

## THE ANTIMUTAGENIC EFFECT OF MONOTERPENES AGAINST UV-IRRADIATION-, 4NQO- AND T-BOOH-INDUCED MUTAGENESIS IN *COLI*

BILJANA NIKOLIĆ, DRAGANA MITIĆ-ĆULAFIĆ, BRANKA VUKOVIĆ-GAČIĆ and  
JELENA KNEŽEVIĆ-VUKČEVIĆ

*Chair of Microbiology, Faculty of Biology, University of Belgrade, 11000 Belgrade, Serbia*

**Abstract** – The aim of this work was to investigate the antimutagenic potential of monoterpenes from sage and basil in *Escherichia coli*. The mutagenic potential of monoterpenes was pre-screened with *Salmonella*/microsome reversion assay in strain TA100 and no mutagenic effect was detected. The antimutagenic potential against UV- 4NQO- and *t*-BOOH-induced mutagenesis was evaluated in *E. coli* K12 and *E. coli* WP2 by reversion assays. The obtained results indicate that camphor and thujone reduce UV- and 4NQO-induced mutations; myrcene reduces *t*-BOOH-induced mutations, while eucalyptol and linalool reduce mutagenicity by all tested mutagens. Considering evolutionary conservation of DNA repair and antioxidative protection, the obtained results indicate that further antigenotoxicity studies should be undertaken in eukaryotes.

**Key words:** Antimutagenesis, monoterpenes, UV-irradiation, 4NQO, *t*-BOOH, *Escherichia coli*

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### INTRODUCTION

In order to protect human health, a relatively new area of research, designated as antimutagenesis/anticancerogenesis, is continuously developing. Different tests, routinely used to detect environmental mutagens, are suitably adapted for identifying agents with antigenotoxic, antimutagenic and anticarcinogenic potential, as well as for elucidating the molecular mechanisms of their action (Kada et al., 1985; Kuroda, 1990; De Flora et al., 2001; De Flora and Ferguson, 2005).

The basic categorization of antimutagens into two major groups, bio-antimutagens and desmutagens, was given by Kada et al. (1986). Bio-antimutagens are modulators of DNA replication and repair which prevent the processing of premutagenic lesions into mutations. Desmutagens are agents that inhibit the conversion of promutagens into mutagens, inactivate

mutagens or prevent their interaction with DNA. Of the desmutagenic agents, antioxidants are especially investigated because reactive oxygen species (ROS) are the causative factors involved in many human degenerative disorders including cancer, and the use of natural antioxidants could be very important for chemoprevention (Kohlimeir et al., 1995; De Flora, 1998).

Terpenes, the largest group of natural substances abundantly found in fruits, vegetables, aromatic and medicinal plants, have been shown to possess cancer chemopreventive and chemotherapeutic activities (for review see Crowell, 1999; Bakkali et al., 2008). The monoterpenes investigated in this study, linalool (Lin), myrcene (Myr), eucalyptol (Euc), camphor (Cam) and thujone (Thu), are present in the essential oils of sage, basil and other aromatic plants that are widely used in our traditional medicine. Numerous biological activities of different extracts of the

*Salvia* and *Ocimum* species, including antimicrobial, anti-inflammatory, antioxidative, antidiarrheal, chemopreventive, blood-sugar lowering, nervous system stimulatory, spasmolytic and cholinergic binding properties, have been described (Cuvelier et al., 1994; Chattopadhyay, 1999; Koga et al., 1999; Offiach and Chikwendu, 1999; Barićević and Bartol, 2000; Klem et al., 2000, Prakash and Gupta, 2000; Zupko et al., 2001; Capasso et al., 2004; Ren et al., 2004; Mitić-Ćulafić et al., 2005).

In our previous work, the antimutagenic effect of the essential oils of sage (*Salvia officinalis* L.) and basil (*Ocimum basilicum* L.) has been detected in prokaryotic and eukaryotic cells (Knežević-Vukčević et al., 2005; Vuković-Gačić et al., 2006a; 2006b; Stajković et al., 2007; Berić et al., 2008; Stanojević et al., 2008). The aim of the present work was to evaluate and compare the bio-antimutagenic and desmutagenic potential of monoterpenes from the essential oils Lin, Myr, Euc, Cam and Thu in *E. coli*. The mutagenic potential of monoterpenes was pre-screened with the *Salmonella*/microsome reversion assay, using the strain TA100 (Maron and Ames, 1983). The antimutagenic potential was determined in *E. coli* K12 (Vuković-Gačić et al., 2006b; Stanojević et al., 2008) and *E. coli* WP2 reversion assays (Urios and Blanco, 1996; Blanco et al., 1998), using strains with a different capacity of DNA repair or antioxidative protection. To assess the bio-antimutagenic potential of monoterpenes, we used UV-irradiation, as well as UV-mimetic 4-nitroquinoline-1-oxide (4NQO) as mutagens. Oxidative mutagen *t*-butyl hydroperoxide (*t*-BOOH) was used to evaluate the desmutagenic effect of monoterpenes based on their antioxidative properties.

