.00	28.75 ± 4.11^{b3}
Car6.25	7.75 ± 2.06	5.00 ± 1.83	1.75 ± 0.85	0.00 ± 0.00	3.50 ± 1.85	0.25 ± 0.25	18.25 ± 3.94^{b1}
Thy12.5	3.50 ± 3.18	5.00 ± 5.00	0.00 ± 0.00	0.25 ± 0.25	1.75 ± 1.75	0.25 ± 0.25	10.75 ± 10.42^{a1}
Thy6.25	10.50 ± 2.40	14.25 ± 3.90	0.75 ± 0.48	0.00 ± 0.00	3.50 ± 0.87	0.15 ± 0.00	29.00 ± 6.47^{b3}
Lin12.5	9.25 ± 3.59	18.25 ± 3.82	0.75 ± 0.48	0.00 ± 0.00	6.00 ± 2.20	0.25 ± 0.25	34.50 ± 5.87^{b3}
Lin6.25	10.25 ± 3.97	8.00 ± 4.69	0.50 ± 0.50	0.00 ± 0.00	3.00 ± 1.78	0.50 ± 0.50	22.25 ± 7.90^{b2}
AP12.5	11.25 ± 3.50	22.25 ± 2.69	0.75 ± 0.75	0.25 ± 0.25	4.50 ± 0.87	0.25 ± 0.25	33.25 ± 4.61^{b3}
AP6.25	8.00 ± 3.49	6.00 ± 3.67	0.00 ± 0.00	0.00 ± 0.00	3.50 ± 2.18	0.40 ± 0.00	17.50 ± 9.08^{b1}
Pre-treatment (mg/L)							
NC	4.00 ± 1.58	7.75 ± 0.85	0.00 ± 0.00	0.00 ± 0.00	2.75 ± 0.47	1.45 ± 0.25^{b3}	10.65 ± 0.49
LN10	15.50 ± 2.06^{a3}	18.00 ± 1.73^{a3}	9.75 ± 2.28^{a3}	0.50 ± 0.50	3.50 ± 2.06	4.75 ± 0.48^{a3}	2.55 ± 0.82^{a3}
EO12.5+LN10	0.00 ± 0.00	1.25 ± 0.75^{a2}	0.25 ± 0.25	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.09$^{a1, b3}$	2.00 ± 0.12^{a3}
EO6.25+LN10	0.00 ± 0.00	3.75 ± 0.75	2.25 ± 0.62	0.00 ± 0.00	0.00 ± 0.00	0.60 ± 0.12^{b3}	2.25 ± 0.51^{a3}
Car12.5+LN10	4.25 ± 1.10	9.50 ± 1.25	0.50 ± 0.28	1.00 ± 0.75^{a1}	2.50 ± 0.50	1.77 ± 0.13^{b3}	5.95 ± 0.38^{a2,b1}
Car6.25+LN10	1.75 ± 1.03	2.00 ± 1.22^{a1}	1.25 ± 0.75	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.23^{b3}	7.42 ± 0.41^{b3}
Thy12.5+LN10	6.50 ± 1.19	7.50 ± 1.44	2.50 ± 0.28	0.00 ± 0.00	2.75 ± 0.85	1.92 ± 0.31^{b3}	6.80 ± 1.25^{a1,b2}
Thy6.25+LN10	0.75 ± 0.25	6.25 ± 1.10	0.75 ± 0.47	0.00 ± 0.00	0.75 ± 0.47	0.85 ± 0.10^{b3}	7.70 ± 0.85^{b2}
Lin12.5+LN10	0.75 ± 0.47	3.00 ± 0.70	2.25 ± 0.94	0.25 ± 0.25	0.75 ± 0.47	0.70 ± 0.23^{b3}	2.75 ± 0.29^{a3}
Lin6.25+LN10	1.25 ± 0.48	3.00 ± 0.70	4.50 ± 1.93^{a1}	0.00 ± 0.00	0.25 ± 0.25	0.90 ± 0.26^{b3}	3.55 ± 0.88^{a3}
AP12.5+LN10	1.00 ± 0.70	12.50 ± 2.17	1.00 ± 0.57	0.00 ± 0.00	1.75 ± 1.10	1.62 ± 0.37^{b3}	9.20 ± 0.46
AP6.25+LN10	5.25 ± 1.37	2.75 ± 1.25	0.50 ± 0.50	0.00 ± 0.00	0.00 ± 0.00	0.85 ± 0.22^{b3}	20.57 ± 1.82$^{a3, b3}$

