

The antimutagenic activity of *Lavandula angustifolia* (lavender) essential oil in the bacterial reverse mutation assay

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Abstract

Essential oils from *Melaleuca alternifolia* (tea-tree oil) and *Lavandula angustifolia* (lavender oil) are commonly used to treat minor health problems. Tea-tree oil possesses broad-spectrum antimicrobial activity, and is increasingly used for skin problems. Lavender oil, traditionally used as an antiseptic agent, is now predominantly used as a relaxant, carminative, and sedative in aromatherapy. Despite their growing use no data are available on their mutagenic potential. In this study, after determining the chemical composition of tea-tree oil and lavender oil, by gas-chromatography and mass spectrometry, we investigated their mutagenic and antimutagenic activities by the bacterial reverse mutation assay in *Salmonella typhimurium* TA98 and TA100 strains and in *Escherichia coli* WP2 *uvrA* strain, with and without an extrinsic metabolic activation system. Neither essential oil had mutagenic activity on the two tested *Salmonella* strains or on *E. coli*, with or without the metabolic activation system. Conversely, lavender oil exerted strong antimutagenic activity, reducing mutant colonies in the TA98 strain exposed to the direct mutagen 2-nitrofluorene. Antimutagenicity was concentration-dependent: the maximal concentration (0.80 mg/plate) reduced the number of histidine-independent revertant colonies by 66.4%. Lavender oil (0.80 mg/plate) also showed moderate antimutagenicity against the TA98 strain exposed to the direct mutagen 1-nitropyrene. Its antimutagenic property makes lavender oil a promising candidate for new applications in human healthcare.

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1. Introduction

Essential oils contain a complex mixture of odorous and volatile compounds from secondary plant metabolism, and are widely used in cosmetics as fragrance components, in the food industry as flavouring additives and in a variety of household products as scenting agents. In addition to their characteristic flavour, many essential oils and their components exhibit muscle relaxant, anti-bacterial and antifungal activities (Mazzanti et al., 1998;

Lu et al., 2002; D'Auria et al., 2001). They are also used as carminative, expectorant, sedative, mucolytic and emmenagogue remedies.

The use of essential oils is not without systemic adverse reactions or toxic effects; oils from sage, hyssop and thuja can cause intoxication with tonic-clonic convulsions (Millet et al., 1981); lemon oil can cause phototoxicity (Naganuma et al., 1985), and intoxications following ingestion of the seeds of *Myristica fragrans* have been ascribed to its volatile oil and some of its pure components (Hallstrom and Thuvander, 1997). Published reports describe the in vivo effects on various animals species (Orafidiya et al., 2004) and the in vitro cytotoxicity against a number of cell lines (Hayes and Markovic, 2002).

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Only few papers contain information on the “latent toxicity” of essential oils, such as mutagenicity. *Ocimum selloi* essential oil was not mutagenic to TA97, TA98 and TA100 tester strains in the *Salmonella*/microsome assay (Padilha de Paula et al., 2003). Essential oil of *Mentha spicata* appeared to be slightly genotoxic in the somatic mutation and recombinant test in *Drosophila melanogaster* (Karpouhtsis et al., 1998). The volatile oil of *Artemisia dracuncululus* showed genotoxic activity in the *Bacillus subtilis* rec-assay, but not in the *Salmonella* test (Zani et al., 1991; Lazutka et al., 2001). Finally, the essential oils extracted from *Helicrysum italicum*, *Ledum groenlandicum* and *Ravensara aromatica* showed no significant genotoxicity in the wing spot test of *Drosophila melanogaster*; conversely, in the same test, they displayed antigenotoxic activity, reducing the mutation ratio induced by urethane, a well-known promutagen (Idaomar et al., 2002).

The antimutagenic properties elicited by some plant species have an array of prospective applications in human care. Recent research has underlined the chemopreventive activity of several secondary plant metabolites (Horn and Ferrao Vargas, 2003). Many investigators have mapped out specific compounds, for example, polyphenols, triterpenoids (Shih et al., 2000), or plant extracts (Ramos et al., 2003) with antimutagenic properties.