Table 1. Bacterial strains

Bacteria	Strain	Relevant marker
<i>S. typhimurium</i>	TA100	<i>hisG46 rfa ΔuvrB bio-/pKM101</i>
<i>E. coli</i> K12	SY252	<i>argE3</i>
	IB105	as SY252 but <i>uvrA::Tn10</i>
<i>E. coli</i> B WP2	IC185	<i>trpE65</i>
	IC202	<i>trpE65 oxyR/pKM101</i>

## MATERIAL AND METHODS

### Bacterial strains

The bacterial strains used in this study are listed in Table 1.

### Chemicals

Linalool (Cas No. 78-70-6, Sigma Aldrich), Myrcene (Cas No. 123-35-3, Fluka), Eucalyptol (Cas No. 207-431-5, Fluka), D, L-Camphor (Cas No. 76-22-2, Alfa Aesar) and  $\alpha, \beta$ -Thujone (Cas No. 76231-76-0, Sigma Aldrich) were freshly dissolved in 96% ethanol. 4-Nitroquinoline-N-oxide (Cas No. N-8141, Sigma Aldrich) was dissolved first in DMSO and then ten-fold diluted in distilled water. *t*-Butyl hydroperoxide (Cas No. 75-91-2, Sigma Aldrich) was dissolved in distilled water immediately before use.

### Media and growth conditions

Bacteria were grown in LB medium at 37°C (Stanojević et al., 2008). All media for *S. typhimurium* reversion assay were as described by Maron and Ames (1983). Semi-enriched minimal medium - SEM (Nikolić et al., 2004) and top agar (5 g NaCl, 6 g agar, 1000 ml distilled water) were used for *E. coli* K12 reversion assay. Media used for WP2 reversion assay were previously described by Blanco et al. (1998).

The S9 fraction was isolated from the liver of Albino Wister male rats (170-180 g) induced with phenobarbital/ $\beta$ -naphthoflavone (Ong et al., 1980). The S9 mixture contained 4% (v/v) S9 fraction, 33 mM KCl, 8 mM MgCl<sub>2</sub>, 5 mM glucose-6-phosphate and 4 mM NADP in 0.1 M phosphate buffer pH 7.4 (Maron and Ames, 1983).

### Evaluation of the mutagenic potential of monoterpenes

The overnight culture of *S. typhimurium* TA100 strain was washed by centrifugation and resuspended in the same volume of 0.01 M MgSO<sub>4</sub>. Samples (0.1 ml) were added to 3 ml of molten top agar with or

without the S9 mixture (0.3 ml), mixed and poured in duplicate onto minimal glucose agar plates with different concentrations of monoterpenes. Ethanol was used as a negative control, while the well-known mutagens benzo[*a*]pyrene (B[*a*]P), 4NQO and UV-irradiation were used as positive controls. After incubation at 37°C for 48h, the number of His<sup>+</sup> revertants was determined and the presence of the bacterial background lawn on all plates was confirmed.

#### *UV-irradiation conditions*

UV-irradiation was carried out with a germicidal lamp (Benda, NU-8 KL) with a maximal output at 254 nm. Dose rates were measured with a Latarjet dosimeter (Latarjet et al., 1953). Cell suspensions in 0.01 M MgSO<sub>4</sub> were irradiated in glass Petri dishes at a thickness of less than 1 mm (Simić et al., 1985). UV-treated cell suspensions were kept in the dark to prevent photoreactivation.

#### *Evaluation of the bio-antimutagenic potential of monoterpenes*

For detection of the antimutagenic potential against UV-induced mutagenesis, the overnight cultures of SY252 and IB105 strains were washed by centrifugation at 4000 rpm for 10 min, resuspended in the same volume of 0.01 M MgSO<sub>4</sub> and UV-irradiated. The applied UV doses were 28 J/m<sup>2</sup> for SY252 and 3 J/m<sup>2</sup> for IB105. Samples (0.1 ml) of unirradiated and UV-irradiated cells, appropriately diluted for determination of cell survival and Arg<sup>+</sup> revertants, were added to 3 ml of molten top agar, mixed and poured in duplicate onto 3% SEM plates with different concentrations of monoterpenes, and incubated at 37°C for 48 h.

