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Antimutagenic Properties of Basil (*Ocimum basilicum* L.) in *Salmonella typhimurium* TA100

Olivera Stajković[1], *Tanja Berić-Bjedov*[2]*, *Dragana Mitić-Ćulafić*[2], *Slaviša Stanković*[2], *Branka Vuković-Gačić*[2], *Draga Simić*[2] *and Jelena Knežević-Vukčević*[2]

[1]Laboratory for Microbiology, Institute of Soil Science, Teodora Drajzera 7, 11000 Belgrade, Serbia

[2]Laboratory for Microbiology, Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia

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Summary

The use of dietary antimutagens and anticarcinogens has been seen as a promising approach to the protection of human health. Basil (*Ocimum basilicum* L.) is a well-known medicinal and aromatic plant, with a range of newly discovered biological activities possibly important for chemoprevention. In the preliminary experiments, toxic and mutagenic potential of essential oil (EO) from basil and pure substances: linalool, β-myrcene and 1,8--cineole were tested using *Salmonella typhimurium* TA98, TA100 and TA102, with and without S9 mix (microsomal fraction of rat liver). No mutagenic effect of basil derivatives was detected in any tested strain. Antimutagenic effects of essential oil from basil and its pure constituents were further evaluated in the Ames test using *S. typhimurium* TA100. UVC irradiation and three chemical mutagens, 4-nitroquinoline-N-oxide (4NQO), 2-nitropropane (2-NP) and benzo(a)pyrene (B(a)P) were used to induce mutagenesis. All tested basil derivatives significantly reduced UV-induced mutations. The maximum inhibition was in the range of 64–77 %. Inhibitory potential against direct acting model mutagen/carcinogen 4NQO was similar to UV (52–67 %). In the presence of S9, EO and 1,8-cineole showed moderate inhibition of 2-NP induced mutagenesis, while the remaining two substances had no effect. Linalool exhibited high co-mutagenic effect with B(a)P, 1,8-cineole showed moderate inhibitory effect against B(a)P-induced mutations, while EO and β-myrcene were ineffective.

Key words: 4-nitroquinoline-N-oxide (4NQO), 2-nitropropane (2-NP), benzo(a)pyrene (B(a)P), UVC irradiation, *Ocimum basilicum*, antimutagens, *Salmonella typhimurium* TA100

Introduction

It has been estimated that around one-third of all human cancers may be related to diet (*1*). It is well known that a number of plant species contain biologically active compounds which can be used in diet as antimutagens and anticancerogens to protect human health. In recent years, there has been great interest in investigating compounds originating from plants and their effects on DNA (*2*). Essential oils (EO) extracted from plants contain complex mixture of odorous and volatile compounds and are widely used in aromatherapy and in traditional medicine. They also found application in food industry (as flavouring additives), perfume industry and agriculture. Many EO and their components exhibit antiviral, antibacterial, antioxidant and antimutagenic activities (*3,4*).

*Corresponding author; Phone/Fax: ++381 11 637 364; E-mail: tanjab@bfbot.bg.ac.yu
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Among various medicinal and culinary herbs, we focused our interest on basil (*Ocimum basilicum*), since it is employed as a folklore remedy for a wide spectrum of ailments in many traditional medicines, including ours. Recent interest in basil has resulted from its inhibitory activity against HIV-1 reverse transcriptase and against platelet aggregation induced by collagen and adenosine-5′-diphosphate (ADP) (*5,6*).

Although there are reports about modulatory effect of basil on Phase I and Phase II enzymes, as well as on elevation of antioxidant level and thus chemopreventive activity (*7,8*), there are no available data about its antimutagenic activity. Therefore, this study was undertaken to evaluate the antimutagenic properties of EO from basil and its constituents: linalool, β-myrcene and 1,8-cineole against different model and environmental mutagens in *Salmonella typhimurium* TA100. UV irradiation and three chemical mutagens, 4-nitroquinoline-N-oxide (4NQO), a model mutagen/carcinogen; 2-nitropropane (2-NP), an industrial solvent and a component of paints, inks and varnishes (*9*), also present in cigarette smoke (*10*); and benzo(a)pyrene (B(a)P), widely spread environmental mutagen, were used to induce reverse mutations.