NC: Negative Control; LN10: Lead Nitrate-10mg/L; Thy6.25 /12.5:Thymol:6.25 mg/L/12.5 mg/L ; Car6.25 /12.5:Carvacrol-6.25 mg/L/12.5 mg/L; EO6.25 /12.5:Essantial Oil-6.25 mg/L/12.5 mg/L; Lin6.25 /12.5: Linalool-6.25 mg/L/12.5 mg/L; AP6.25 /12.5:*α*-Pinene; 6.25 mg/L/12.5 mg/L; Irreg. Meta: Irregular Metaphase; MN: Micronucleus; Vag. Chr: Vagrant Chromosome; MI: Mitotic Index; T.Ab.C.; Total Aberrant Cells; MI: Mitotic Index. a^1:(*) $P \leq 0.05$; a^2:(**) $P \leq 0.01$; a^3:(***) $P \leq 0.001$ when compared with negative control. b^1:(*) $P \leq 0.05$; b^2:(**) $P \leq 0.01$; b^3:(***) $P \leq 0.001$ when compared with lead nitrate.

As a result of this study, it can be said that lead has a serious toxic effect (Figure 2E-J). The lead nitrate reduces the MI values in *A. cepa* root tip cells can be explained by the disruption of DNA integrity in the cells because of chromosomal damage induced by lead nitrate and the possible negative effects of these damages on the cell cycle. Considering the results in Table 2, it is seen that MI levels increased in all treatments except essential oil compared to the positive control (lead nitrate; 10 mg/L). This result reveals the protective effect of components of *O. onites*-EOs. The protective effect of *O. onites*-EO components against the damage caused by lead nitrate in cells can be listed as AP6.25>AP12.5>Thy6.25>Thy12.5>Car6.25>Car12.5>Lin6.25>Lin12.5 (Table 2 and Figure 1). It appears that the protective effect is higher at lower dosages. Furthermore, AP6.25 significantly increased the MI rate. It was observed that the MI value in the AP6.25 treatment increased approximately 8 times

when compared to the positive control. As a result of this study, it was determined that *α*-pinene (AP6.25-AP12.5) had the strongest protective effect among all essential oil components tested.