For detection of the antimutagenic potential against 4NQO-induced mutagenesis, the overnight cultures of SY252 and IB105 strains were pre-treated with a water solution of 4NQO for 40 min at 37°C with aeration at 150 rpm. The final concentration of 4NQO was 50 µg/ml for SY252 and 5 µg/ml for IB105. After 4NQO-treatment, the cells were washed by centrifugation at 4000 rpm for 10 min and re-suspended in the same volume of 0.01 M MgSO<sub>4</sub>.

4NQO-treated and untreated cell suspensions, appropriately diluted for determination of cell survival and Arg<sup>+</sup> revertants, were added to 3 ml of molten top agar, mixed and poured in duplicate onto 3% SEM plates with different concentrations of monoterpenes and incubated at 37°C for 48h.

#### *Evaluation of the desmutagenic potential of monoterpenes*

For detection of the desmutagenic potential in *E. coli* K12, the overnight culture of SY252 (100 µl), solution of *t*-BOOH (100 µl), and appropriate monoterpene dilution (100 µl) were added to 3 ml of molten top agar, mixed and poured onto 3% SEM. The concentration of *t*-BOOH was 250 µg/plate. The number of Arg<sup>+</sup> revertants was scored after incubation for 48 h at 37°C. The number of surviving cells was determined by plating appropriate dilutions onto 3% SEM.

For detection of the desmutagenic potential in *E. coli* WP2, the overnight cultures of IC185 or IC202 (100 µl), solution of *t*-BOOH (100 µl), and appropriate monoterpene dilution (100 µl) were added to 3 ml of molten top agar, mixed and poured onto minimal ET4 plates (minimal medium, supplemented with 0.5 µg/ml tryptophan). The concentration of *t*-BOOH was 25 µg/plate for IC202 and 250 µg/plate for IC185 strain. The number of Trp<sup>+</sup> revertants was scored after incubation for 48 h at 37°C. The number of surviving cells was determined by plating appropriate dilutions on LA plates (LB containing 15g/l agar).

#### *Calculation of inhibition of mutagenesis*

The inhibition of the mutagenic effect was calculated according to the equation: % I (% of inhibition) = (1 - TM/M) x 100, where TM is the number of revertants per plate in the presence of mutagen and tested monoterpene, and M is the number of revertants per plate in the presence of mutagen alone. The anti-mutagenic effect was considered strong when inhibition of mutagenesis was higher than 40%, moderate when it was in the range between 25% and 40%, and

weak or absent when the inhibitory effect was less than 25% (Ikken et al., 1999).

### Statistical analysis

The Student's t-test was used for statistical analysis of the data obtained in bacterial mutagenicity and antimutagenicity tests. The significance was tested at  $p < 0.05$  level.

## RESULTS AND DISCUSSION

### Mutagenic potential of monoterpenes

The mutagenic effect of monoterpenes was tested in the *Salmonella*/microsome mutagenicity assay with

TA100 strain (Maron and Ames, 1983). The well known mutagens B[a]P, 4NQO and UV-irradiation were used as positive controls. None of the tested monoterpenes, with or without metabolic activation by the S9 fraction, induced an increase in the number of His<sup>+</sup> revertants, compared with the corresponding solvent controls, while all tested mutagens induced a mutagenic response (Tab. 2). This is in agreement with previous studies that did not observe mutagenicity of Lin, Myr, Euc, Cam and Thu in *Salmonella*/microsome and WP2 reversion tests (Ishidate et al., 1984; Yoo, 1986; Heck et al., 1989; Gomes-Carneiro et al., 1998; Letizia et al., 2003), as well as in mammalian *in vitro* genotoxicity assays (Kauderer et al., 1991; Letizia et al., 2003; Di Sotto et al., 2010).

**Table 2.** Mutagenicity testing of monoterpenes in the *Salmonella*/microsome TA100 assay

Conc. <sup>1</sup>	His <sup>+</sup> revertants/plate (mean ± SE) <sup>2</sup>									
	$\alpha+\beta$ Thujone		Eucalyptol		Linalool		Camphor		Myrcene	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0	122±15	134±13	110±13	118±11	134±7	138±4	134±11	104±13	110±13	118±11
Et <sup>3</sup>	122±11	124±13	114±12	120±11	138±8	128±9	111±4	104±12	125±11	125±7
0.25	nt <sup>4</sup>	nt	nt	nt	nt	nt	118±5	96±11	nt	nt
0.5	nt	nt	nt	nt	110±1	117±9	114±9	90±14	nt	nt
1.0	109±17	120±14	104±15	120±8	124±5	121±15	114±8	108±14	94±11	132±10
2.0	134±13	130±10	111±10	116±14	120±12	125±13	nt	nt	nt	nt
5.0	127±17	128±15	114±7	110±5	128±11	135±3	146±23	94±5	115±8	106±12
10.0	121±8	130±13	100±5	110±13	nt	nt	91±16	102±17	110±5	106±9
B[a]P <sup>5</sup>	139±3	842±12								
4NQO <sup>6</sup>	996±17	nt								
UV-irradiation <sup>7</sup>	1180±53	1364±93								

