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Genotoxicity and antigenotoxicity of some essential oils evaluated by wing spot test of *Drosophila melanogaster*

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Abstract

Essential oils extracted from the three medicinal plants; *Helichrysum italicum*, *Ledum groenlandicum* and *Ravensara aromatica,* together with their mixture were tested for their genotoxic and antigenotoxic activities against urethane, a well-known promutagen. We have adopted the somatic mutations and recombination test (SMART) in the wings of *Drosophila melanogaster*. Three days old larvae, trans-heterozygous for two genetic markers *mwh* and *flr*, were treated by essential oil and/or urethane. A negative control corresponding to solvent was also used. Our results do not show any significant effect of the oils tested but they reduce the mutation ratio resulting from urethane. The mixture of the three oils at equal volume seems to be the most effective. The antimutagenic effect of these oils could be explained by the interaction of their constituents with cytochrome P-450 activation system leading to a reduction of the formation of the active metabolite. The effect could also be attributed to certain molecules that are involved in these oils.

Keywords: *Helichrysum italicum*; *Ledum groenlandicum*; *Ravensara aromatica*; Essential oils; SMART assay

1. Introduction

During the last years, the use of medicinal plants in the cancer treatment has become more and more promising. However, the available scientific data regarding their properties, their efficiency and their chemical composition are very limited. The possibility of moderating the response of cells to a particular mutagen, by natural substances, opens new horizons in cancer control. On this basis, the research for antimutagens could bring about surprises in the discovery of new anticarcinogenic substances. Antimutagens are classified into two major groups according to their mechanisms of actions: interceptors regrouping inhibitors, which act directly on mutagens or their precursors to inactive them, and bioantimutagens, which inhibit the mutagenesis processes or repairing DNA damage [1,2]. Genotoxicity tests allowed the identification of several plants, whose oils or some of their compounds might have antimutagenic and/or anticarcinogenic effects [3–5]. Tests on *Drosophila melanogaster* have been used for more than 50 years

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in the identification of carcinogen products and in the study of their mechanisms of action. Nevertheless, the tests of antigenotoxicity date back to the last decade. In fact, there are many reasons why *D. melanogaster* is suitable for developing reliable essay systems. Among them, its potential capacity to biotransform certain procarcinogens to their reactive metabolites [6]. Recently, *Drosophila* genome sequencing, carried out by the "*Drosophila* Genome Project", has revealed that half of the identified protein sequences have shown their similarity to the mammalian proteins. Furthermore, the fly presents an orthology of 61% of human disease genes and 68% of the cancer genes that control cancers [7,8]. Somatic mutations and recombination test (SMART) in the wings of *D. melanogaster* [9] is among the most used tests with this fly. It is based on the loss of heterozygoty for two recessive markers *mwh* (3–0.3) and *flr* (3–38.8). This test is able to detect a wide spectrum of alterations, including the point mutations, deletions, mitotic recombinations, chromosomal loss and non-disjunction [10]. SMART detects also the activity of promutagens, using strains with high capacity of transforming some carcinogens to their active metabolites [11].

The aim of the present study is the evaluation of genotoxic and antigenotoxic activities of the three essential oils extracted from *Helichrysum italicum*, *Ledum groenlandicum* and *Ravensara aromatica* and their mixtures. The positive control used is urethane, a promutagen which could be found in many fermented foods and beverages.

Table 1
Chemical constituents (%) contained in *Helichrysum*, *Ledum* and *Ravensara*