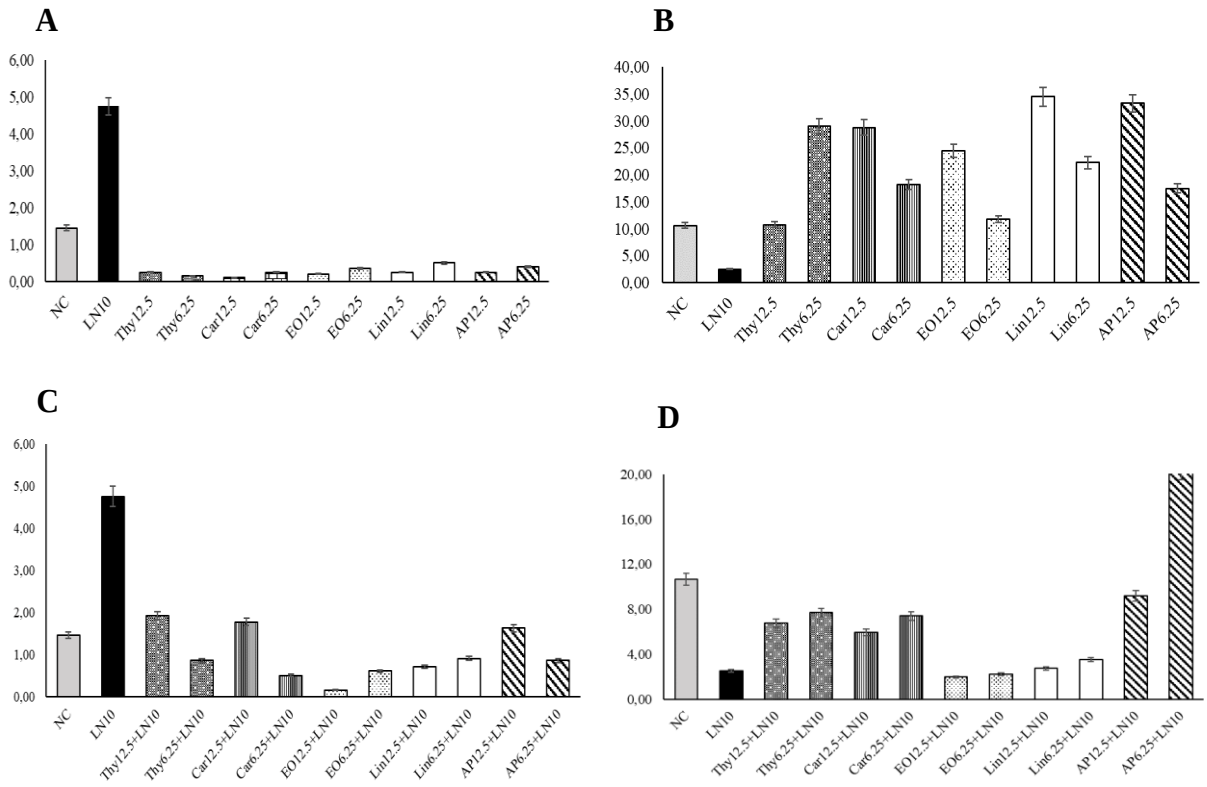

Figure 1. The percentages of Total abnormality (A for treatment and C for pre-treatment) and Mitotic index (B for treatment and D for pre-treatment) in *A. cepa* treated with *O. onites*-EO/components of *O. onites*-EO

Furthermore, the protective effect of the essential oil, which contains all the components, could not be determined. It has been observed that the components of essential oil have different effects, and it can be said that these components suppress the effects of each other in the mixture where they are found together. Considering that the component dosages in the essential oil have decreased, it can be thought that the phytochemicals are not in protective dosage, that is, they are insufficient (Table 2 and Figure 1). However, this study generally shows that the most important chromosome abnormality in *A. cepa* root cells is the formation of sticky chromosomes (Table 2 and Figure 2F). Sticky chromosome abnormality is associated with DNA depolymerization, partial dissolution of nucleoproteins, and increased chromosomal condensation. Chromosomal stickiness also causes irreversible cell damage [53]. Chromosomal abnormalities observed in *A. cepa* indicate a toxic effect on the cell, and serious abnormalities have been observed in lead nitrate treatments. This result reveals the genotoxic effect of lead nitrate. It can be said that this genotoxicity is suppressed by *Origanum*- EO components, mainly *α*-pinene. In conclusion, this study shows that *Origanum*-EO components have a protective effect on lead nitrate-induced *A.cepa* root tip cells.

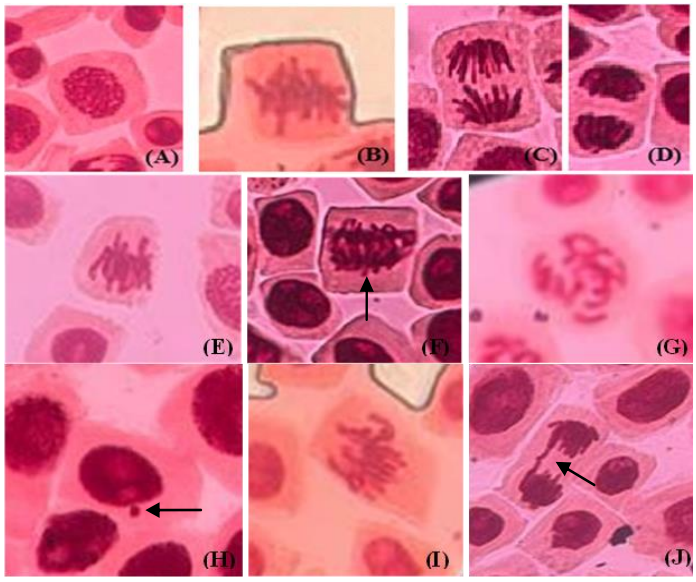

Figure 2. Stages of mitosis in negative control groups (A–D): (A) Prophase, (B) Metaphase, (C) Anaphase, (D) Telophase; Mitotic division abnormalities determined in Lead nitrate groups (E-J): (E)Irregular metaphase, (F) Stickiness, (G) C-mitosis, (H) Micronucleus, (I) Vagrant chromosome, (J) Bridge