Two plants whose essential oils are used in health remedies are tea-tree oil and lavender oil. Tea-tree oil is the essential oil extracted from the leaves of the Australian native plant *Melaleuca alternifolia* Maiden (*Myrtaceae*). Owing to its broad-spectrum antimicrobial activity (Banes-Marshall et al., 2001; D’Auria et al., 2001), tea-tree oil is often used as a natural remedy for a variety of skin problems. Of particular interests are its beneficial effects in the treatment of *acne vulgaris*, *tinea pedis* and other dermatological diseases (Bassett et al., 1990; Ernest and Huntley, 2000; Satchell et al., 2002). When tested on human epithelial and fibroblast cells, the cytotoxic activity induced by tea-tree oil does not increase along with the time of exposure (Soderberg et al., 1996). A recent study described the in vitro antioxidant property of tea-tree oil but failed to attribute it to the major component, terpinen 4-ol (Kim et al., 2004); others showed that tea-tree oil and terpinen 4-ol interfere with the growth of human melanoma cells thus inducing programmed cell death (Calcabrini et al., 2004).

Lavender oil, the essential oil obtained by the aerial part of *Lavandula angustifolia* Mill. (*Lamiaceae*), is predominantly used in aromatherapy as a relaxant, carminative and sedative agent (Cavanagh and Wilkinson, 2002). It was traditionally used as an antiseptic agent in swabbing of wounds, for burns and insect bites, and in veterinary practice to kill lice and other animal parasites. One study nevertheless reported an in vitro cyto-

toxic activity of lavender oil and its main components, linalyl acetate and linalool, on human skin cells (Prashar et al., 2004); the same study found linalyl acetate more cytotoxic than the whole oil. Like tea-tree oil, lavender oil also has antioxidant properties (Hohmann et al., 1999).

As their range of uses widens and consumers grow in number the world trade in essential oils is expected to expand. Because many countries have no regulatory controls for essential oils and because humans are increasingly exposed to these compounds their toxicological profile now calls for adequate assessment. No published data are available on the mutagenicity and antimutagenicity of tea-tree oil or lavender oil.

In this study we first assessed the mutagenic potential of tea-tree oil and lavender oil and, if they proved negative, we investigated their antimutagenic activity. After determining the chemical composition of the two essential oils by gas-chromatography and gas-chromatography–mass spectrometry, we evaluated their mutagenicity or antimutagenicity, using the bacterial reverse mutation assay on *Salmonella typhimurium* TA98 and TA100 strains and *Escherichia coli* WP2 *uvrA* strain, with and without an extrinsic metabolic activation system.

2. Materials and methods

2.1. Chemicals

Tea-tree oil (batch 2B2705) was kindly supplied by Aboca (Sansepolcro, Arezzo, Italy) and lavender oil (batch JL055000) by Janousec (Muggia, Trieste, Italy). All pure reference substances, mutagens included, were obtained from Sigma–Aldrich Co (St. Louis, MO, USA).

2.2. Gas-chromatography and gas-chromatography–mass spectrometry

The two essential oils were analysed by gas-chromatography and gas-chromatography–mass spectrometry as described elsewhere (Ghelardini et al., 1999). Chemical components were identified by co-gas chromatography of the essential oils with authentic substances, and by comparing the spectra with the NIST/EPA/MSDC Mass Spectral Database, as well as by calculating their retention indices and comparing them with those of Adams (1995).

2.3. S9-based metabolic activation system

Rat liver post-mitochondrial supernatant (S9) induced by phenobarbital/5,6-benzoflavone, was pur-

chased from Moltax (Molecular Toxicology, Boone, NC, USA). The S9 mixture was prepared, just before use, by adding 500 μ l phosphate buffer (0.2 M), 130 μ l deionised water, 100 μ l KCl (0.33 M), 80 μ l MgCl₂ (0.1 M), 100 μ l S9 fraction, 50 μ l glucose-6-phosphate (0.1 M) and 40 μ l NADP (0.1 M). The mixture was kept on ice during testing.

2.4. Bacterial strains

S. typhimurium strain TA98 (frameshift mutation), *S. typhimurium* strain TA100 and *E. coli* WP2 *uvrA* strain (base-pair substitution), were kindly provided by the Research Toxicological Centre (Pomezia, Rome, Italy). For all assays, fresh cultures were prepared from frozen permanent cultures, and incubated and shaken overnight at 37 °C to a concentration of approximately 1×10^9 bacteria/ml.