<sup>1</sup> Concentrations are expressed in µg/plate for Camphor and µl/plate for other monoterpenes

<sup>2</sup> Data are from three independent experiments performed in duplicates

<sup>3</sup> Ethanol was used as solvent

<sup>4</sup> Not tested

<sup>5</sup> benzo[a]pyrene in DMSO (15 µg/plate)

<sup>6</sup> 4-Nitroquinoline-N-oxide in distilled water (0.15 µg/plate)

<sup>7</sup> UV-dose 6 J/m<sup>2</sup>

### Bio-antimutagenic potential of monoterpenes

The bio-antimutagenic potential of monoterpenes was studied in *E. coli* K12 strains possessing differing DNA repair capacity. Strain SY252 is repair proficient, while its counterpart IB105 is deficient in nucleotide excision repair (NER) due to *uvrA::Tn10* mutation (Simić et al., 1998; Vuković-Gačić et al., 2006b). Both strains contain *argE3* mutation (ochre), leading to auxotrophy and can revert to prototrophy by base substitutions of AT base pairs (Tood et al., 1979).

To avoid the possibility of interaction between the mutagen and antimutagen, i.e. to avoid detection of the desmutagenic effect of monoterpenes, we used UV-irradiation (254 nm) or pre-treatment with 4NQO. The main DNA lesions induced by UV and 4NQO are pyrimidine dimers and stable 4NQO-induced adducts, respectively, both repaired by NER (Homme et al., 2000; Friedberg et al., 2006). In our preliminary experiments, the doses of mutagens induced a high mutagenic response, with about 50% of lethality. They were 28 J/m<sup>2</sup> of UV and 50 µg/ml of 4NQO for the repair proficient strain, and 3 J/m<sup>2</sup> of UV and 5 µg/ml of 4NQO for NER for the deficient strain.

In the range of non-toxic concentrations (Cam up to 20 µg/plate, Myr up to 3 µl/plate, Lin up to 10 µl/plate, Euc and Thu up to 15 µl/plate), none of the tested monoterpenes had any significant effect on the spontaneous revertants number in both SY252 and IB105 strains (data not shown).

After UV and 4NQO treatment of the repair proficient strain SY252, a significant reduction of revertant number was detected in the presence of Thu, Euc and Cam (Fig. 1 A, B). Inhibition of UV- and 4NQO-induced mutagenesis with Cam reached a maximum at 10 µg/plate, and was 44% and 49%, respectively. Maximum inhibition with Euc was at 10 µl/plate for UV (45%) and at 7.5 µl/plate for 4NQO (41%). Similar results were obtained with Thu, with maximum inhibition at 5 µl/plate for UV (44%) and at 10 µl/plate for 4NQO (41%). In contrast, the anti-