In recent years, there has been a growing interest in the utilization of medicinal and aromatic plants [54]. These plants yield essential oils containing a wealth of biologically active compounds renowned for their antioxidant and radical scavenging properties [55,56]. Recently, the biological activity of *O. onites*-EO and its components has become a focus of interest for researchers [11]. However, no study has been found on the use of *O. onites* essential oil and its components in the protection of *A. cepa* root tip cells induced by lead nitrate. In this context, in this study, the protective effect of *O. onites*-EO and essential oil components (Thymol, Carvacrol, Linalool, and *α*-Pinene) on lead nitrate-induced *A. cepa* root tip cells were investigated. As a result of the study, the protective effect of all components except essential oil was determined. Alpha pinene showed the highest protective effect. The protective effect of essential oil components can be listed as *α*-pinene, thymol, carvacrol, and linalool. *α*-Pinene, which is among the monoterpene compounds, is found in the essential oils of many plants. Numerous pharmacological activities have been documented, encompassing antibiotic resistance modulation, anticoagulant, antitumor, antimicrobial, antimalarial, antioxidant, anti-inflammatory, anti-Leishmania, and analgesic effects [57]. In this study, although *alpha*-pinene is not very high (0.16%-0.18%), it is contained in the essential oil of *O. onites*. As in this study, there are some studies in the literature regarding its protective effect against cytotoxicity. The protective effect of *α*-pinene against aspirin-induced cytotoxicity was detected in IEC-6 cells (small intestinal epithelial cells) of rats [58]. In a different study, the protective effect of *α*-pinene on UVA-induced oxidative imbalance in human skin epidermal keratinocytes (HaCat cells) was determined [59]. In this study, the high protective effect of *α*-pinene on *A. cepa* root tip cells against the cytotoxic/genotoxic effects of lead nitrate was detected for the first time. Thymol, a phenolic monoterpene, is found in the essential oils of many plants and is known for its versatile activity, including antioxidant, antimicrobial, and anti-inflammatory properties [60,61]. In a study, biochemical results and histological findings revealed that thymol may have a protective effect against cisplatin ototoxicity by increasing antioxidant levels and reducing oxidative stress parameters [62]. A different study suggests that thymol protects the liver against toxicity caused by carbon tetrachloride (CCl_4) and that this protection may be mediated through its ability to inhibit lipid peroxidation [63]. Carvacrol, another *O. onitum*-EO component whose protective effect was tested in this study, is present in the essential oil at a very high rate (83.21%). Various studies have shown that carvacrol has antioxidant, anti-inflammatory, antitumor, analgesic, antihepatotoxic, antimicrobial, and insecticidal activities [64]. Carvacrol has strong antioxidant properties and may be effective in preventing and preventing various diseases [65]. A study revealed the protective effect of carvacrol against oxidative stress caused by chronic stress in the brain, liver, and

kidney of rats [66]. Linalool, another molecule whose protective activity was tested in this study, is known to have various biological activities such as antimicrobial, anti-inflammatory, anticancer, and antioxidant properties [67]. Linalool has been found to induce apoptosis of cancer cells and protect normal cells [68]. The protective effect of linalool has been proven by previous studies and it has been shown that linalool reduces neurodegeneration and neural damage that may occur due to rifampicin [69]. As a result, the protective effects of the *O. onites*-EO components (*α*-pinene, thymol, carvacrol, and linalool) tested in this study on *A. cepa* root tip cells against the cytotoxic/genotoxic effect of lead nitrate were determined, in parallel with other studies.