2.5. Mutagenicity assay

Mutagenicity of the two oils was assayed with the bacterial mutagenicity test (main test) using the plate incorporation method, as reported by Maron and Ames (1983) and Green and Muriel (1976), without pre-incubation. In the preliminary experiment, toxicity was assessed either as a reduction in the number of revertant colonies and as a change in the auxotrophic background growth (background lawn) (OECD, 1997). To ensure that cytotoxicity did not interfere with inhibitory responses, in all subsequent assays, the upper limit of the test-concentration range was either the highest non-toxic concentration or the lowest toxic concentration determined in the preliminary toxicity test.

Solutions of tea-tree oil and lavender oil were prepared in serial dilutions using dimethylsulfoxide (DMSO). Positive controls consisted of 2-nitrofluorene (2NF in DMSO, 2 μ g/plate for TA98 without S9), 2-aminoanthracene (2AA in DMSO, 1 μ g/plate for TA98 and TA100 with S9 and 10 μ g/plate for WP2 *uvrA* with S9), sodium azide (SA in deionised water, 1 μ g/plate for TA100 without S9) and methyl methane sulfonate (MMS in DMSO, 500 μ g/plate for WP2 *uvrA* without S9). Nutrient broth, bacteriological and nutrient agar were obtained from Oxoid (Basingstoke, Hampshire, England). A 100 μ l aliquot of bacterial suspension from an overnight culture, 100 μ l of test concentrations, and 500 μ l of S9 mixture or phosphate buffer (0.1 M), were added to 2 ml of top agar containing 10% of histidine/biotin (0.5 mM) for TA98 and TA100 or 10% of tryptophan (0.5 mM) for WP2 *uvrA*. Histidine- or tryptophan-independent revertant colonies and viable cells were scored on plates after incubation at 37 °C for 72 h. The experiments were repeated at least twice and each concentration was determined in triplicate. A positive response in the main test was defined as an increase

(at least two-fold above the control), in histidine- or tryptophan-independent revertant colonies in every strain, with or without metabolic activation (Ames et al., 1975).

2.6. Antimutagenicity assay

Antimutagenicity was assayed as previously described by Edenharter et al. (1993) following the procedure reported by Maron and Ames (1983), with minor modifications. Suitable concentrations of known mutagens for TA98, TA100 and WP2 *uvrA* strains, with and without S9 mixture, were chosen from the linear part of a concentration–response curve: 2-nitrofluorene (2NF in DMSO, 6 μ g/plate), 1-nitropyrene (1NP in DMSO 2.0 μ g/plate), 3-nitrofluoranthene (3NFA in DMSO 0.12 μ g/plate) and 4-nitroquinoline 1-oxide (4NQO in DMSO 0.5 μ g/plate) for TA98 without S9; 2-aminoanthracene for TA98 and TA100 with S9 (2AA in DMSO, 5 μ g/plate) and for WP2 *uvrA* with S9 (2AA in DMSO 25 μ g/plate); sodium azide for TA100 without S9 (SA in deionised water, 5 μ g/plate); methyl methane sulphinate for WP2 *uvrA* without S9 (MMS in DMSO, 1700 μ g/plate). A 100 μ l aliquot of bacterial suspension from an overnight culture, 50 μ l of mutagen, 50 μ l of test concentrations, and 500 μ l of S9 mixture or phosphate buffer (0.1 M), were added to 2 ml of top agar containing 10% of histidine/biotin (0.5 mM) for both *Salmonella* strains or 10% of tryptophan (0.5 mM) for WP2 *uvrA*. Histidine- or tryptophan-independent revertant colonies and viable cells were scored on plates after incubation at 37 °C for 72 h. The experiments were repeated at least twice and each concentration was determined in triplicate. The number of revertant colonies grown in plates containing the mutagen without essential oils was defined as 100%. Control plates were prepared with vehicle alone. The percentage of inhibition was calculated according to the formula: $100 - [(T/M) \times 100]$ where *T* is the number of revertant colonies per plate in the presence of mutagen and essential oils, and *M* is the number of revertant colonies per plate in plates containing mutagen without essential oils: 25–40% inhibition was defined as moderate antimutagenicity; 40% or more inhibition as strong antimutagenicity; and 25% inhibition as no antimutagenicity (Ikken et al., 1999; Negi et al., 2003).