Compound	*Helichrysum*	*Ledum*	*Ravensara*
Bornyl acetate	–	0.83	–
Gamma cadinene	4.16	–	0.86
Camphre	–	–	0.08
beta *trans*-caryophyllene	8.83	1.50	0.62
Cineole 1.8	0.55	–	56.45
Citronellal	1.16	–	–
Gamma curcumene	21.31	–	–
Delta 3-carene	–	–	0.86
Geranyl acetate	1.32	–	–
D-Germacrene	–	9.01	–
Alpha humulene	–	0.10	0.71
Ledol	–	0.15	–
Limonene	1.75	36.16	2.67
Linalol	0.52	–	0.33
Myrcene	–	0.45	1.35
Myrtenal	–	1.40	–
Nerol	0.19	5.42	–
Nerolidol	0.67	–	–
Neryl acetate	3.04	–	–
Neryl butyrate	6.15	–	–
Neryl isobutyrate	4.24	–	–
Paracymene	–	5.00	–
Alpha phellandrene	–	2.61	0.09
Alpha Pinene	28.67	0.94	4.51
Beta pinene	0.43	0.94	3.40
Sabinine	–	1.44	12.99
Terpinene-1-ol-4	0.15	8.69	2.48
Gamma terpinene	0.37	0.62	1.30
Alpha terpineol	0.29	1.17	7.63
Terpinolene	0.25	1.13	0.33
Beta thujone	–	1.15	–
Not determined	11.72	17.77	2.40

2. Materials and methods

2.1. Extraction and identification of essential oils

The used part of *Helichrysum* (flower head), *Ledum* (bough) and *Ravensara* (leaf) were subjected to a water vapour distillation. This was performed at a low pressure without chemical descalers. The essential oil obtained was analysed by gas chromatography (FISONS GC 8180 chromatograph) with a flame ionisation detector. Helium (1 ml/min) was used as a carrier gas. The essential oil diluted 1/25 in hexane and directly injected (0.5–1.0 μl) in two glass capillary columns (0.3 mm × 60 m), respectively, filled with DB-I and DB-wax. The oven temperature was programmed from 80 to 250°C at a rate of 2°C/min [12,13]. A volume of 88, 82, and 98%, of the oil composition were identified in *Helichrysum*, *Ledum* and *Ravensara*, respectively. Table 1 lists only the major components of each of the oils obtained by gas chromatography analysis.

2.2. Compounds

PRANAROM International Company, Belgium, provided our laboratory with essential oils, *Helichrysum*, *Ledum* and *Ravensara*. Their mixture was

prepared by adding equal volume of the three oils. Urethane (51-79-6) was a Sigma product.

2.3. Stocks and crossing

Males of genotype *NORR/NORR*; *NORR/NORR*; *mwh/mwh* were crossed with virgin females of genotype *NORR/NORR*; *NORR/NORR*; *flr*^{3}*/TM3*, *Bd*^{s}. NORR strains (new ORR) were constructed by Pacella et al., [14] according to the procedure described by Frolich and Würgler [15] in the construction of the ORR strains. Detailed information on genetic markers is given by Lindsey and Zimm [16].

2.4. Experimental protocol

Eggs were collected for 8 h in cultivating bottles containing extra live yeast. Three days later, larvae were collected using a 20% sodium chloride solution. The larvae were then transferred into tubes containing 840 mg of *Drosophila* Instant Medium (Formulated 4–24, Carolina Biological Supply, Burlington, NC) mixed with 4 ml of the solution to be tested. All the tested compounds were dissolved in Tween-80 and were vigorously agitated. The tubes containing the treated larvae and those of control were incubated at 25°C until the emergence of adults. The surviving flies trans-heterozygous (*mwh+/flr+*) were collected from the treatment vial on days 10–12 after egg laying and were stored in the ethanol 70%. The wings were put on the slides. Following the procedure described by Graf et al. [9], they were analysed under a photonic microscope for the occurrence of mutant spots on the phenotypically wild type wing blade. Wings were scored for (1) small (one to two cells) simple spots, (2) large (more than two cells) single spots, both with *mwh* or *flr*^{3} phenotype, and (3) twin spots (phenotypes *mwh* and *flr*^{3} in adjacent clone).

For the statistical analysis, we used the Chi-square test to compare the rate of mutations induced in the treated series and those of controls. The percentage of inhibition was also calculated as following:

$$\frac{100(a-b)}{a}$$

where a is the frequency of spots induced by urethane alone and b the frequency of spots induced by urethane in the presence of essential oil.