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References

[1] M. Boran and I. Altınok (2010). A Review of heavy metals in water, sediment and living organisms in the Black Sea, *Turk. J. Fish. Aquat. Sci.* **10**, 565-572.
[2] T. Özer, C. Caner , E. Altıntığ and H. Altundağ (2022). Determination of some heavy metal deposits in gluten-free foods in Turkish market with ICP-OES, *J. Chem. Metrol.* **16**,135-146.
[3] K. Thürmer, E. Williams and J. Reutt Robey (2002). Autocatalytic oxidation of lead crystallite surfaces, *Science* **297**, 2033-2035.
[4] G. Flora, D. Gupta and A. Tiwari (2012). Toxicity of lead: A review with recent updates, *Interdiscip. Toxicol.* **5**, 47-58.
[5] M. Ahamed and M. Siddiqui (2007). Low level lead exposure and oxidative stress: current opinions, *Clin. Chim. Acta.* **383**, 57-64.
[6] M. Ahamed, M. J. Akhtar and M. A. M. Khan (2020). Single-walled carbon nanotubes attenuate cytotoxic and oxidative stress response of Pb in human lung epithelial (A549) cells, *Int. J. Environ. Res. Public Health.* **17**, 8221.
[7] M. Jaishankar, T. Tseten, N. Anbalagan, B. B. Mathew and K. N. Beeregowda (2014). Toxicity, mechanism and health effects of some heavy metals, *Interdiscip. Toxicol.* **7**, 60-72.
[8] U. Najeeb, W. Ahmad, M. H. Zia, Z. Malik and W. Zhou (2014). Enhancing the lead phytostabilization in wetland plant *Juncus effusus* L. through somaclonal manipulation and EDTA enrichment, *Arab. J. Chem.* **10**, 3310-3317.
[9] J. Checa and J. M. Aran (2020). Reactive oxygen species: drivers of physiological and pathological processes, *J. Inflamm. Res.* **2**, 1057-1073.
[10] B. L. Zaric, M. T. Macvanin and E. R. Isenovic (2023). Free radicals: relationship to human diseases and potential therapeutic applications, *Int. J. Biochem. Cell Biol.* **154**, 106346.
[11] A. Aykac, E. Becer, D. Özbeyli, G. Şener and K. H. C. Başer (2020). Protective effects of *Origanum onites* essential oil in the methotrexate-induced rat model: role on apoptosis and hepatoxicity, *Rec. Nat. Prod.* **14**, 395-404.
[12] B. Tepe, A. Cakir and A. Sihoglu Tepe (2016). Medicinal uses, phytochemistry, and pharmacology of *Origanum onites* (L.): A review, *Chem. Biodivers.* **13**, 504-520.
[13] S. El-Gengaihi, H. S. Taha and A. M. Kamel (2006). In vivo and in vitro comparative studies of *Origanum* species, *J. Food Agric. Environ.* **4**, 127-134.
[14] K. H. C. Başer (2001). Her derde deva bitki kekik, Bilim ve Teknik. 74-77.
[15] Z. Özer, S. Çarıkçı, H. Yılmaz., T. Kılıç, T. Dirmenci and A. C. Gören (2020). Determination of secondary metabolites of *Origanum vulgare* subsp. *hirtum* and *O. vulgare* subsp. vulgare by LC-MS/MS, *J. Chem. Metrol.* **14**, 25-34.

[16] S. Çarıkçı, S. Kılıç, T. Özer, Z. Dirmenci, T. Arabaci and A. C. Gören (2018). Quantitative determination of some phenolics in *Origanum laevigatum* boiss. extracts via validated LC-MS/MS method and antioxidant activity, *J. Chem. Metrol.* **12**, 121-127.

[17] S. Çarıkçı, T. Kılıç, T. Dirmenci and A. C. Gören (2022). Phenolic compounds from section Majorana (Mill.) Benth of Origanum L. species extracts via validated LC-MS/MS method, *J. Chem. Metrol.* **16**, 147-151.

[18] A. Altintas, N. Tabanca, E. Tyihák, P. G. Ott, Á. M. Móricz, E. Mincsovics and D. E. Wedge (2013). Characterization of volatile constituents from *Origanum onites* and their antifungal and antibacterial activity, *J. AOAC Int.* **96**, 1200-1208.

[19] V. Lagouri, G. Blekas, M. Tsimidou, S. Kokkini and D. Boskou (1993). Composition and antioxidant activity of essential oils from *Oregano* plants grown wild in Greece, *Z. Lebensm. Unters. Forsch.* **197**, 20-23.

[20] A. Ayvaz, O. Sagdic, S. Karaborklu and I. Ozturk (2010). Insecticidal activity of the essential oils from different plants against three stored product insects, *J. Insect Sci.* **10**, 1-13.

[21] A. Cetin, U. Arslanbas, B. Saraymen, O. Canoz, A. Ozturk and O. Sagdic (2011). Efects of grape seed extract and *Origanum onites* essential oil on cisplatin-induced hepatotoxicity in rats, *UHOD Uluslararasi Hematol. Derg.* **21**, 133-140.

[22] A. Erdogan and A. Ozkan (2013). A comparative study of cytotoxic, membrane and DNA damaging efects of *Origanum majorana*'s essential oil and its oxygenated monoterpene component linalool on parental and epirubicin-resistant H1299 cells, *Biol.* **68**, 754-761.