2.7. Statistical analysis

All values are expressed as mean \pm SE. An analysis of variance (ANOVA), followed by Holm-Sidak method when appropriate, was used to verify the significance of a positive response. *p* Values less than or equal to 0.05 were considered to indicate statistical significance.

3. Results

Chemical identification and quantitative estimation of tea-tree oil and lavender oil showed that tea-tree oil contained four main components: terpinen-4-ol (39.1%), γ -terpinene (20.4%), α -terpinene (9.2%), and 1,8-cineole (4.1%). The content of terpinen-4-ol and 1,8-cineole met the ISO 4730 requirements. Lavender oil predominantly contained linalyl acetate (43.1%), linalool (32.7%), caryophyllene (4.9%) and terpinen-4-ol (3.1%) (Table 1).

When incubated without the metabolic activator S9, tea-tree oil proved toxic on both TA strains (at

0.28 mg/plate on TA98 and at 0.88 mg/plate on TA100): when the S9 mixture was added, tea-tree oil was less toxic (toxicity began at 2.78 mg/plate). At concentration up to 2 mg/plate in *E. coli* WP2 *uvrA* strain, with and without added S9, tea-tree oil induced no measurable toxicity. When incubated with and without S9, lavender oil was less toxic than tea-tree oil: on TA98 its toxicity became apparent at 2.78 mg/plate. On the TA100 strain, lavender oil displayed the same toxicity as tea-tree oil. With and without metabolic activation, lavender oil at the highest concentration tested (2.5 mg/plate) induced no toxicity on *E. coli* WP2 *uvrA* strain.

Table 1

Main components of the essential oils of *Melaleuca alternifolia* (tea-tree oil) and *L. angustifolia* (lavender oil) along with their retention indices (RI)

Tea-tree oil	%	RI	Lavender oil	%	RI
Terpinen-4-ol	39.1	1187	Linalyl acetate	43.1	1257
γ -Terpinene	20.4	1062	Linalool	32.7	1098
α -Terpinene	9.2	1018	Caryophyllene	4.9	1404
1,8-Cineole	4.1	1033	Terpinen-4-ol	3.1	1180
Terpinolene	3.5	1088	2-Octanone/myrcene	2.4	988
<i>p</i> -Cymene	3.3	1026	Trans-ocimene	1.5	1050
α -Terpineol	3.0	1189	A-Terpineol	1.0	1189
α -Pinene	2.6	939	Borneol	0.8	1165
Limonane	1.9	1031	B-Farnesene	0.8	1443
α -Thujene	0.9	931	1,8-Cineole	0.6	1033
Myrcene	0.9	991	Camphor	0.5	1143
β -Pinene	0.7	980	Caryophyllene oxide	0.5	1581
Octanal	0.4	1001	A-Humulene	0.4	1454
Sabinene	0.3	976	Limonene	0.3	1031
Fenchyl alcohol	0.2	1125			
Aromadendrene	0.2	1439			

Table 2

Antimutagenicity of the essential oils of *Melaleuca alternifolia* (tea-tree oil) and *Lavandula angustifolia* (lavender oil) to *Salmonella typhimurium* (TA98, TA100) and *Escherichia coli* (WP2 *uvrA*) with and without metabolic activation (S9) ($n = 6$ or 9 plates)

