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ANTIGENOTOXIC EFFECT OF PLANT EXTRACTS

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This report reviews our study of antigenotoxic compounds from medicinal and aromatic plants performed over several years. The studies of this type are aimed at understanding the protective mechanisms which may be relevant for the primary prevention of cancer and other mutation-related diseases.

Antigenotoxic potential in this study is estimated with prokaryotic and eukaryotic tests measuring spontaneous and induced mutations, recombination, mutagenic repair, chromosomal aberrations and micronuclei. Our results indicate that monoterpenoids from sage act as modulators of DNA repair pathways, whereas sage antioxidants interfere with metabolic activation enzymes. The potential use of sage extracts in cancer prevention is discussed.

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INTRODUCTION

The integrity of cellular DNA is continuously attacked by agents that chemically modify its structure. Major sources of DNA lesions are DNA damaging agents from the environment, cellular intermediates of metabolism, spontaneous chemical reactions of DNA (deamination, depurination), incorporation of foreign or damaged nucleotides etc. (LIVNEH *et al.,* 1993). Unrepaired DNA lesions may block replication and transcription, potentially leading to cell death, or may give miscoding information, generating mutations. As a response to DNA damage, essentially all organisms have developed numerous repair mechanisms to preserve the integrity of their genetic material: reversion, excision or tolerance of a lesion. These mechanisms are largely conserved among prokaryotes and eukaryotes, including human cells (FRIEDBERG *et al.*, 1995).

DNA repair is a dynamic process and can be modulated by many factors, such as the rate of DNA replication and cell proliferation, the level of expression of damaged genes, the inactivation of certain repair enzymes etc. (SIMIĆ, 1995; 1997). Hypothetically, all factors that improve the fidelity of DNA repair and replication may be considered antimutagenic. Many important human diseases, including atherosclerosis, autoimmune diseases and some types of diabetes are associated with mutations (MARNETT, 2000; OLINSKI *et al.,* 2002). Moreover, epidemiological studies indicate that many types of cancer are dependent on multiple mutational etiology, as well as on inherited mutator phenotype (RADMAN *et al.,* 1995). In the relatively new area of research, under the dual name antimutagenesis/anticarcinogenesis, all mechanisms relevant to prevention of genotoxic effects and cancer are studied.

The antimutagenic effect of certain naturally occurring compounds extracted from plants has been well established in bacteria and mammalian cells (KURODA, 1990, BOOTMAN *et al.*, 1988). It is known or suspected that many antimutagens act by multiple mechanisms, which implies a discriminative approach in antimutagenesis studies (selection of the test system, mutagen, end point, experimental conditions etc.), as well as careful interpretation of the results. Moreover, comparative studies in prokaryotic and eukaryotic models are necessary to identify mechanisms relevant for human protection.

Bacterial short-term tests, routinely used to detect environmental mutagens, are recommended for identifying antimutagens (KURODA, 1990, KADA *et al.,* 1985). In addition to their rapidity and low costs, they provide considerable information about cellular mechanisms of mutagenesis and antimutagenesis. Combined with mammalian enzymes, they provide information about the kind of metabolic activation or detoxification that the agent may undergo *in vivo*. Bacterial short-term tests represent the first step in screening for antimutagens.

According to KADA *et al.,* (1982; 1985) and DE FLORA and RAMEL (1988), antimutagens are placed in two major categories: bioantimutagens and desmutagens. Desmutagens are factors which inactivate mutagens or prevent their interaction with DNA, whereas bioantimutagens modulate DNA replication and repair, preventing mutation fixation. The aim of our research is to identify antimutagenic substances from traditional medicinal and aromatic plants and to elucidate the mechanisms of their action. The results obtained in our study with newly constructed, as well as other selected prokaryotic and eukaryotic test systems, are reviewed in the light of the idea that dietary antimutagens may be used in the primary prevention of cancer and other mutation-related diseases.

THE NEW E. coli K12 TEST SYSTEM FOR BIOANTIMUTAGENS

Protective effect of bioantimutagenic can be obtained by: (1) the increase of fidelity of DNA replication, (2) the stimulation of error-free repair of DNA damage and (3) the inhibition of error-prone repair systems (KADA et al., 1982). With long experience in the field of DNA repair, we designed a new and more refined E. coli K12 assay system to detect bioantimutagens and to identify their mechanisms of action (KNEŽEVIĆ-VUKČEVIĆ, 1995; VUKOVIĆ-GAČIĆ, 1995; VUKOVIĆ-GAČIĆ and SIMIĆ, 1993; SIMIĆ et al., 1994; VUKOVIĆ-GAČIĆ et al., 2006). The bacterial assay is composed of four tests (Table 1) measuring different end-points at the DNA level. The repair proficient strain was chosen from the laboratory collection for detection of induced mutagenesis (Test A) and its uvrA counterpart was constructed to elucidate whether bioantimutagenic effect involves increased capacity for excision repair. For detection of spontaneous mutagenesis (Test B), the isogenic mismatch repair deficient strains were constructed to amplify the sensitivity of the assay. As mismatch repair deficient strains are incapable of correcting replication errors (FRIEDBERG et al., 1995), agents affecting the fidelity of DNA replication can be detected. In both tests, the reversion of argE3 ochre mutation, which can occur by base substitution, is monitored.