[23] R. B. Bostancioglu, M. Kurkcuoglu, K. H. C. Baser and A.T. Koparal (2012). Assessment of anti-angiogenic and anti-tumoral potentials of *Origanum onites* L. essential oil, *Food Chem. Toxicol.* **50**, 2002-2008.

[24] K. Spyridopoulou, E. Fitsiou, E. Bouloukosta, A. Tiptiri Kourpeti, M. Vamvakias, A. Oreopoulou, E. Papavassilopoulou, A. Pappa and K. Chlichlia (2019). Extraction, chemical composition, and anticancer potential of *Origanum onites* L. essential oil, *Molecules* **18**, 2612.

[25] R. Liman, Y. Acikbas and I. H. Cigerci (2019). Cytotoxicity and genotoxicity of cerium oxide micro and nanoparticles by *Allium* and comet tests, *Ecotoxicol. Environ. Saf.* **168**, 408-414.

[26] E. P. D. Vale Junior, M. V. R. Ferreira, B. C. S. Fernandes, T. T. D. Silva, F. A. Martins and P. M. Almeida (2022). Protective effect of kavain in meristematic cells of *Allium cepa* L. *An. Acad. Bras. Cienc.* **13**, e20200520.

[27] E. Bonciu, P. Firbas, C. S. Fontanetti, J. Wusheng, M. C. Karaismailoğlu, D. Liu, F. Menicucci, D. S. Pesnya, A. Popescu, A.V. Romanovsky, S. Schiff, J. Ślusarczyk, C. P. de Souza, A. Srivastava, A. Sutan and A. Papini (2018). An evaluation for the standardization of the Allium cepa test as cytotoxicity and genotoxicity assay, *Caryologia.* **71**, 191-209.

[28] S. Carikci, A. C. Goren, T. Dirmenci, B. Yalcinkaya, A. Erkucuk and G. Topcu (2020). Composition of the essential oil of Satureja metastasiantha: a new species for the flora of Turkey. *Z. Naturforsch*. **75**, 271-277.

[29] V. Morales, A. C. Goren, A. Held, M. Bilsel, S. Gunduz and H. Yilmaz (2015). Validation of a GC–IDMS method for the metrologically traceable quantification of selected FAMEs in biodiesel, *Accredit. Qual. Assur.* **20**, 411-419.

[30] A. C. Gören, T. Kilic, T. Dirmenci and G. Bilsel (2006). Chemotaxonomic evaluation of Turkish Salvia species: fatty acid compositions of seed oils. *Biochem. Syst. Ecol.* **34**, 160-164.

[31] I. T. Oyeyemi (2020). The protective effect of the aqueous extract of *Sida acuta* BURM.F on lead nitrate-induced genotoxicity, *Nova Biotechnol. Chim.* **19**, 192-199.

[32] C. D. Darlington and L. F. Lacour (1979). The handling of chromosome. George Allen and Unwin Ltd., London.

[33] A. Bakare., A. Mosuro. and O. Osibanjo (2000). Effect of simulated leachate on chromosomes and mitosis in roots of *Allium cepa* (L.) *J. Environ. Biol*. **21,** 263-271

[34] L.T. Huong, L.D. Linh, D.N. Dai and I.A. Ogunwande (2023). Essential oil compositions and antimicrobial activity of the leaves of *Alphonsea monogyma* Merr. & Chun and *Goniothalamus banii* B. H. Quang, R. K. Choudhary & V.T. Chinh from Vietnam, *Rec. Nat. Prod*. **17**(**3**), 571-576.

[35] Y. Tang , X. Hu, F. Xu and X. Xing (2023). Chemical composition, antibacterial, synergistic antibacterial and cytotoxic properties of the essential oil from *Gelsemium elegans* (Gardner & Champ.) Benth, *Rec. Nat. Prod*. **17**(**6**), 1074-1079.

[36] X. Li, W. Zhang, Y. Qin and X. Xing (2022). Essential oil from *Hedyotis chrysotricha*: chemical composition, cytotoxic, antibacterial properties and synergistic effects with streptomycin, *Rec. Nat. Prod*. **16**(**4**), 376-381.

[37] A. Musa, K.F. El-Massry, A.H. El-Ghorab, A.Farouk, H.M. Ali, M.A Abdelgawad, I.A. Naguib and E.M. Mostafa (2021). Volatile constituents of *Cistanche tubulosa* and their antioxidant and antimicrobial potentials, *Rec. Nat. Prod*. **15**(**4**), 301-311.