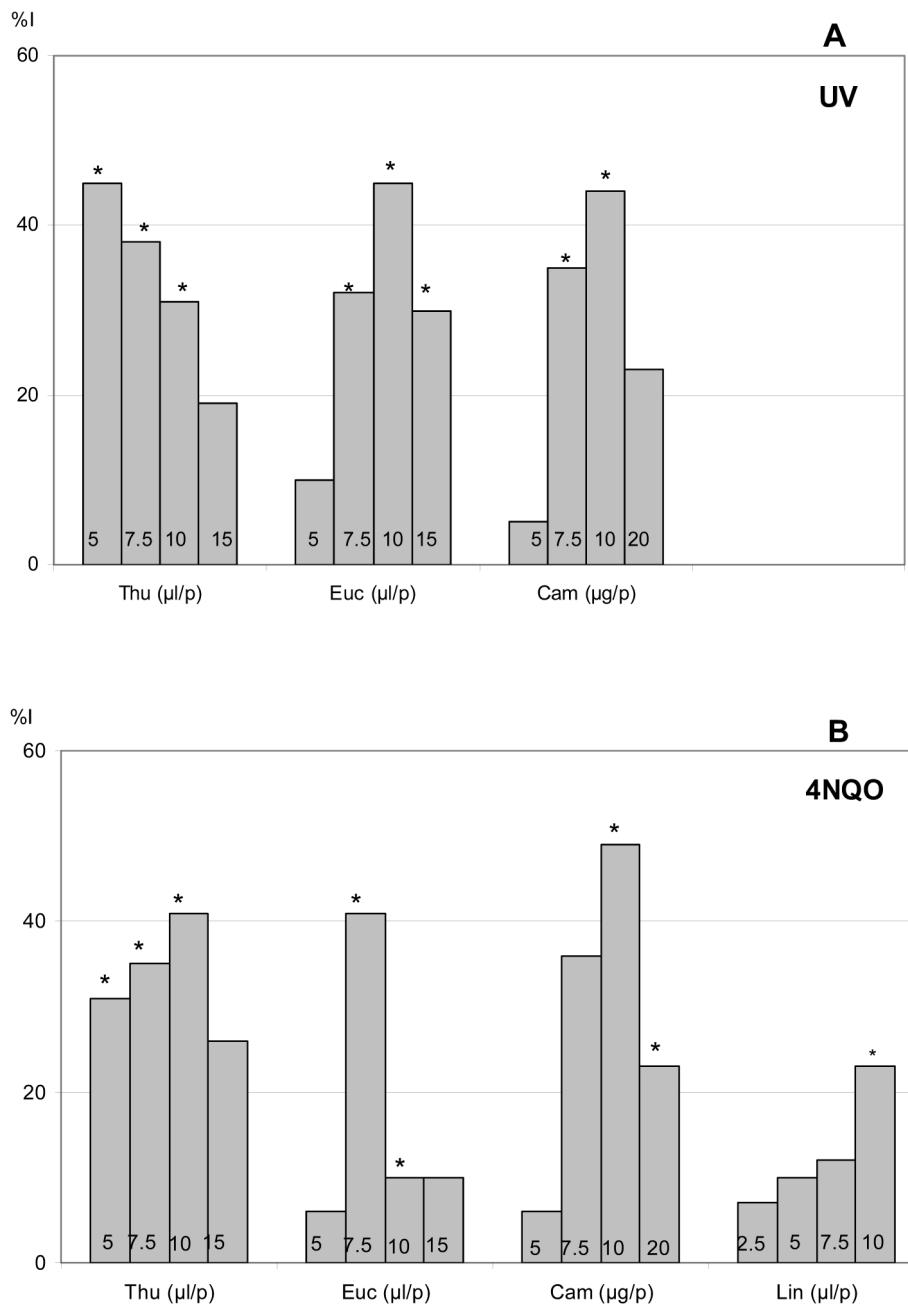
mutagenic effect of Lin was weak. Maximum inhibition against 4NQO-induced mutagenesis was 23% at 10 µl/plate (Fig. 1B). Under slightly different experimental conditions, Stanojević et al., (2008) reported that Lin reduced UV-induced mutagenesis in SY252 for 24% at 10 µl/plate. In the range of tested concentrations, Myr did not show a protective effect against either UV- or 4NQO-induced mutagenesis (data not shown).

The protective effect of Euc, Lin, Cam and Thu against UV- and 4NQO-induced mutagenesis, observed in the repair proficient SY252, was diminished in the NER deficient strain IB105 (data not shown), indicating that a possible mechanism of their bio-antimutagenic action could be the modulation of NER. A similar mechanism of bio-antimutagenesis has already been proposed for Lin (Stanojević et al., 2008). However, the involvement of other mechanisms of bio-antimutagenesis is not excluded.

### Desmutagenic potential of monoterpenes

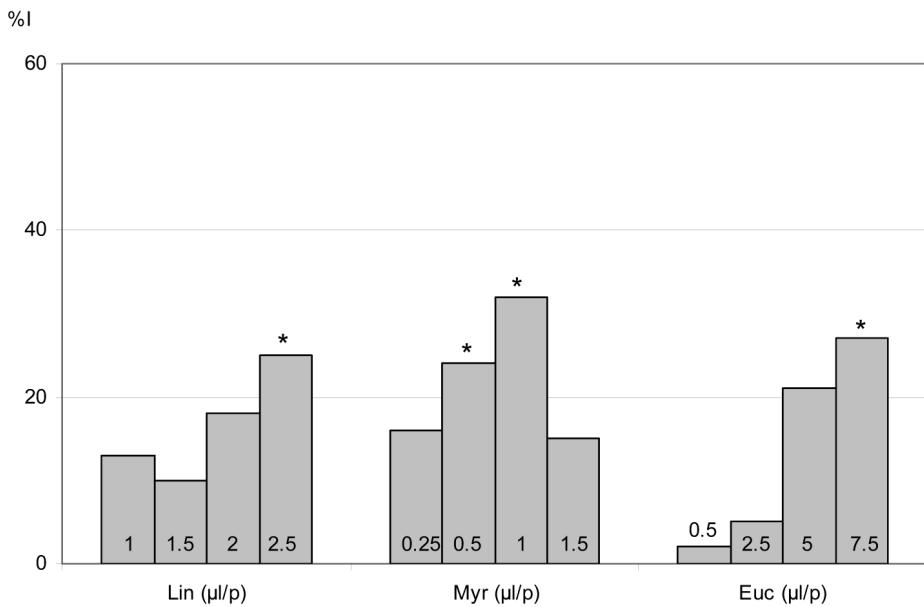
A protective effect against oxidative DNA damage and mutagenesis was determined for Lin, Myr and Euc; their protective effect against lipid peroxidation has been confirmed by TBA assay (Mitić-Ćulafić, 2009). The desmutagenic potential against *t*-BOOH-induced mutagenesis was measured in strains SY252 and IC185, both proficient in the induction of antioxidative enzymes. In the IC185 strain (*E. coli* WP2) reversion of the *trpE65* (ochre) mutation to Trp<sup>+</sup> was monitored (Urios and Blanco, 1996; Blanco et al., 1998). In our preliminary experiments, the concentration of *t*-BOOH was chosen to give a high mutagenic response, with about 50% of lethality, and it was 250 µg/plate for both strains (Mitić-Ćulafić, 2009).