In order to measure the level of SOS induction (Test C), corresponding to the induction of mutagenic SOS repair (WALKER, 1984), the repair proficient strain was lysogenized with non-inducible λ phage carrying sfiA::lacZ fusion. In the resulting strain IB100, the level of SOS induction is measured by monitoring the level of β-galactosidase. To simultaneously assess the effect on overall protein synthesis this strain was made constitutive for alkaline phosphatase (BERIĆ-BJEDOV, 2003).

To measure homologous recombination (Test D), we use the strains with two non-overlapping deletions in duplicated lac operon (KONRAD, 1977) in which intrachromosomal recombination results in the formation of Lac^+ recombinants. The strains carry different recA alleles and thus have different capacities for both recombination and SOS induction (ENNIS et al., 1989). Test D is the most refined one because it can be used to identify the complex situation when the bioantimutagen is acting by multiple mechanisms.

Tabela 1.Escherichia coli K12 assay for bioantimutagenesis

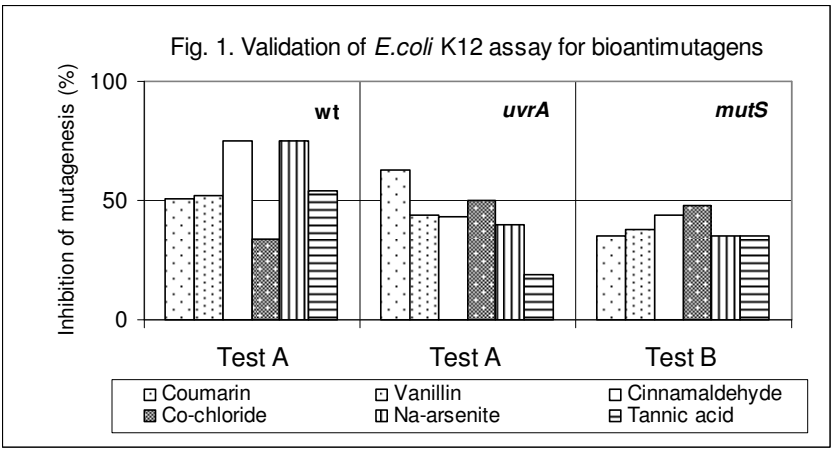
	Strain	Relevant marker
TEST A	SY252	*argE3*
	IB105	SY252 *uvrA*::Tn*10*
	IB112	SY252 *lpcA*
TEST B	IB101	SY252 *mutH471*::Tn*5*
	IB102	SY252*mutL218*::Tn*10*
	IB103	SY252*mutS215*::Tn*10*
	IB113	IB103 *lpcA*
	IB104	SY252 *uvrD260*::Tn*5*
	IB106	SY252 *mutT*::Tn*5*
TEST C	IB100	SY252^{a}λp(*sfiA::lacZ*)c**I***ind1*°
	IB111	IB100 PHO^{C}
	IB107	IB100 *uvrA*::Tn*10*
TEST D	GY7066	*lacMS286*Φ80dII*lacBK1*Δ*recA306 srl*::Tn*10*
	GY8281	GY7066/miniF*recA*$^{+}$
	GY8252	GY7066/miniF*recA730*
	GY7811	GY7066/miniF*recA430*

Specificity of detection of bioantimutagens is achieved by using UV-irradiation as a mutagen, excluding possible interaction between chemical mutagen and antimutagen. Since it is known that many chemical mutagens and carcinogens share the cellular mechanisms of mutation avoidance and mutation fixation with UV-irradiation (WALKER, 1984), bioantimutagens detected in this way may prove effective in inhibition of chemically induced mutagenesis.