Materials and Methods

Plant material and preparation of essential oil from basil

Basil (*Ocimum basilicum* L.) was cultivated in the experimental field of the Institute for Medicinal Plant Research »Dr Josif Pancic« in Pancevo, Serbia. Voucher specimen was confirmed and deposited in the herbarium of the Institute in Belgrade. Essential oil was prepared according to National Pharmacopoeia IV, by distillation of dried aerial parts (*basilici herba*) in 2-m^3 steam distiller (Hromil) for 2 h, at the pressure of 3–4 bar and at 135–145 °C. The composition of the essential oil was determined using analytical GC-FID and GC-MS techniques and Wiley/NBS library of mass spectra (*11*), shown in Table 1, and the oil was stored at 4 °C. The quality of the essential oil meets the standards of National Pharmacopoeia IV and ISO 9909.

Table 1. Chemical composition (in %) of the essential oil from basil (*Ocimum basilicum* L.)

Monoterpenes		**Sesquiterpenes**	
α-Terpinene	0.005	β-Burbonene	0.080
Camphene	0.006	α-Murolene	0.090
α-Pinene	0.100	Naphthalene	0.270
β-Myrcene	0.300	α-Copaen	0.400
Limonene	0.900	α-Humulene	0.500
Monoterpenoids		β-Caryophyllene	0.560
p-Cimen-8-ol	0.025	Zingiberene	0.600
Terpinen-4-ol	0.040	β-Elemene	0.800
Carvone	0.060	α-Bergamotene	1.020
trans-β-Ocimene	0.100	β-Selinene	1.040
Endoborneol	0.270	α-Guaiene	1.110
Endobornylacetate	0.300	δ-Cadinene	1.130
Camphor	0.300	α-Selinene	1.670
Nerol	0.400	δ-Guaiene	2.100
cis-β-Ocimene	0.400	γ-Cadinene	2.500
α-Terpinolene	0.400	**Sesquiterpenoids**	
Thiogeraniol	0.560	Nerodiol	0.110
α-Terpineol	0.700	*cis*-Farnesol	0.180
1,8-Cineole	0.800	*trans*-Murolol	0.430
Geraniol	1.900	α-Cadinol	2.560
Linalool	69.200		
Aromatic compounds			
Eugenol	1.400		
Estragole	2.400	**Identified in total**	97.716 %

Substances

All pure reference substances, mutagens included, were obtained from Sigma-Aldrich Co (St. Louis, MO, USA) and dissolved in dimethyl sulfoxide (DMSO). Purity of all tested substances was 95 % or more.

Mutagenicity assay

The Ames test was performed by the plate incorpoation method as described by Maron and Ames (*12*) using *S. typhimurium* TA98, TA100 and TA102. Different concentrations of EO or pure substances were added in top agar with or without S9 mix (microsomal fraction of rat liver). The number of revertant colonies formed in each plate was counted after 48 h of incubation at 37 °C. As a positive control 4NQO, 2-NP and B(a)P were used.

Antimutagenicity assay

Various concentrations of EO or pure substances and 4NQO (0.15 μg/plate, without S9 mix), 2-NP (14.9 mg/plate, with S9) and B(a)P (5 μg/plate, with S9) used as mutagens, were added in top agar with TA100 (*12*). UV irradiation was carried out with a germicidal lamp (Camag) having maximum output at 254 nm (UVC). The applied dose was 6 J/m^2. Cell suspensions in 0.01 M $MgSO_4$ were irradiated in a glass Petri dish of less than 1 mm thickness and added into top agar containing basil derivatives. Metabolic activation was provided by S9 liver homogenate from male Wistar rats induced with phenobarbital and β-naphthoflavone (for 2-NP) or Aroclor 1254 (for B(a)P) (*12*). The number of revertant colonies formed in each plate was counted after 48 h of incubation at 37 °C. The percentage of inhibition (I/%) was calculated according to the formula: I/%=[1-(number of His^+ revertants in the presence of basil derivatives/number of His^+ revertants in the presence of solvent)]×100.