Lin, Myr and Euc slightly reduced *t*-BOOH-induced mutagenesis in both strains, while no significant effect on the spontaneous revertants number was observed. The highest obtained inhibition in SY252 was 32% with 1 µl/plate of Myr, 27% with 7.5 µl/plate of Euc and 25% with 2.5 µl/plate of Lin (Fig. 2). In strain IC185 the obtained inhibition was simi-



**Fig. 1.** Bio-antimutagenic effect of monoterpenes against UV- (A) and 4NQO- (B) induced mutagenesis in repair proficient SY252 strain.

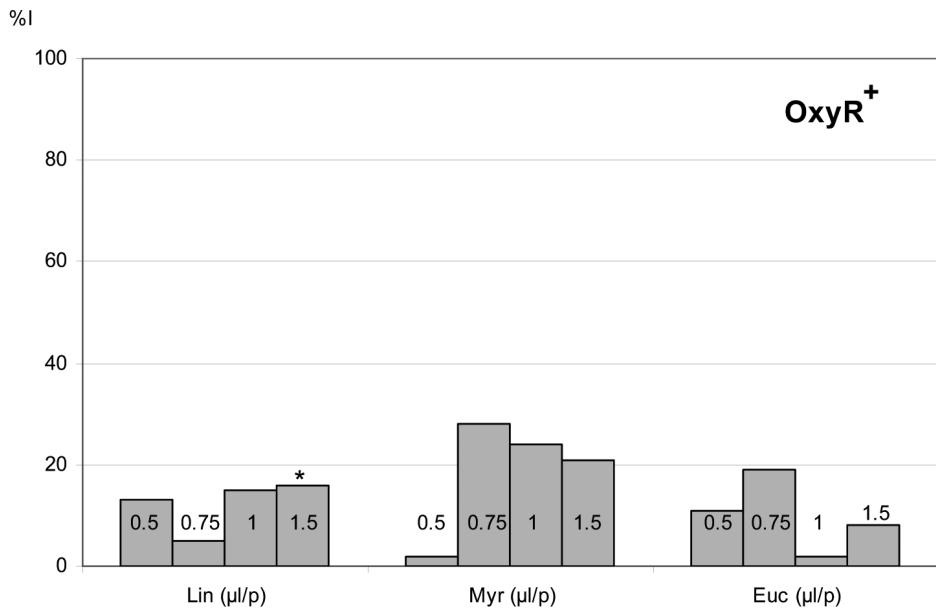
The effect of Lin on UV-induced mutagenesis was reported previously (Stanojević et al., 2008). The number of spontaneous revertants was 20–50/plate. The number of UV-induced revertants/plate in solvent control was  $162 \pm 10$  for Thu,  $156 \pm 7$  for Euc and  $194 \pm 10$  for Cam. The number of 4NQO-induced revertants/plate in solvent control was  $148 \pm 8$  for Thu,  $183 \pm 5$  for Euc,  $247 \pm 9$  for Cam and  $131 \pm 3$  for Lin.  
\*  $p < 0.05$  compared with corresponding solvent control



**Fig. 2.** Desmutagenic effect of monoterpenes against *t*-BOOH-induced mutagenesis in SY252 strain.

The number of spontaneous revertants was 20-50/plate. The number of *t*-BOOH-induced revertants/plate in solvent control was  $114 \pm 2$  for Lin,  $168 \pm 7$  for Myr and  $114 \pm 10$  for Euc.