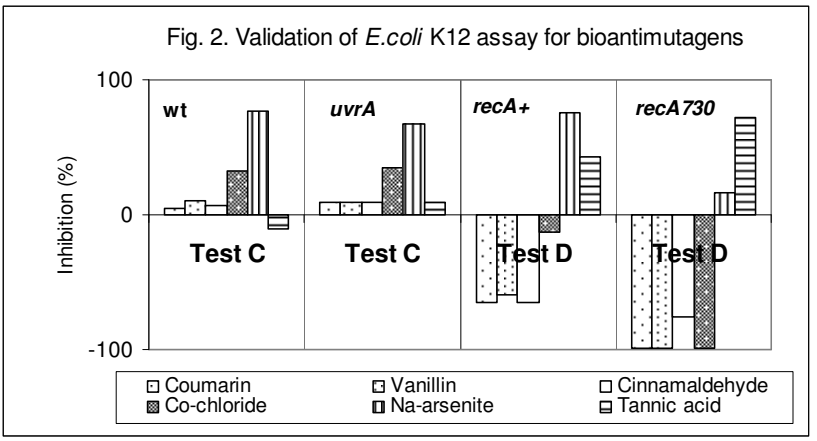
VALIDATION OF THE TEST SYSTEM

To validate our test system, pure substances with proposed mechanisms of bioantimutagenesis were tested: tannic acid, an enhancer of excision repair (SHIMOI et al., 1985) coumarin, vanillin and cinnamaldehyde, all three promoters of recombinational repair (OHTA et al., 1983a; 1983b; 1988), cobaltous chloride, reported to increase the fidelity of DNA replication and enhance RecA protein recombinational activity in vitro, (DE FLORA and RAMEL, 1988, KURODA and INOUE, 1988) and sodium arsenite reported both to increase and decrease UV-induced mutagenesis in E. coli. The effect of sodium arsenite seems to be concentration dependent: at low concentrations it increases UV-induced mutations by inhibiting excision repair of pyrimidine dimers (ROSSMAN, 1981), whereas at higher concentrations it decreases UV-induced mutagenesis by inhibiting the induction of the SOS error-prone repair (NUNOSHIBA and NISHIOKA, 1987).

Obtained results in validation study (VUKOVIĆ-GAČIĆ, 1995; SIMIĆ et al., 1997; 1998) are summarized in Figs. 1 and 2. The inhibitory effect of model bioantimutagens on UV-induced mutagenesis is confirmed in Test A, both in repair proficient and excision deficient strains (Fig. 1). The only exception is tannic acid, which showed no bioantimutagenic potential in uvrA mutant, confirming previous data (SHIMOI et al., 1985) that the enhancement of excision repair is its most important mechanism of mutagenesis inhibition. The inhibition of recombination by tannic acid and the lack of the effect on SOS induction (Fig. 2, Tests D and C) are all in support of the proposed mechanism.



On the other hand, the bioantimutagenic effect of coumarin, vanillin and cinnamaldehyde could be attributed to the enhancement of recombinational repair (Test D), as no effect on SOS induction was observed in Test C (Fig. 2). Similarly, in Salmonella/microsome test (MARON and AMES, 1983) there was no inhibition of UV-induced His^+ reversions by tannic acid in the excision deficient strain TA100, while coumarin showed the same extent of mutagenesis inhibition as in the repair proficient E. coli.



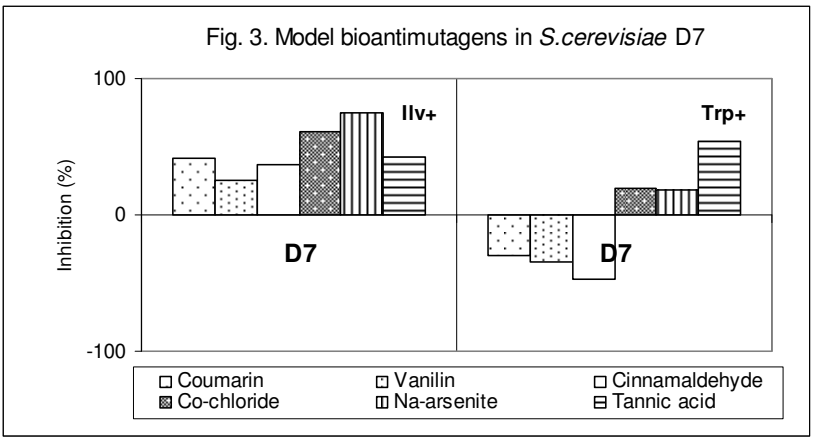
From the pattern of responses obtained with sodium arsenite and cobaltous chloride it can be concluded that their protective effect against UV-induced mutations is mainly due to the inhibition of SOS induction (Test C). However, this effect is probably caused by the inhibition of protein synthesis, as there is a similar inhibition (66% for sodium arsenite and 30% for cobaltous chloride) of both β-galactosidase and alkaline phosphatase synthesis in the strain IB111 (BERIĆ-BJEDOV, 2003). The inhibitory effect of both substances on RecA protein synthesis following DNA damage could also explain the absence of increased recombination in the UV-irradiated $recA^+$ strain (Fig. 2, Test D). This idea is further supported, at least for cobaltous chloride, by increased recombination obtained in recA730 mutant constitutively expressing high levels of RecA protein (WALKER, 1985).