3. Results

The four essential oils were tested, at least, at two concentrations. The pooled data of genotoxicity are summarised in Table 2; those of the antigenotoxicity are reported in Table 3. All four oils tested were toxic at a concentration higher than 0.3%. *Ledum* showed the highest toxicity, and the maximum tolerated concentration was around 0.2%. To compare the spot size distributions, data of single spots from urethane alone and in combination with the essential oils were showed in Fig. 1.

3.1. Controls

The compounds tested were dissolved in Tween-80 at 0.1%. The frequency of total mutations obtained (0.49 spots per wing) did not deviate significantly from historical water control of our laboratory (0.30). Urethane, as a positive control, was tested in all experiments at 5 mM. This promutagen increased significantly the small single spots, large simple spots, and the total of spots ($P < 0.001$). The frequency of twin spots was insignificant. The induction of the small size clone was more important than that of the large clones. Repetitions implying urethane were homogeneous; however, its results were not grouped so as not to influence the rate of inhibition expressed by each co-treatment.

3.2. H. italicum

The essential oil extracted from *Helichrysum* was tested at three different concentrations. The maximum tolerated concentration was 0.3%. The frequencies obtained for each type of the mutation did not differ from those obtained in solvent (Table 2). The co-incubation of the larvae with *Helichrysum* and urethane showed a significant reduction of mutations ($P < 0.001$). The frequency of total spots were reduced from 1.63 in the case of urethane to 0.72, 0.70 and 0.75, when it was combined with 0.1, 0.2 and 0.3% of oil, respectively. This represents a reduction of 56, 57 and 54% (Table 3). The effect was not dose-dependent (Fig. 1a). An increased toxicity was observed when urethane was combined with 0.3% of oil as compared to the oil alone.

Table 2
Summary of results obtained with the *Drosophila* wing spot test in the presence of different concentrations of the essential oils of *Helichrysum*, *Ledum*, *Ravensara* and their mixture[a]

Compounds concentration (%)	Number of wings	Spots per wing (number of spots)			
		Small single spots (one to two cells)	Large single spots (more than two cells)	Twin spots	Total spots
Control (Tween 80)					
0.1	240	0.45 a (107)	0.03 a (07)	0.01 a (03)	0.49 a (117)
H. italicum					
0.1	80	0.51 a (41)	0.03 a (02)	00 a (00)	0.54 a (43)
0.2	80	0.45 a (36)	0.01 a (01)	00 a (00)	0.46 a (37)
0.3	80	0.34 a (27)	0.04 a (03)	00 a (00)	0.38 a (30)
L. groenlandicum					
0.1	79	0.51 a (40)	0.05 a (04)	0.01 a (01)	0.57 a (45)
0.2	77	0.58 a (45)	0.03 a (02)	0.00 a (00)	0.61 a (47)
R. aromatica					
0.1	84	0.38 a (32)	0.01 a (01)	0.01 a (01)	0.40 a (34)
0.2	86	0.50 a (43)	0.09 b (08)	0.00 a (00)	0.59 a (51)
0.3	80	0.40 a (32)	0.08 a (06)	0.01 a (01)	0.49 a (39)
Mixture					
0.1	81	0.37 a (30)	0.00 a (00)	0.09 b (07)	0.46 a (37)
0.2	40	0.45 a (18)	0.13 b (05)	0.00 a (00)	0.58 a (23)
0.3	80	0.36 a (29)	0.05 a (04)	0.03 a (02)	0.44 a (35)

[a] Values with different letters are significantly different at least at $P < 0.05$.

Table 3
Inhibitory effect of the essential oils of *Helichrysum*, *Ledum, Ravensara* and their mixture on the somatic mutation induced by urethane[a]