Statistical analysis

The Student's *t*-test was employed for statistical analysis. The significance was tested at $p<0.05$ level. The results presented in tables are expressed as the means obtained in three independent experiments, with the standard error of the mean.

Results and Discussion

After determining the composition of EO from basil (Table 1), monoterpenoids linalool (69.2 %) and 1,8-cineole (0.8 %), as well as monoterpene β-myrcene (0.3 %)

were included in our investigation. In the preliminary experiments, toxic and mutagenic potential of EO and pure substances were tested using *S. typhimurium* TA98, TA100 and TA102. Toxicity assay was performed on TA100, with and without S9 mix, as recommended by Mortelmans and Zeiger (*13*). The concentrations of EO and its pure constituents resulting in thinner auxotrophic background lawn were considered toxic (*12*). In control experiment the influence of cell density on the number of spontaneous revertants in TA100 was examined. The results showed that if the number of plated cells was reduced by half or more, the reduction of spontaneous revertants was statistically significant (data not shown). According to that, the concentrations of EO and its pure constituents that significantly reduce the number of spontaneous revertants compared to the solvent controls were also considered potentially toxic and excluded from further experiments.

The range of non-toxic concentrations of EO and its pure constituents, determined as described above, was applied in mutagenicity tests using TA98, TA100 and TA102. No mutagenic effect of basil derivatives was detected in any tested strain, with or without S9 mix; there was no increase in the number of spontaneous revertants compared to the corresponding solvent controls, while positive controls induced mutagenic response (Table 2).

The results of antimutagenicity tests in TA100 are presented in Table 3. It can be seen that all the studied basil derivatives reduce UV- and 4NQO-induced mutagenesis. Number of UV-induced revertants decreased with increasing of concentrations of all tested substances. Maximum of inhibition was 76 % for EO and 64 % for linalool, both at 2 μL/plate. Significant inhibition was also detected with 1,8-cineole (70 % at 3 μL/plate), and β-myrcene (74 % at 0.4 μL/plate). When 4NQO was applied as a mutagen, similar results were obtained. EO from basil and 1,8-cineole exhibited the same inhibition (52 %) at the highest applied concentrations, while linalool and β-myrcene were slightly more effective (66 and 67 %, respectively).

Effect of basil derivatives was also tested against 2-NP (with S9 mix). Moderate inhibitory influence of EO and 1,8-cineole was obtained (30 and 35 %, respectively), while linalool and β-myrcene showed no effect.

Table 2. Mutagenicity tests of basil derivatives in *S. typhimurium* TA98, TA100 and TA102

V(substance)/(μL/plate)	TA98		TA100		TA102	
	without S9	with S9	without S9	with S9	without S9	with S9
0	35±4	32±4	170±9	168±4	257±9	249±11
S	33±2	31±3	162±14	167±17	237±8	240±36
EO						
0.5	33±2	27±0	177±20	210±11	236±16	233±33
1.0	21±4	33±2	183±5	199±11	225±3	245±6
1.5	26±3	32±2	177±7	186±23	228±29	243±28
2.0	20±0*	36±2	176±11	192±18	232±44	242±9
Linalool						
0.5	32±4	37±6	181±31	178±16	267±4	270±13
1.0	31±2	35±1	184±17	153±20	190±7	236±16
1.5	28±2	30±3	180±16	151±11	218±6	254±11
2.0	24±1	28±1	171±4	161±14	258±21	241±12
β-myrcene						
0.05	37±4	34±6	146±4	140±18	230±14	236±4
0.1	46±5	46±6	165±13	156±2	234±13	229±19
0.25	31±2	41±2	140±15	131±9	194±31	240±12
0.4	30±2	37±3	134±15	134±3	203±8	211±2
1,8-cineole						
1.0	42±2	40±4	158±30	154±3	273±36	232±14
2.0	50±3	40±4	158±14	157±5	215±34	219±10
3.0	31±6	35±4	140±11	156±4	213±38	209±11
4NQO	229±11	nt	996±7	312±11	1700±27	nt
2-NP	86±5	119±4	720±7	807±11	582±7	807±11
B(a)P	35±4	115±20	139±3	842±12	239±3	948±12