Inhibition of spontaneous mutagenesis by model bioantimutagens was only detected in the assay with amplified sensitivity (Fig. 1, Test B), indicating that the incidence of formation of mismatches during DNA replication is reduced.

Taken together, the results of validation study showed that our assay successfully detected model bioantimutagens and was able to distinguish multiple proposed mechanisms.

The antimutagenic activity of model bioantimutagens was also tested in the lower eukaryote *Saccharomyces cerevisiae*. Diploid strain D7 (ZIMMERMANN *et al.,* 1975) was used and UV-induced reversions of the *ilv-92* mutation and mitotic gene conversions at the *trp5* locus were monitored (VUKOVIĆ-GAČIĆ *et al.,* 2001).

All prokaryotic bioantimutagens reduce the number of UV-induced revertants, but to a different extent compared with *E. coli*, and the inhibitory effect is observed with lower concentrations applied. UV-induced mitotic gene conversion is stimulated by cinnamaldehyde, coumarin and vanillin, but inhibited to a different extent by tannic acid, cobaltous chloride and sodium arsenite (Fig 3.).



As already mentioned, the inhibition of mutagenesis by coumarin, vanillin and cinnamaldehyde in bacteria is ascribed to the stimulation of recombinational repair. The enhanced mitotic gene conversion in *S. cerevisiae* might point to the similar mechanism. This hypothesis is further strengthened by the fact that coumarin is antimutagenic and co-recombinogenic in *S. cerevisiae* following treatment with TEM, an agent having recombinogenic effects in eukaryotic cells (FAHRIG, 1984; 1992). *E. coli* RecA protein has a true homologue Rad51 in *S. cerevisiae,* which is essential for damage-induced mitotic gene conversion (PETES *et al.,* 1991). By stimulating some of the common catalytic activities of the RecA and Rad51 proteins (formation of the helical filament on DNA, homologous pairing, strand exchange, ATP hydrolysis) antimutagens with co-recombinogenic activity might channel the repair of premutagenic lesions into recombination (FAHRIG, 1996). It is interesting to note that coumarin, vanillin and cinnamaldehyde increase the frequency of SCEs after MMC treatment in Chinese hamster ovary cells. Furthermore, vanillin increases SCEs after EMS, ENNG, ENU and MNU indicating that it may directly or indirectly modify some enzymes involved in the rejoining of DNA strands during S phase (SASAKI *et al.,* 1987a). Moreover, vanillin suppresses the breakage-type chromosome aberrations induced by MMC, which arise from unrepaired DNA-strand breaks in G2 phase, indicating that it may also modify the activity of G2 repair enzymes (SASAKI *et al.,* 1987b).

On the contrary, tannic acid inhibits UV-induced intrachromosomal recombination and mitotic gene conversion, excluding increased recombination as a mechanism. Excision repair is very important for removing DNA lesions induced by a number of agents, including UV, MMC and MMS, both in bacteria and mammalian cells. As tannic acid inhibited chromosomal aberrations induced by those agents in Chinese hamster ovary cells (SASAKI *et al.,* 1988), it is possible that it also promotes excision repair in eukaryotes.

According to our results, sodium arsenite is antimutagenic and anti-recombinogenic following UV-irradiation of *S. cerevisiae* cells (Fig. 3). However, in the same model sodium arsenite is co-mutagenic and anti-recombinogenic following treatment with TEM (FAHRIG, 1984). Sodium arsenite increases UV- and N-NO-AAF-induced chromosome aberrations in Chinese hamster ovary cells and UV-induced mutations to 6-thioguanine resistance, which results from deletions and translocations. However, it has no effect on mutations to quabain resistance, resulting from base substitutions (LEE *et al.,* 1985, LIN and TSENG, 1992). Similar effect i.e. increased UV-induced genotoxicity (HPRT mutations and SCEs) in mammalian cells were observed in the presence of Co(II) (HARTWIG *et al.,* 1991). From these and our results, it seems that sodium arsenite and cobaltous chloride affect both recombination and mutagenic translesion replication.