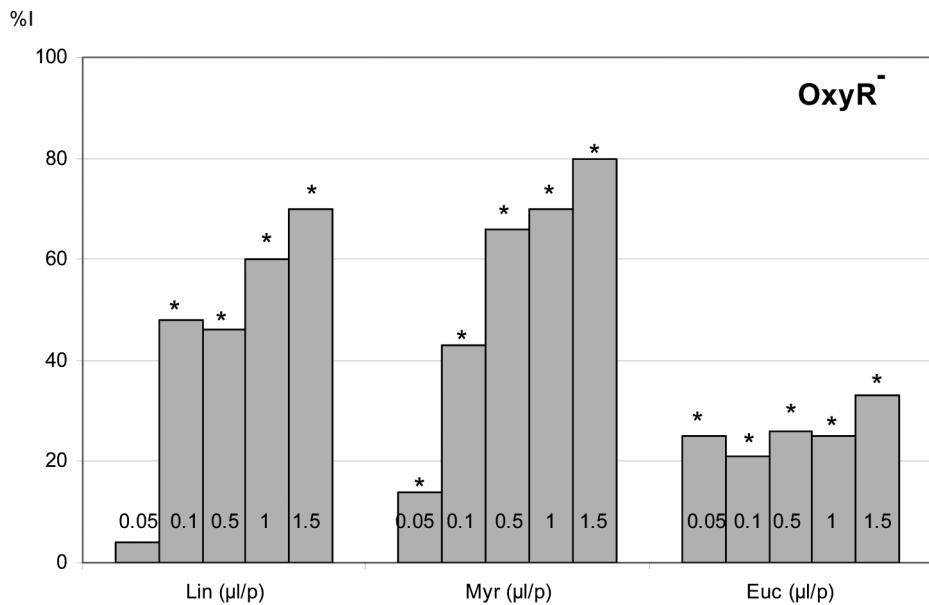
\*  $p < 0.05$  compared with corresponding solvent control



**Fig. 3.** Desmutagenic effect of monoterpenes against *t*-BOOH-induced mutagenesis in OxyR proficient IC185 strain.

The number of spontaneous revertants was 18-33/plate. The number of *t*-BOOH-induced revertants/plate in solvent control was  $172 \pm 17$  for Lin,  $173 \pm 3$  for Myr and  $125 \pm 16$  for Euc.

\*  $p < 0.05$  compared with corresponding solvent control



**Fig. 4.** Desmutagenic effect of monoterpenes against *t*-BOOH-induced mutagenesis in OxyR<sup>-</sup> deficient IC202 strain. The number of spontaneous revertants was 40–52/plate. The number of *t*-BOOH-induced revertants/plate in solvent control was 254±4 for Lin, 343±7 for Myr and 273±10 for Euc.  
\* p<0.05 compared with corresponding solvent control

lar: 28%, 19% and 16% for Myr, Euc and Lin, respectively (Fig. 3). As expected, the maximum inhibitory concentrations of monoterpenes in IC185 were lower than in SY252, due to the higher permeability of the WP2 cell line (Berić et al., 2008). The obtained results indicated that in spite of a strong antioxidative potential of Lin and Myr, their protective effect against oxidative mutagenesis was low, presumably due to the efficient antioxidative defense in wild type strains.

To increase the sensitivity of the assay, an *E. coli* WP2 strain IC202 *oxyR*, deficient in the induction of antioxidative enzymes, was included in the study (Urios and Blanco, 1996; Blanco et al., 1998). Since IC202 is deficient in removing ROS, it allows for a more sensitive detection of the antimutagenic potential of antioxidants. In preliminary experiments we determined that in this strain 25  $\mu\text{g}/\text{plate}$  of *t*-BOOH induced a high mutagenic response with about 50% of lethality (Mitić-Ćulafić, 2009).

All of the tested monoterpenes reduced *t*-BOOH-induced mutagenesis in the IC202 strain to a significantly higher extent than in its OxyR<sup>+</sup> counterpart IC185 (Fig. 4). The strongest reduction was obtained with Myr and Lin, which decreased the *t*-BOOH-induced revertants number for 80% and 70%, respectively, without any effect on cell viability. Neither Myr nor Lin exhibited any protective effect against spontaneous mutagenesis (data not shown).

The results obtained with Euc were different in comparison with the two other monoterpenes. Euc exerted only a moderate inhibition of *t*-BOOH-induced mutagenesis (33%, Fig. 4), but it significantly reduced the number of spontaneous revertants (49%, data not shown). It should be noted that the IC202 strain, unlike SY252 and IC185, harbors plasmid pKM101 (Tab. 1) encoding error-prone DNA polymerase R1, which increases both spontaneous and induced mutation rates (Goldsmith et al., 2000). Since Euc suppressed spontaneous mutations only in

IC202, it might suppress DNA pol R1 mediated replication over DNA damage. This idea is supported by previous studies demonstrating the antimutagenic effect of Euc in *S. typhimurium* TA100 strain, which also carries pKM101 (Kim et al., 1992; Stajković et al., 2007).