The presented values are averages of duplicate samples from three independent experiments with the standard error of the mean. Positive controls (per plate): 4NQO 0.17 μg (TA100) and 0.5 μg (TA98, TA102), 2-NP 14.9 mg (TA98, TA100) and 19.8 mg (TA102), B(a)P 15 μg (TA98) and 5 μg (TA100, TA102)
0 – spontaneous revertants
S – solvent DMSO
nt – not tested
*$p<0.05$

Table 3. The effect of basil derivatives against different mutagens in *S. typhimurium* TA100

V(substance)/ (μL/plate)	UV dose=6 J/m^2		*m*(4NQO)=0.15 μg/plate		*m*(B(a)P)=5 μg/plate		*m*(2-NP)=14.9 mg/plate	
	Rev/plate	I/%	Rev/plate	I/%	Rev/plate	I/%	Rev/plate	I/%
EO								
S	1210±58		1227±69		834±33		715±28	
0.5	941±31*	22	942±25	23	932±42	–12	655±18	8
1.0	672±25*	50	972±38	21	982±28	–18	544±7*	16
1.5	495±27*	59	740±26*	40	848±99	–17	504±5*	23
2.0	292±37*	76	597±67*	52	933±95	–12	452±77*	30
Linalool								
S	538±60		1025±92		901±14		634±80	
0.5	409±80*	24	1139±49	–11	952±51	–6	605±45	5
1.0	269±13*	50	889±52	13	1076±2	–19	588±72	7
1.5	233±20*	57	760±77	24	1231±12*	–37	592±25	6
2.0	192±23*	64	350±33*	66	1359±26*	–51	534±32	16
β-myrcene								
S	976±112		1249±17		838±76		479±54	
0.05	740±28	24	1164±36	7	803±23	4	493±20	–3
0.1	744±83	24	1177±40	6	822±54	2	458±45	4
0.25	700±24	28	985±65	21	917±49	–9	386±20*	20
0.4	225±52*	77	408±13*	67	739±49	–12	407±7*	15
1,8-cineole								
S	1491±92		1391±83		838±76		603±16	
1.0	1145±78*	28	848±11*	39	704±34	16	522±84	13
2.0	716±73*	52	764±5*	45	595±33	19	523±22	13
3.0	447±17*	70	677±27*	52	573±73*	32	392±5*	35

The presented values are averages of duplicate samples from three independent experiments with the standard error of the mean. The number of spontaneous revertants was from 142±14 to 187±7 without S9 and from 133±3 to 176±23 with S9. DMSO used as a solvent (S) had no effect on the number of spontaneous and induced revertants
I/% – percentage of inhibition
*$p<0.05$

As shown in Table 3, linalool significantly increased mutagenic effect of B(a)P (51 %). In the case of 1,8-cineole, moderate inhibitory effect was detected (32 %), while EO and β-myrcene had no effect on B(a)P-induced mutagenesis.