Compounds concentration (%)	Number of wings	Spots per wing (number of spots)				Inhibition (%)
		Small single spots (one to cells)	Large single spots (more than two cells)	Twin spots	Total spots[b]	
Tween 80 (0.1)	240	0.45 (107)	0.03 (07)	0.01 (03)	0.49 (117)	
Ure	81	1.00 (81)	0.58 (47)	0.05 (04)	1.63 a (132)	
Ure and Hel 0.1	81	0.59 (48)	0.11 (09)	0.03 (01)	0.72 b (58)	56
Ure and Hel 0.2	40	0.60 (24)	0.08 (03)	0.02 (01)	0.70 b (28)	57
Ure and Hel 0.3	32	0.69 (24)	0.06 (02)	0.00 (00)	0.75 b (24)	54
Ure	81	1.00 (81)	0.58 (47)	0.05 (04)	1.63 a (132)	
Ure and Led 0.1	80	0.75 (60)	0.25 (20)	0.04 (03)	1.04 b (83)	36
Ure and Led 0.2	40	0.7 (28)	0.20 (08)	0.03 (01)	0.93 b (37)	43
Ure	80	1.41 (113)	0.26 (21)	0.00 (00)	1.67 a (134)	
Ure and Rav 0.1	80	1.13 (90)	0.23 (19)	0.04 (03)	1.40 a (112)	16
Ure and Rav 0.2	82	0.85 (70)	0.21 (17)	0.01 (01)	1.07 b (88)	36
Ure and Rav 0.3	57	0.82 (47)	0.09 (05)	0.00 (00)	0.91 b (52)	46
Ure	78	1.63 (127)	0.36 (28)	0.01 (01)	2.00 a (156)	
Ure and Mix 0.1	80	0.75 (60)	0.21 (17)	0.03 (0.3)	1.00 b (80)	50
Ure and Mix 0.2	67	1.00 (67)	0.15 (10)	0.06 (04)	1.21 b (81)	40
Ure and Mix 0.3	39	0.36 (14)	0.13 (05)	0.05 (02)	0.54 c (21)	73

[a] Ure, urethane; Hel, *Helichrysum*; Led, *Ledum*; Rav, *Ravensara*; and Mix, mixture.
[b] For the total spots and for each oil, values with different letters are significantly different at least at $P < 0.05$.