In *E. coli* the inhibitory effect of cobaltous chloride and sodium arsenite on UV-induced mutagenesis is most probably due to the inhibition of RecA-mediated SOS induction (Fig. 2), which prevents both recombinational repair and translesion replication. Although inducible response, like SOS induction, does not exist in eukaryotes, it has been suggested that yeast may assemble a specialized complex that mediates translesion replication in a manner similar to that postulated for UmuD'C in *E. coli.* Moreover, *S. cerevisiae* Rev1 protein involved in mutagenesis is 25% identical with the *E. coli* UmuC protein (LARIMER *et al*., 1989; PETES *et al.,* 1991; NELSON *et al.,* 1996; LAWRENCE, 2002). Being a salts, cobaltous chloride and sodium arsenite may affect many cellular enzymes, including ones necessary for recombination and damage induced mutagenesis.

Obtained results in validation study, in spite of some differences, indicate that antimutagens identified in prokaryotic tests can be effective in eukaryotic cells.

ANTIMUTAGENIC EFFECT OF TERPENOIDS FROM SAGE

The *E. coli* K12 assay, validated with model bioantimutagens, was used to detect the antimutagenic potential of nine plant extracts: sage, lime-tree, mint, nettle, camomile, aloe, thyme, St. John's wort and the plant mixture "X-tea". Analysis of the obtained data showed heterogeneous response, depending on the extract, concentration applied and genetic end-point monitored. Comparison of obtained data promoted St. John's wort, mint and sage as potential source of bioantimutagens (VUKOVIĆ-GAČIĆ and SIMIĆ 1993).

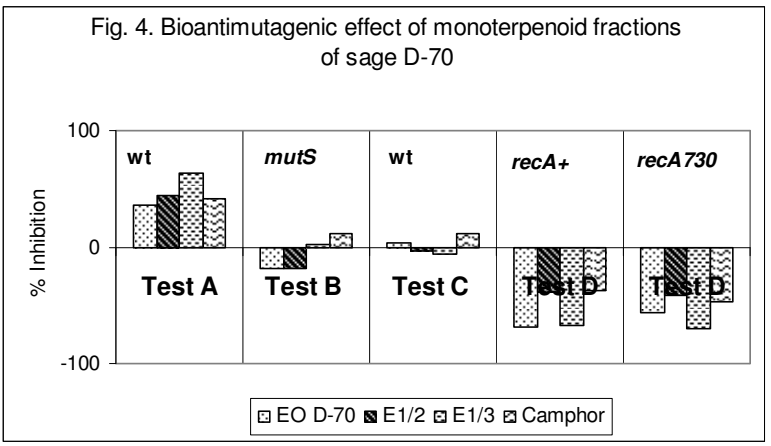
Given the possibility to obtain large quantities of chemically characterized extracts from different varieties of sage, selected for industrial cultivation in our continental climate, we focused our research on this plant. We screened fractionated extracts of wild and cultivated sage, containing terpenoids. The wild sage originated from Pelješac, Dalmatia, and cultivated sage (variety D-70) was selected and grown at the Institute for Hop, Sorghum and Medicinal Plants, Bački Petrovac (DARMATI *et al.,* 1991). The most striking difference between the two plants is the composition of essential oils (EO). While both plants contain thujone,

camphor is present only in traces in the wild sage, whereas it represents 1/5 of the monoterpenoids in variety D-70 (DARMATI *et al.,* 1994).

The most interesting results were obtained with fraction E1/3 of cultivated sage, re-extracted with CO_2 at the pressure of 300 bars. This fraction reduces UV-induced mutagenesis in repair proficient strain (Test A), and has no effect on the spontaneous mutation frequency in mismatch repair deficient strains (Test B) (SIMIĆ *et al.,* 1998). Fractions obtained at the same CO_2 pressure from the same plant devoid of essential oils, or from wild sage, showed no inhibition of mutagenesis, indicating the role of volatile terpenoids, especially camphor (SIMIĆ *et al.,* 1994).

In subsequent comparative study (SIMIĆ *et al.,* 1997) monoterpenoid-rich fractions of sage D-70 (EO, E1/2, E1/3) and camphor produced a similar pattern of responses in all tests (Fig. 4). The results supported a protective effect of sage monoterpenoids *via* enhanced recombinational repair. Additional study showed that the extent of mutagenesis inhibition by E1/3 was reduced in *uvrA* strain (30%), indicating the participation of excision repair in the protection mechanisms. Nevertheless, significant antimutagenic effect of E1/2 and E1/3 is found in excision deficient *S. typhimurium* TA100, both with and without metabolic activation (STEVIĆ *et al.,* 1994). In *S. cerevisiae* D7 E1/3 reduced UV-induced mutations by 35%, but inhibited UV-induced gene conversions (30%), which may indicate the participation of excision mechanism rather than recombination.