Test item	Concentration mg/plate (used for WP2 <i>uvrA</i>)	Number of revertant colonies (mean \pm SE)					
		TA98		TA100		WP2 <i>uvrA</i>	
		–S9	+S9	–S9	+S9	–S9	+S9
Tea-tree oil	0.80 (2.00)	548.8 \pm 38.4	499.6 \pm 6.9 ¹	643.0 \pm 26.9 ¹	1556.0 \pm 51.7 ¹	162.0 \pm 9.0	256.0 \pm 29.2
	0.66 (1.50)	440.2 \pm 21.7	535.3 \pm 24.8	619.0 \pm 23.1	1661.3 \pm 36.3 ¹	151.0 \pm 10.8	264.0 \pm 4.2
	0.50 (1.00)	392.1 \pm 50.2	535.3 \pm 29.1	647.3 \pm 39.2	1293.3 \pm 70.5	165.3 \pm 13.5	270.8 \pm 14.5
	0.40 (0.75)	438.0 \pm 27.1	563.0 \pm 20.4	630.6 \pm 26.1	1769.6 \pm 73.9	186.7 \pm 15.6	231.5 \pm 14.9
	0.25 (0.50)	417.8 \pm 18.0	547.0 \pm 9.6	641.6 \pm 22.5	1796.3 \pm 17.8	275.3 \pm 26.2	228.7 \pm 9.9
	0.13 (0.25)	457.7 \pm 11.0	543.6 \pm 32.1	656.6 \pm 32.8	1763.3 \pm 38.1	302.0 \pm 21.1	220.7 \pm 20.3
Lavender oil	0.80 (2.50)	176.6 \pm 12.8*	572.9 \pm 23.6	652.3 \pm 15.0	1702.3 \pm 32.3 ¹	270.0 \pm 0.0	232.0 \pm 21.8
	0.66 (1.25)	206.7 \pm 16.5*	567.8 \pm 21.6	688.2 \pm 22.1	1752.3 \pm 19.4	248.7 \pm 31.9	308.3 \pm 3.7
	0.50 (0.90)	234.0 \pm 28.9*	535.9 \pm 30.1	685.0 \pm 23.3	1922.1 \pm 40.0	260.0 \pm 31.0	300.0 \pm 0.0
	0.40 (0.60)	278.3 \pm 31.5*	547.8 \pm 18.2	692.4 \pm 30.3	1828.5 \pm 18.3	202.7 \pm 21.3	288.7 \pm 41.5
	0.25 (0.40)	372.3 \pm 29.1*	528.3 \pm 22.3	688.2 \pm 18.5	1790.2 \pm 35.2	264.7 \pm 36.6	233.3 \pm 41.7
	0.13 (0.20)	414.3 \pm 22.3*	532.6 \pm 25.3	695.5 \pm 20.3	1758.2 \pm 23.0	199.3 \pm 10.1	237.3 \pm 36.3
	Vehicle	30.6 \pm 1.2	34.3 \pm 0.8	88.6 \pm 8.6	88.3 \pm 15.3	25.7 \pm 4.0	22.4 \pm 5.3
	Mutagen	525.3 \pm 27.7 ^a	606.3 \pm 9.5 ^b	678.6 \pm 14.1 ^c	1810.6 \pm 32.6 ^b	225.7 \pm 11.2 ^d	263.2 \pm 19.5 ^e

Vehicle = DMSO 100 μ l; Mutagen: ^a2-nitrofluorene (6 μ g/plate); ^b2-aminoanthracene (5 μ g/plate); ^csodium azide (5 μ g/plate); ^dmethyl methane sulfonate (1700 μ g/plate); ^e2-aminoanthracene (25 μ g/plate).

¹ Low toxicity: low reduction of the background lawn and revertant colonies.

* $p < 0.05$ vs mutagen (Holm-Sidak Method).

In the mutagenicity test, neither tea-tree oil nor lavender oil, either with or without added S9 significantly increased the number of revertants in the two *Salmonella* strains and in the *E. coli* WP2 *uvrA* strain (data not shown).

Tea-tree oil, with and without metabolic activation, induced no antimutagenic activity, on TA98 or TA100 or *E. coli* WP2 *uvrA* strains (Table 2). Conversely, when assayed without S9-based metabolic activation, lavender oil exerted significant antimutagenic activity against the 2-nitrofluorene mutagen ($p < 0.05$ vs mutagen by Holm-Sidak method) and also reduced mutant colonies in the TA98 strain. Lavender oil-induced inhibition was concentration-dependent: 0.25 mg/plate induced moderate antimutagenic activity, reducing histidine-prototrophic revertant colonies by 29.1% whereas higher concentrations (from 0.40 mg/plate to 0.80 mg/plate) reduced his-