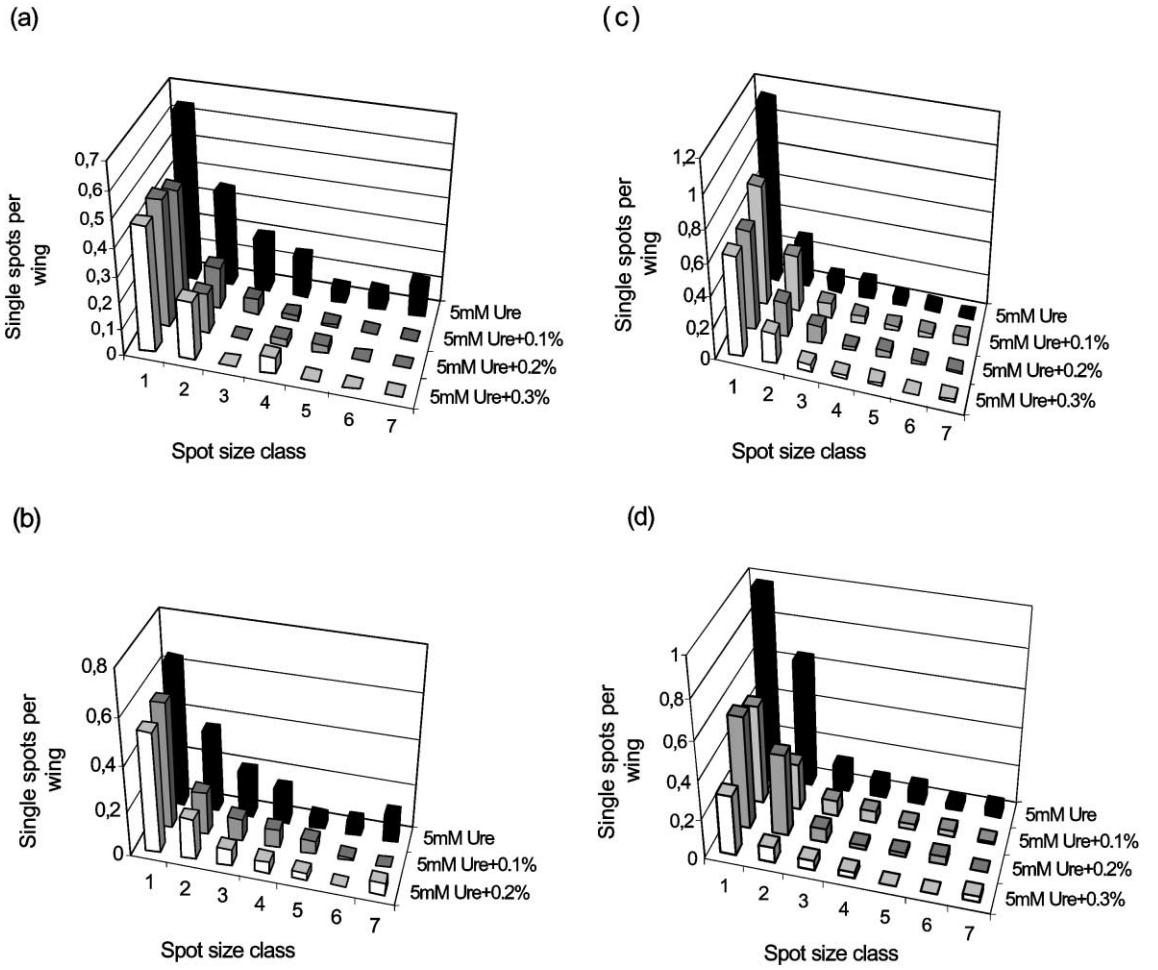

Fig. 1. Spot size distributions and single spot induction frequencies after larvae fed with urethane alone and in combination with (a) *Helichrysum*, (b) *Ledum*, (c) *Ravensara*, and (d) their mixture in equal volume. Clone size classes: 1, one cell; 2, two cells; 3, three to four cells; 4, five to eight cells; 5, 9–16 cells; 6, 17–32 cells; and 7, more than 32 cells.

3.3. L. groenlandicum

The essential oil of this plant showed the highest toxicity as compared to the last one. The maximum tolerated concentration was 0.2%. With respect to genotoxicity results, no significant increase above control level could be observed (Table 2). However, its co-incubation at 0.1 and 0.2% with urethane showed an inhibition of 36 and 46%, respectively ($P < 0.001$). These reduction ratios remained lower than those expressed by *Helichrysum* (Table 3). The reduction was important in the induction of large spots (Fig. 1b).

3.4. R. aromatica

The essential oil of this plant was less toxic, than the two first ones. Except for a weak effect for the large single spot ($P < 0.05$) at 0.2%, the frequency of mutations induced by this compound alone did not show any difference of the value obtained in the negative control (Table 2). The antigenotoxic effect was very significant at 0.2% ($P < 0.01$) and 0.3% ($P < 0.001$), where an overall reduction of 36 and 46% in the number of spots was observed. However, at 0.1%, the value was about 16% (Table 2).

A dose-antigenotoxic effect was also observed with *Ravensara* (Fig. 1c).

3.5. Mixture

The toxicity of mixture, at equal volume of the three oils, was lower than that of *Ledum* oil. The mixture did not induce any increase in the number of mutations over negative control (Table 2). However, a drastic reduction of mutations was observed when the mixture was co-administered with urethane (Table 3). This reduction reached its maximum ($P < 0.001$) at 0.3% (Fig. 1d) where an inhibition of 73% was noted.