Fig. 4. Bioantimutagenic effect of monoterpenoid fractions of sage D-70



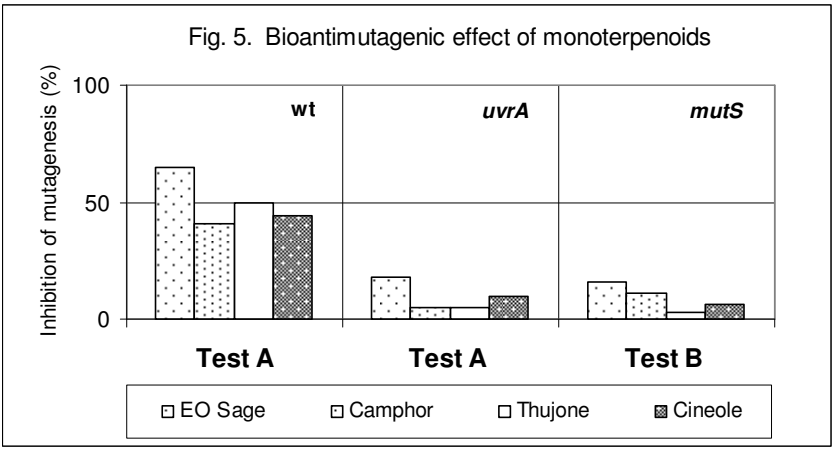
MONOTERPENOIDS FROM SAGE

Additional evidence for bioantimutagenic effect of sage monoterpenoids came from the study of EO of sage, grown for industrial purposes by the Institute for Medicinal Plant Research "Dr. Josif Pančić", Belgrade. In addition to thujone

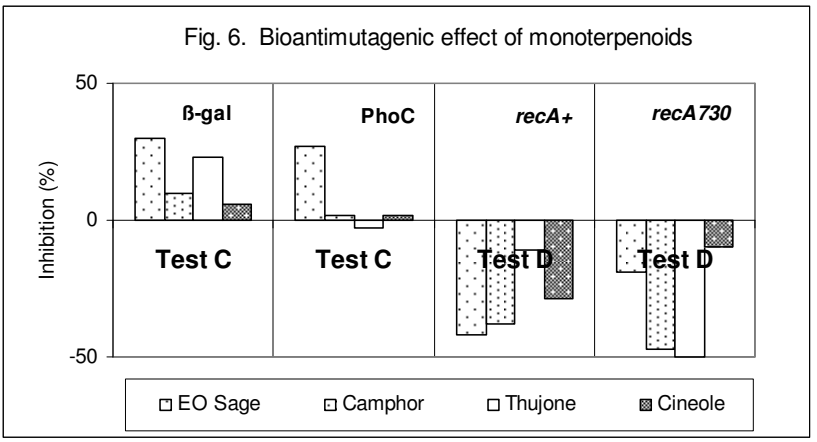
and camphor, this plant contains 1,8-cineole, which is not present in sage D-70. EO was fractionated by vacuum rectification to yield 5 fractions (F1-F5).

F1 and F2 contain only monoterpenoids, F3 and F4 contain small proportion of sesquiterpenoids in addition to monoterpenoids, and F5 contains mainly sesquiterpenoids (BRKIĆ *et al.,* 1999). Antimutagenic effect was studied with *E. coli* K12, *Salmonella/*microsome and *S. cerevisiae* D7 reversion assays. EO and fractions containing only monoterpenoids (F1 and F2) exhibited antimutagenic potential in all tests. Fractions F3 and F4 produced toxic, mutagenic or antimutagenic response, depending on the test organism used (KNEŽEVIĆ-VUKČEVIĆ *et al.,* 2005). The reduction of spontaneous and UV-induced mutations by F5 was detected only in permeable strains of *E. coli* (IB112 and IB113), indicating bioantimutagenic potential of sesquiterpenoids.

Fig. 5 presents the results obtained with pure monoterpenoids from sage: camphor, α+β thujone and 1,8-cineole. Significant antimutagenic effect against UV-induced mutations obtained in wild type strain was diminished in *uvrA* mutant, confirming the importance of excision repair in the protective mechanism.



The results obtained with Tests C and D indicates that EO also modulates mutagenesis by enhanced recombination and inhibition of SOS induction, which is probably caused by inhibition of protein synthesis (Fig. 6). From the pattern of responses of major monoterpenoids it can be concluded that the protective effect of EO is probably due to their combined effect, possibly aided by minor constituents.