[38] M. Kulić, D. Drakul, D. Sokolović, J. Kordić-Bojinović, S. Milovanović and D. Blagojević (2023). Essential oil of *Satureja montana* L. from Herzegovina: assessment of composition, antispasmodic, and antidiarrheal effects, *Rec. Nat. Prod*. **17**(**4**), 536-548.

[39] M. Akdeniz, I. Yener, A. Ertas, M. Firat, B. Resitoglu, N. Hasimi, S. Irtegun-Kandemir, M. A. Yilmaz, A. Barla Demirkoz, U. Kolak and S. Oksuz (2021). Biological and chemical comparison of natural and cultivated samples of *Satureja macrantha* C.A.Mey, *Rec. Nat. Prod*. **15**(**6**), 568-584.

[40] L.J. Mao, X.Y. Xie, Y. Gao, P.X. Lai and Q.L. Ma (2019). Chemical composition, antibacterial and antioxidant activities of essential oil from *Leonurus pseudomacranthus* Kitag, *Rec. Nat. Prod*. **13**(**1**), 91-95.

[41] J. Liang, Y. Shao, H. Wu, Y. An, J. Wang, J. Zhang, and W. Kong (2021). Chemical constituents of the essential oil extracted from *Elsholtzia densa* and their insecticidal activity against *Tribolium castaneum* and *Lasioderma serricorne*, *Foods* **10**, 2304. doi.org/10.3390/ foods10102304.

[42] R.P. Adams (2000). Systematics of Juniperus section Juniperus based on leaf essential oils and random amplified polymorphic DNAs (RAPDs)*, Biochem. Syst. Ecol.* **6**,515-528.

[43] R.P. Adams and S. Nguyen (2005). Infra-specific variation in *Juniperus deppeana* and *F. Sperryi* in the Davis Mountains of Texas: variation in leaf essential oils and random amplified polymorphic DNAS (RAPDS)*, Phytologia,* **87**(**2**), 96-108.

[44] R. Baranauskiene, R.P. Venskutonis and J.C.R. Demyttenaere (2003). Sensory and instrumental evaluation of catnip *(Nepeta cataria* L.) aroma**,** *J. Agric. Food. Chem***. 51** (1**3**), 3840-3848.

[45] O. Tzakou and T. Constantinidis (2005). Chemotaxonomic significance of volatile compounds in Thymus samius and its related species *Thymus atticus* and *Thymus parnassicus*, *Biochem. Syst. Ecol.* **33**(**11**), 1131-1140.

[46] A. Mohagheghzadeh, M. Shams-Ardakani and A. Ghannadi (2000). Volatile constituents of callus and flower-bearing tops of *Zataria multiflora* Boiss. (Lamiaceae)*, Flavour Fragr. J.* **15**(**6**)*,* 373-376.

[47] J.A. Pino, R. Marbot and F. Fuentes (2003). Characterization of volatiles in Bullock's heart *(Annona reticulata* L.*)* fruit cultivars from Cuba, *J. Agric. Food Chem*. **51**(**13**), 3836-3839.

[48] A.C. Mesa-Arango, L. Betancur-Galvis, J. Montiel, J.G. Bueno, A. Baena, D.C. Duran, J.R. Martinez and E.E. Stashenko (2010). Antifungal activity and chemical composition of the essential oils of *Lippia alba* (Miller) N.E. Brown grown in different regions of Colombia, *J. Essential Oil Res.* **22**(**6**), 568-574.

[49] K. Morteza-Semnani, M. Azadbakht, and Goodarzi (2004). The essential oils composition of *Phlomis herba-venti* L. leaves and flowers of Iranian origin**,** *Flavour Fragr. J.* **19**(**1**), 29-31.

[50] R.P. Adams, J.A. Morris, R.N. Pandey and A.E. Schwarzbach (2005). Cryptic speciation between *Juniperus deltoides* and *Juniperus oxycedrus* (Cupressaceae) in the Mediterranean, *Biochem. Syst. Ecol.* **33**(**8**), 771-787.

[51] I.H.Cho, H.J.Namgung, H.K. Choi and Y.S. Kim (2008). Volatiles and key odorants in the pileus and stipe of pine-mushroom *(Tricholoma matsutake Sing.)*, *Food Chem.* **106**(**1**), 71-76,

[52] R. Sudhakar, N. Gowda and G. Venu (2001). Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*, *Cytologia*. **66**, 235-239.