tidine-independent revertant colonies (by 47.0% and 66.4%) (Table 2 and Fig. 1). To verify whether the antimutagenic activity on the TA98 strain was specific for the chemical class of nitroarenes, the same concentrations of lavender oil were assayed against two tetracyclic nitroarenes (1-nitropyrene, 3-nitrofluoranthene) and 4-nitroquinoline 1-oxide, a nitroazarene *N* oxide. At the highest concentration tested (0.80 mg/plate), lavender oil exerted a protective effect against 1-nitropyrene; it moderately though significantly reduced the number of histidine-independent revertant colonies (by 33% vs mutagen, $p < 0.001$ by Holm-Sidak method) (Table 3).

4. Discussion

These findings obtained by the bacterial reverse mutation assay clearly demonstrate that *M. alternifolia* and *L. angustifolia* essential oils, with or without metabolic activation, have no mutagenic activity either in the TA98 and TA100 *S. typhimurium* strains and or in the *E. coli* WP2 *uvrA* strain.

Under our experimental conditions, lavender oil clearly and selectively protected against the mutagenicity induced by the 2-nitrofluorene-induced frameshift mutation (C-G base-pair deletion) in the TA98 strain. Co-incubation of the TA98 strain with mutagen and lavender oil significantly reduced the 2-nitrofluorene-induced mutation. 2-Nitrofluorene is a direct-acting mutagen that induces mutations of frameshift type due not to simple intercalation but to adduct formation (Rosenkranz and Mermelstein, 1983). Lavender oil probably protects against mutation by intercepting rather than by altering enzymatic activation (Horn and Ferrao Vargas, 2003). It could do so by directly deactivating 2-nitrofluorene thus blocking the DNA-damaging mutagen. Our in vitro experiments showing that lavender oil exerted its antimutagenic effect in the absence

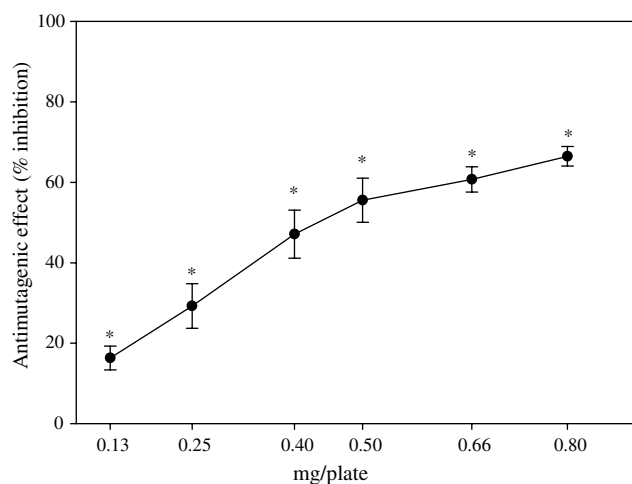


Fig. 1. Inhibitory effect of *L. angustifolia* essential oil against 2-nitrofluorene (6 µg/plate) mutagenicity in *Salmonella typhimurium* TA98 without metabolic activation (–S9). Points represent the mean number of revertant colonies per plate ($n = 9$) ± standard error. * $p < 0.05$ vs mutagen (Holm-Sidak method).

Table 3

Antimutagenicity of *Lavandula angustifolia* (lavender oil) essential oils to *Salmonella typhimurium* TA98 without metabolic activation (–S9) against three different direct mutagens ($n = 6$ or 9 plates)

Test item	Concentration mg/plate	Number of revertant colonies (mean ± SE)		
		TA98-S9		
		1NP ^a	3NFA ^b	4NQO ^c
Lavender oil	0.80	684.0 ± 40.2*	940.5 ± 34.2	802.6 ± 35.0
	0.66	900.2 ± 28.3	895.8 ± 40.3	798.6 ± 24.8
	0.50	954.3 ± 31.0	990.4 ± 28.6	712.8 ± 31.6
	0.40	895.4 ± 28.6	925.2 ± 30.4	760.4 ± 24.4
	0.25	974.4 ± 34.2	971.9 ± 31.9	748.9 ± 27.9
	0.13	924.5 ± 31.9	958.7 ± 31.9	773.5 ± 30.7
	Vehicle	31.0 ± 0.9	35.2 ± 1.0	33.8 ± 1.2
Mutagen	1020.7 ± 35.1	983.3 ± 40.6	750.0 ± 28.9	

Vehicle = DMSO 100 µl; Mutagen: ^a1-nitropyrene (2.0 µg/plate); ^b3-nitrofluoranthene (0.12 µg/plate); ^c4-nitroquinoline 1-oxide (0.5 µg/plate).