ANTIMUTAGENIC EFFECT OF PLANT ANTIOXIDANTS

Among desmutagenic agents, antioxidants are of special interest because they are implicated in inhibition of tumor initiation, promotion and progression. The mechanisms of inhibition of mutagenesis and the initiation step of carcinogenesis by antioxidants include scavenging of reactive oxygen species, inhibition of certain enzymes involved in metabolism of xenobiotics and inhibition of mutagen/carcinogen binding to DNA (DE FLORA, 1998).

Since antioxidative potential of sage extracts, as well as diterpenoids isolated from wild sage and cultivated variety D-70 was established by lipid peroxidation test (DARMATI *et al.,* 1994), we tested antimutagenic potential of sage fractions involving their antioxidative properties. To test protective capacity against DNA lesions caused by reactive oxygen species, the newly constructed *E. coli* K12 *mutT* strain was used (Table 1). Due to the deficiency in removing 8-oxo-G from the cell pool, this strain shows increased level of spontaneous mutagenesis (FOWLER *et al.,* 2003). Screening for modulation of the enzymes involved in metabolism of xenobiotics was performed with *Salmonella*/microsome TA98 strain treated with DNA intercalating agent ethidium bromide (EtBr). Metabolic activation of EtBr is required for its genotoxic activity (http://metadata.nbii.gov/).

As model antioxidants we used vitamin E and synthetic antioxidant butylated hydroxytoluene (BHT), reported to inhibit genotoxic effects of EtBr (WATERS *et al.,* 1990). We also tested extracts of *Maclura pomifera* Rob. and *Ginkgo biloba* L., which also possess high antioxidative potential (DARMATI *et al.,* 1998; BRIDI *et al.,* 2001).

Obtained results with studied antioxidants (IVKOVIĆ, 1998; MITIĆ, 1999; MITIĆ *ET AL.,* 1998; 2001; NIKOLIĆ, 2004; STANOJEVIĆ, 2005) are summarized in Table 2. Antimutagenic effect against EtBr is found in all fractions of sage tested. By comparing mutagenesis inhibition by fractions E1/2-E1/5, it can be concluded

that their antimutagenic potential increases with the increase of CO_2 pressure used in extraction. This indicates that antimutagenic compounds belong to the class of high molecular weight terpenoids. Phenolic diterpenoids rosmanol-9-ethyl ether and 6-methyl-ether-γ-lactone carnosic acid, present in high proportion in E2/5 and A2/5 (DARMATI *et al.,* 1993), are probably responsible for the high antimutagenic effect observed.

M. pomifera extract and its major constituent pomiferin also show significant inhibition of mutagenesis induced by EtBr. Another major constituent of the extract, osajin, shows only mild inhibition, consistent with its low antioxidative potential, caused by the lacks of catehol unit in comparison with pomiferin (DARMATI *et al.,* 1998). The obtained results indicate that protection against EtBr-induced mutagenesis involves antioxidative activity. The results obtained with *G. biloba* extract EGb-761 support this hypothesis.In IB106 *mutT* strain only vitamin E, E2/5 and A2/5 show suppression of spontaneous mutagenesis (Table 2), indicating that high antioxidative potential is needed to prevent spontaneous oxidative DNA damage.

Table 2. Antimutagenic effect of plant antioxidants

	Inhibition of mutagenesis (%)		
	S. typhimurium	*E. coli* K12	
	TA98[a]	IB106[b]	SY252
Vitamin E	44	32	26
BHT	65	13	17
S. officinalis			
E1/2[d]	30	nt	44
E1/3[d]	42	29	63
E1/4[d]	33	nt	22
E1/5[d]	58	nt	19
E2/3[d]	45	nt	26
E2/5[d]	71	37	33
E3/3[d]	45	nt	34
A2/5[d]	72	34	32
M. pomifera			
Extract	55	18	15
Pomiferin	44	24	nt
Osajin	29	13	nt
G. biloba	57	11	nt

Mutagens: [a]EtBr; [b]spontaneous mutagenesis; [c]UV-irradiation

[d]CO_2 pressure (bars)/100; A2/5 acetone extract of cultivated sage nt – not tested

The experiments aimed at elucidating the mechanisms of antimutagenicity against EtBr indicate that protective effect of BHT and sage fraction E2/5 mainly results from the inhibition of metabolic activation of EtBr (Fig 7.A). In addition, BHT shows mild inhibitory effect on activated mutagen (Fig. 7.B), while E2/5 has modulatory effect on the repair of DNA damage induced by EtBr (Fig. 7.C). The modulatory effect of E2/5 is consistent with its inhibitory potential on UV-induced mutagenesis in *E. coli* K12 Test A (Table 2).