[53] Turkoglu S. (2007), Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L., *Mutat. Res.* **626**, 4-14.

[54] B. Kiraz Kınoğlu, T. Dirmenci, S. H. Alwasel, İ. Gulcin and A. C. Goren. (2023). Quantification of main secondary metabolites of *Satureja icarica* P.H. Davis (Lamiaceae) by LC-HRMS and evaluation of antioxidant capacities, *J. Chem. Metrol.* **17**, 199-214.

[55] D. Yiğit Hanoğlu, A. Hanoğlu, B. Demirci and K. H. C. Başer (2023). The essential oil compositions of *Teucrium* spp. belonging to the section *Polium Schreb.* (Lamiaceae) growing in Cyprus. *Rec. Nat. Prod.* **17**, 113-124.

[56] B. Demirci, K. H. C. Başer, Z. Aytaç, S. I. Khan, M. R. Jacob and N. Tabanca (2018). Comparative study of three *Achillea* essential oils from eastern part of Turkey and their biological activities, *Rec. Nat. Prod*. **12**, 195-200.

[57] B. Salehi, S. Upadhyay, I. Erdogan Orhan, A. Kumar Jugran, S. L. D. Jayaweera, A. D. Dias, F. Sharopov, Y. Taheri, N. Martins, N. Baghalpour, W. C. Cho and J. Sharifi-Rad (2019). Therapeutic potential of α- and β-Pinene: A miracle gift of nature, *Biomolecules* **14**, 738.

[58] H. Bouzenna, N. Hfaiedh, M. A. Giroux Metges, A. Elfeki and H. Talarmin (2017). Potential protective effects of alpha-pinene against cytotoxicity caused by aspirin in the IEC-6 cells, *Biomed. Pharmacother.* **93**, 961-968.

[59] R. Karthikeyan, G. Kanimozhi, N. R. Prasad, B. Agilan, M. Ganesan and G. Srithar (2018). Alpha pinene modulates UVA-induced oxidative stress, DNA damage and apoptosis in human skin epidermal keratinocytes, *Life Sci.* **212**, 150-158.

[60] F. Bakkali, S. Averbeck, D. Averbeck and M. Idaomar (2008). Biological effects of essential oils a review, *Food Chem. Toxicol.* **46**, 446-475.

[61] M. Sobczak, D. Kalemba, B. Ferenc and L. Zylinska (2014). Limited protective properties of thymol and thyme oil on differentiated PC12 cells with downregulated Mgst1, *J. Appl. Biomed.* **12**, 235-243.

[62] I. Koçak, O. F. Unal, E Aydogan, R. Doğan, D. Akakin, K. Koroglu and O. F. Ozer (2017). The protective effect of thymol against cisplatin-induced ototoxicity: an experimental animal study, *KBB. Forum.* **16**, 43-52.

[63] K. Alam, M. N. Nagi, O. A. Badary, O. A. Al-Shabanah, A. C., Al-Rikabi and A. M. Al-Bekairi (1999). The protective action of thymol against carbon tetrachloride hepatotoxicity in mice, *Pharmacol Res.* **40**, 159-163.

[64] M. Melusova, D. Slamenova, K. Kozics, S. Jantova and E. Horvathova (2014). Carvacrol and rosemary essential oil manifest cytotoxic, DNA-protective and pro-apoptotic effect having no effect on DNA repair, *Neoplasma.* **61**, 690-699.

[65] W. Z. Liang and C. H. Lu (2012). Carvacrol-induced [Ca^{2+}] rise and apoptosis in human glioblastoma cells, *Life Sci.* **90**, 703-711.

[66] S. Samarghandian, T. Farkhondeh, F. Samini and A. Borji (2016). Protective effects of carvacrol against oxidative stress induced by chronic stress in rat's brain, liver, and kidney, *Biochem. Res. Int.* **2016**, 2645237.

[67] G. Kamatou and A. Viljoen (2008). Linalool-a review of a biologically active compound of commercial importance, *Nat. Prod. Commun.* **3**, 1183-1192.

[68] Q. An, J. N. Ren, X. Li, G. Fan, S. S. Qu, Y. Song, Y. Li and S. Y. Pan (2021). Recent updates on bioactive properties of linalool, *Food Funct.* **12**, 10370-10389.

[69] M. Ozgocmen and S. Azırak (2023). The curative role of linalool in rifampicin-induced brain tissue damage, *Med. J. SDU.* **30**, 362-370.

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