Our results indicate that the observed antimutagenic potentials of monoterpenes against *t*-BOOH-induced mutagenesis in the *oxyR* strain are in correlation with their antioxidantivity. Myr and Lin possess strong antioxidative potential (Mitić-Ćulafić, 2009) and consequently significantly reduce *t*-BOOH-induced mutagenesis, while the weak antioxidantivity of Euc corresponds to its moderate antimutagenic potential. The obtained results are in agreement with the previously reported data showing that Lin reduced H<sub>2</sub>O<sub>2</sub>-induced DNA damage in the yeast Comet test (Stanojević et al., 2004). Moreover, Lin, Myr and Euc reduced the *t*-BOOH-induced genotoxicity determined with Comet assay in HepG2 and NC-NC human cell lines (Mitić-Ćulafić, 2009). It is interesting that monoterpenes were less efficient in HepG2 cells which possess a higher capacity of antioxidantive defense than NC-NC cells. Considering both the results obtained in human cell lines and in bacteria, it can be proposed that in cells with a high level of antioxidative enzymes, the relative contribution of the monoterpenes to the intrinsic radical scavenging potential of cells is significantly lower.

The obtained results are in agreement with other data on the antimutagenic and antigenotoxic features of the tested monoterpenes. Euc and Cam reduced the mutagenic effect of aflatoxin B<sub>1</sub> in *S. typhimurium* TA100 (Kim et al., 1992), while Euc also showed an inhibitory potential against environmental mutagens 2-nitropropane and B[a]P (Stajković et al., 2007). Cam antagonized a  $\gamma$ -radiation-induced increase in sister-chromatid exchanges in mice bone marrow cells (Goel et al., 1989) and Myr reduced cyclophosphamide-induced sister-chromatid exchanges in V79 cells and in the hepatic tumor cell line (Röscheichen et al., 1991). Available data also indicate that Euc and Cam increase the levels and activities of hepatic carcinogen metabolizing

enzymes (Banerjee et al., 1995). In addition, Euc is cytotoxic against different human tumor cell lines (Hayes et al., 1998), while Cam has a radiosensitizing effect on transplantable mammary adenocarcinoma in mice (Goel and Roa, 1988).

To our knowledge, there is only one study on the antimutagenic effect of Thu against aflatoxin B<sub>1</sub> in *S. typhimurium* TA100 (Kim et al., 1992). The authors noticed damaged colonies on plates with Thu and explained this as resulting from probable mutagenic activity. Bearing in mind that a preincubation test was used, and in the absence of data on bacterial background lawn, we speculate that the authors observed the toxic effect of Thu. Although the neurotoxic effect of Thu in mammals is well established (Höld et al., 2000), reported data indicate that Thu is not genotoxic (<http://ntp-apps.niehs.nih.gov/>). Moreover, essential oils containing Thu have been widely used in traditional medicine and there are indications of their antimutagenic properties (Minnunni et al., 1992; Vuković-Gačić et al., 2006a; Patenković et al., 2009; [www.ema.europa.eu/pdfs/vet/mrls/060299en.pdf](http://www.ema.europa.eu/pdfs/vet/mrls/060299en.pdf)).

In conclusion, our present and previous studies (Knežević-Vukčević et al., 2005; Vuković-Gačić et al., 2006a; 2006b; Stajković et al., 2007; Berić et al., 2008; Stanojević et al., 2008; Mitić-Ćulafić, 2009) indicate that the significant antimutagenic potential of sage and basil essential oils and their constitutive monoterpenes are due to multiple mechanisms. Since Cam and Thu significantly reduce UV- and 4NQO-induced mutagenesis only in repair proficient, but not in NER deficient strains, their bio-antimutagenic potential could be attributed to the modulation of NER. On the other hand, the obtained results for Lin and Myr indicate that their strong potential to reduce *t*-BOOH-induced mutagenesis is based on their antioxidative (radical scavenging) potential. According to our results, the antimutagenic effect of Euc and Lin is based on both bio-antimutagenic and desmutagenic (antioxidative) properties.

Taken together, our results recommend the essential oils and monoterpenes from sage and basil for

further antimutagenicity and antigenotoxicity studies. Bearing in mind the evolutionary conservation of many cellular functions, including DNA repair and antioxidative protection, the obtained results are a valuable basis for the further evaluation of the protective potential of sage and basil in eukaryotes, and ultimately in humans.

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