	A	**B**	**C**
	EtBr+ S9 + AO	EtBr+ S9	EtBr+ S9 +TA98
	↓ 20 min.	↓ 20 min.	↓ 40 min.
			-EtBr
	+TA98 + AO ↓	+ AO ↓20 min.	↓
	plate		plate
		+TA98 ↓	
		plate	
BHT	**I= 60%**	**I=26%**	**I=15%**
E2/5	**I= 66%**	**I=18%**	**I=29%**

Figure 7. Desmutagenic effect of antioxidants (AO)

PROSPECTS FOR CANCER PREVENTION

It is well established that cancer results from the accumulation of genetic changes in somatic cells during lifetime. All stages of tumorogenesis i.e. initiation, promotion, conversion, progression and metastasis are associated with mutations and increased genetic instability. It has been calculated that if the mutation rate could be reduced by half, the onset of most important human cancers would be delayed beyond the life span (SUGIMURA, 1998). Dietary use of plant antimutagens has been seen by many authors as the most practical way of primary chemoprevention.

The construction of appropriate assay systems for antimutagen detection, and estimation of molecular mechanisms involved, is very important to establish their value for chemoprevention strategies (DE FLORA and FERGUSON, 2005).In order to study antimutagens from medicinal and aromatic plants, we designed and validated the *E. coli* K12 assay for detection of bioantimutagens, modulators of DNA metabolism and repair.

In our assay, monoterpenoid fractions of sage obtained by different extraction procedures, as well as their major monoterpenoids: camphor, α+β thujone and 1,8-cineole, reduced UV-induced mutagenesis by modulation of DNA repair processes. Comparative study with other microbial short term tests, *Salmonella*/microsome and *S. cerevisiae* D7, confirmed bioantimutagenic potential of sage. On the other hand, sage fractions containing high molecular weight terpenoids with antioxidative properties, reduced EtBr-induced mutagenesis mainly by inhibition of metabolic activation of the mutagen.

Collaborative study in higher eukaryotic models showed that EO of sage reduced UV-induced male sterility in *D. melanogaster* (SAVKOVIĆ *et al.,* 1997), and MMC-induced chromosome aberrations in mice bone marrow cells (VUJOŠEVIĆ and BLAGOJEVIĆ, 2004). EO reduced micronuclei formation in mice polychromatophylic erythrocytes after treatment with Na-selenite (Rusov, personal communication) and in human lymphocytes after γ-irradiation (BERIĆ-BJEDOV, 2003). In WISH cell culture terpenoid fractions of sage, E1/2-E1/5, E2/5 and E3/3, induced apoptosis-like morphology changes (ŠMIDLING, 1998). Obtained data strongly suggest protective effect of sage terpenoids in eukaryotes, although the mechanisms remain to be elucidated.

Studies of other authors report that camphor antagonizes γ-radiation-induced increase in SCE frequency in mice bone marrow cells (GOEL *et al.,* 1989). A number of sage terpenoids: α-pinene, 1,8-cineole, borneole, camphor, myrcene, α-humulene, α-humulene epoxide, β-cariofilene and β-cariofilene oxide, increase the levels of one or more detoxification enzymes of the cytochrome P450 superfamily (HIROI *et al.,* 1995). In addition, 1,8-cineole, α-terpineol, terpinene-4-ol and carnosic acid are cytotoxic in several human cancer cell lines (AHN *et al.,* 1998; HAYES *et al.,* 1998) and camphor has radiosensitizing effect on transplantable mammary adenocarcinoma in mice (GOEL and ROA, 1988). From these and our data it can be concluded that sage terpenoids show a wide spectrum of activities, possibly relevant for cancer prevention.

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ANTIGENOTOKSIČNI EFEKAT BILJNIH EKSTRAKATA

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I z v o d

Ovaj rad predstavlja pregled višegodišnje studije antigenotoksičnog efekta sastojaka lekovitih i aromatičnih biljaka. Studije ove vrste su usmerene ka razumevanju protektivnih mehanizama koji mogu biti značajni za primarnu prevenciju kancera i drugih bolesti povezanih sa mutacijama.

U ovoj studiji antigenotoksč~ni potencijal je praćen pomoću prokariotskih i eukariotskih testova, određivanjem spontanih i indukovanih mutacija, rekombinacije, mutagene reparacije, hromozomskih aberacija i mikronukleusa. Naši rezultati ukazuju da monoterpenoidi iz žalfije moduliraju procese DNK reparacije, dok antioksidanti iz žalfije deluju na enzime uključene u metaboličku aktivaciju. Diskutovana je mogućnost primene ekstrakta žalfije u prevenciji kancera.

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