The results from this study indicate that basil derivatives were not mutagenic in *S. typhimurium* TA98, TA100 and TA102. The application of basil derivatives with four tested mutagens resulted in different ranges of inhibition or even enhancement of mutagenic activity, as assessed by Ames assay in TA100. The different actions depended on the mutagen-antimutagen combination. Our findings clearly demonstrate that essential oil from basil and all pure tested constituents have strong (above 50 %) antimutagenic effect on UV-, as well as 4NQO-induced mutations. Similar results for these two mutagens were expected, since 4NQO is a UV-mimic mutagen/carcinogen, mainly producing alkali-stable DNA lesions (*14*). It is possible that basil derivatives decrease mutagenesis due to modulation of *mucAB*-mediated error-prone repair, responsible for mutagenicity of lesions induced by UV and 4NQO (*15*). In contrast, 2-NP-induced mutagenesis was less affected by basil derivatives, since only EO and 1,8-cineole showed moderate antimutagenic effect.

In many previous studies it has been noted that reduction of B(a)P-induced mutagenesis was usually caused by inhibition of its metabolic activation (*16–18*). In this study slight antimutagenic effect was detected only with 1,8-cineole. Linalool produced significant co-mutagenic effect with B(a)P, which may be a consequence of an increased metabolic activation caused by linalool. Available data demonstrate that linalool administrated to rats for longer periods increases the level of cytochrome P450 enzymes (*19*). Moreover, a precursor of linalool, linalyl acetate, applied with B(a)P to the skin of female mice caused increase in the incidence of tumors, effect which was not observed when linalyl acetate or B(a)P were administrated independently (*20*). β-myrcene did not show any effect and this agrees with previously reported data that β-myrcene did not reduce B(a)P induced genotoxicity in mammalian cells *in vitro* (*21*). It has been noted that β-myrcene is a potent inhibitor of pentoxyresorufin-*o*-dependentilase (PROD), a marker of CYP2B1/2, but it has no effect on ethoxyresorufil-*o*-deethilase (EROD), a marker of CYP1A1, which is responsible for activation of B(a)P (*22*).

Conclusion

Although there are limitations in extrapolation of bacterial antimutagenicity data on mammalian cells, the results presented in this study clearly demonstrate antimutagenic potential of basil derivatives and make EO from basil and its components promising candidates for future antimutagenicity and anticarcinogenicity studies. It is however necessary to stress out that our results demonstrating co-mutagenic effect of linalool with B(a)P, together with literary findings, indicate that uncontrolled consumption of basil might be dangerous under certain environments rich in aromatic hydrocarbons.