* $p < 0.001$ vs mutagen (Holm-Sidak method).

of rat liver fraction S9, exclude an interaction between this essential oil and components of the exogenous mammalian metabolic system. Alternatively, lavender oil might promote the excision repair system even if both TA98 and TA100 are plasmid-containing strains with an enhanced error-prone DNA repair, and WP2 *uvrA* is not a repair proficient strain. Lavender oil did not inhibit the base-pair substitution induced by the other direct mutagens, sodium azide and methyl methane sulphonate for *S. typhimurium* TA100 and *E. coli* WP2 *uvrA*, and the indirect mutagen 2-aminoanthracene.

Lavender oil effectively antagonized the mutagenic effect of the tricyclic nitroarene 2-nitrofluorene but left mutagenicity of the tetracyclic nitroarene 3-nitrofluoranthene, and the nitroazarene *N* oxide, 4-nitroquinoline 1-oxide, unchanged. It also exerted only a moderate antimutagenic effect against the other tetracyclic we tested, nitroarene 1-nitropyrene, and did so only at the highest non-toxic essential oil concentration assayed. Lavender oil may have failed to reverse the mutagenicity exerted by tetracyclic nitroarenes because these are the most mutagenic mononitroarenes so far reported (Rosenkranz and Mermelstein, 1983). Our data nevertheless seem to argue against the finding that flavonoids, coumarins and various phenolic compounds show a more effective protective action against tetracyclic nitroarenes than against the tricyclic 2-nitrofluorene (Edenharder and Tang, 1997).

Among several environmental compounds possessing mutagenic properties, nitroarenes, the reference positive control we used in this study, are well-known agents generated by the reaction of nitrogen oxides with polycyclic aromatic hydrocarbons during incomplete combustion of organic materials. They are found in particulate emissions from diesel engines, in urban air, and in river sediments (Rosenkranz and Mermelstein, 1983). The tricyclic linear 2-nitrofluorene is the dominating atmospheric nitroarene. Besides being mutagenic, 2-nitrofluorene is among the few nitroarenes shown to have carcinogenic activity in rodents (Moller et al., 1989); and is possibly carcinogenic in humans (IARC, 1989). As well as being inhaled, nitroarenes can also be ingested through the gastrointestinal tract because they form in food during combustion or via oxidation of the respective amines (Edenharder and Tang, 1997). Several natural substances, including plant constituents or extracts, reportedly exert antimutagenic effects and may be of value in preventing cancer or other diseases linked with mutation occurrence: plant polyphenols (flavonoids, tannins) are known to inhibit various enzymatic activities responsible for xenobiotic metabolism, or to act as antioxidant agents (Wall et al., 1990; Yen and Chen, 1994). Essential oils generally have a complex composition, owing to the presence of a large variety of highly functional chemical entities, belonging to different chemical classes and skeletal types.

Considering the increasing use of these essential oils, and their frequent presence in products for personal care bought without prescription, their safety profile must be carefully assessed. Hence their lack of mutagenicity is reassuring and our findings may help to understand better how two commonly used essential oils interact with the heritable material.

The antimutagenic activity of lavender oil is an especially interesting finding. Further studies are required to identify the components responsible for its antimutagenic actions and understand the underlying mechanisms of action.

The growing interest in the use of essential oils now makes it advisable to assess their genotoxic potential and identify the mutagenic components. Plant species represent a great source of biologically active compounds whose effects on heritable material are mostly unknown. Research focussed on investigating the safety and possible new activities of herbal products is a welcome advance that could lead to the development of new products with favourable risk-benefit profiles (Barnes, 2003).

The antimutagenic property of lavender oil we report in this paper could make lavender oil a promising candidate for future applications in human healthcare.

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