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References

1. *Food, Nutrition and the Prevention of Cancer: A Global Perspective*, American Institute for Cancer Research, Washington, DC, USA (1997) pp. 1–670.
2. R.C. Horn, V.M.F. Vargas, Antimutagenic activity of extracts of natural substances in the *Salmonella*/microsome assay, *Mutagenesis, 18* (2003) 113–118.
3. B. Tepe, E. Donmez, M. Unlu, F, Candan, D. Daferera, G. Vardar-Unlu, M. Polissiou, A. Sokmen, Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret *et* Aucher *ex* Benth.) and *Salvia multicaulis* (Vahl), *Food Chem. 84* (2004) 519–525.
4. M. Idaomar, R. El Hamss, F. Bakkali, N. Mezzoug, A. Zhiri, D. Baudoux, A. Muñoz-Serrano, V. Liemans, A. Alonso--Moraga, Genotoxicity and antigenotoxicity of some essential oils evaluated by wing spot test of *Drosophila melanogaster*, *Mutat. Res. 513* (2002) 61–68.
5. K. Yamasaki, M. Nakano, T. Kawahata, H. Mori, T. Otake, N. Ueba, I. Oishi, R. Inami, M. Yamane, M. Nakamura, H. Murata, T. Nakanishi, Anti-HIV-1 activity of herbs in *Labiatae*, *Biol. Pharm. Bull. 21* (1998) 829–833.
6. K. Okazaki, S. Nakayama, K. Kawazoe, Y. Takaishi, Antiaggregant effects on human platelets of culinary herbs, *Phytother. Res. 12* (1998) 603–605.
7. T. Dasgupta, A.R. Rao, P.K. Yadava, Chemomodulatory efficacy of basil leaf (*Ocimum basilicum*) on drug metabolizing and antioxidant enzymes, and on carcinogen-induced skin and forestomach papillomagenesis, *Phytomedicine, 11* (2004) 139–151.
8. J. Prakash, S.K. Gupta, Chemopreventive activity of *Ocimum sanctum* seed oil, *J. Ethnopharmacol. 72* (2000) 29–34.
9. *Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Some Industrial Chemicals and Dyestuffs*, *Vol. 29*, IARC, Lyon, France (1982) pp. 331–343.
10. D.H. Hoffmann, G. Rathkamp, Chemical studies of tobacco smoke. III. Primary and secondary nitroalkanes in cigarette smoke, *Beitr. Tabakforsch. 4* (1968) 124–134 (in German).
11. B. Marinković, P.D. Marin, J. Knežević-Vukčević, M.D. Soković, D. Brkić, Activity of essential oils of three *Micromeria* species (*Lamiaceae*) against micromycetes and bacteria, *Phytother. Res. 16* (2002) 336–339.
12. D.M. Maron, B.N. Ames, Revised methods for the *Salmonella* mutagenicity test, *Mutat. Res. 113* (1983) 173–215.
13. K. Mortelmans, E. Zeiger, The Ames *Salmonella*/microsome mutagenicity assay, *Mutat. Res. 455* (2000) 29–60.
14. M.C. Paterson, N.E. Gentner, M.V. Middlestadt, M. Weinfeld, Cancer predisposition, carcinogen hypersensitivity, and aberrant DNA metabolism, *J. Cell. Physiol. 3* (1984) 45–62.
15. B. Vuković-Gačić, D. Simić: Identification of Natural Antimutagens with Modulating Effects on DNA Repair. In: *Antimutagenesis and Anticarcinogenesis Mechanisms, III*, G. Bronzetti, H. Hayatsu, S. de Flora, M.D. Waters, D.M. Shankel (Eds.), Plenum Press, New York, USA (1993) pp. 269–277.
16. S. Muto, K. Fujita, Y. Yamazaki, T, Kamataki, Inhibition by green tea catechins of metabolic activation of procarcinogens by human cytochrome P450, *Mutat. Res. 479* (2001) 197–206.
17. M.R. Gomes-Carneiro, D.M.M. Dias, A.C.A.X. De-Oliveira, F.J.R. Paumgartten, Evaluation of mutagenic and antimutagenic activities of α-bisabolol in the *Salmonella*/microsome assay, *Mutat. Res. 585* (2005) 105–112.
18. R. Thapliyal, S.S. Deshpaned, G.B. Maru, Mechanism(s) of turmeric-mediated protective effects against benzo(a)pyrene-derived DNA adducts, *Cancer Let. 175* (2002) 79–88.
19. Linalool, *SIDS Initial Assessment Report For SIAM 14*, OECD SIDS, Paris, France (2002).
20. B. Van Duuren, T. Blazey, B. Goldschmidt, C. Katz, S. Melchionne, A. Sivak, Cocarcinogenesis studies on mouse skin and inhibition of tumor induction, *J. Natl. Cancer Inst. 45* (1971) 1039–1044.
21. C. Roescheisen, H. Zamith, F. Paumgartten, G. Speit, Influence of beta-myrcene on sister-chromatid exchanges induced by mutagens in V79 and HTC cells, *Mutat. Res. 264* (1991) 43–49.
22. A.C.A.X. De-Oliveira, L.F. Ribeiro-Pinto, F.J.R. Paumgartten, *In vitro* inhibition of CYP2B1 monooxygenase by β-myrcene and other monoterpenoid compounds, *Toxicol. Lett. 92* (1997) 39–46.