4. Discussion

The secondary plant compounds such as terpenes, sterols, alkaloids, phenolics and quinone are implicated in plant defence mechanisms against herbivores including insects. Against this situation, a number of insects that are in permanent contact with these plants developed their enzymatic system cytochrome P-450 [17,18]. P-450s are involved in oxidative, peroxidative, and reductive metabolism of numerous pesticides, herbicides, environmental pollutants and plant toxins [19,20]. In *D. melanogaster*, P-450 is related to insecticide resistance, in other words, resistant strains have higher P-450 activity and greater P-450 content than susceptible strains [21,22]. These data could explain the high toxicity obtained in our results when the larvae were exposed directly to these essential oils. The study of the genotoxicity of *Helichrysum, Ledum, Ravensara* and their mixture did not reveal any mutagenic effect. This is in line with the results of other studies carried out on certain constituents of these oils. Thus, the limonene and the cineole, which are the major elements of *Ledum* and *Ravensara*, respectively, had been shown by Ames test to be non-genotoxic [23]. The same result was obtained for other minor elements [23–25]. Urethane is a promutagen which can occur naturally in some fermented food [26]. Its mutagenic activity was studied during several years [27,28]. Many authors had proposed the metabolic way which leads to the formation of the vinylcarbamate, and after peroxidation leads to ADN and ARN adducts [29,30]. This assumption was supported by a study of Gupta and Dani [31] who identified *N*-hydroxyvinyl-carbamate, *N*-hydroxyethyl-carbamate and epoxyethyl-carbamate after incubation of ethyl-carbamate with the microsomes of rat liver. Yet, urethane remains without effect in Ames test due to the absence of S9 [32]. These data show the importance of the biotransformation in the mutagenesis of this compound. Our obtained results with NORR strains are consistent with our previous studies carried out with the same strain [33]. They confirm the thesis of a biotransformation of urethane in the sense that the response of the standard strains of *D. melanogaster* was much more inferior [34].

Co-incubation of larvae with urethane and our essential oils leads to a significant reduction of the ratio of induced mutations. This effect could be caused by the major components of these oils. In this sense, the limonene, alpha pinene and cineole were found to be potent chemopreventive agents in the rat mammary carcinogenesis induced by the 7,12 dimethylbenz[*a*]anthracene [35]. The limonene proved to be effective against rodent skin, liver and lung cancer [36]. Myrcene-inhibited sister-chromatid exchanges induced by several mutagens [37]. *Helichrysum, Ledum* and *Ravensara* constituents interact probably with cytochrome P-450 system leading to a reduction of the formation of the ultimate metabolites. This suggestion is supported by the works of Kuroda et al. [38] who had postulated that when we are dealing with co-treatment, the antimutagen acts as desmutagen which chemically or enzymatically can inactivate the mutagen, or inhibit the metabolic activation of promutagen. In fact, some authors [25,39] demonstrated that the alpha pinene, the limonene, the citronellal and the beta-myrcene inhibit the activity of mono-oxygenase Cyp2B1 necessary for the activation of genotoxins in the rat. Likewise, we find that for each oil studied, there is more than one constituent that was shown to be antimutagen. This leads to the suggestion of the synergetic effect of several inhibitors which is known as a "combined chemoprevention". [40]. This synergy could explain the importance of the inhibition observed in the case of the mixture with regard to the three oils tested separately.

In summary, the present study indicates that these essential oils possess an antigenotoxic activity in *Drosophila* wing spot test. It may involve the interaction of their constituents with cytochrome P-450. However, the precise mechanisms are not

well-understood because of the limited data reported in the literature with respect to the inhibitory effects of these oils or its constituents. Antimutagenesis studies tackling these oils against inactivated mutagens (by cytochrome P-450) and against direct acting mutagens are needed to over-clarify further the mechanisms of action.

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References

[1] K. Morita, M. Hara, T. Kada, Studies on the natural desmutagens: screening for vegetable and fruit factors active in inactivation of mutagenic pyrolysis products from amino acids, Agric. Biol. Chem. 42 (1978) 1235–1238.
[2] T. Kada, Environmental and biological factors suppressing induction of mutagens, Toxicol. Forum 6 (1983) 580–589.
[3] A.P. Guevara, C. Lim-Sylianco, F. Dayrit, P. Finch, Antimutagens from *Momordica charantia*, Mutat. Res. 230 (1990) 121–126.
[4] I.S. Grover, S. Bala, Studies on antimutagenic effect of guava (*Psidium guavaga*) in *Salmonella typhimurium*, Mutat. Res. 300 (1993) 1–3.
[5] I.M. Villaseñor, P. Lemon, A. Palileo, J.B. Bremner, Antigenotoxic spinasterol from *Cucurbita maxima* flowers, Mutat. Res. 360 (1996) 89–93.
[6] A.J. Baars, W.G.H. Blijeven, G.R. Mohn, A.T. Natarajan, D.D. Brieimer, Preliminary studies on the ability of *Drosophila* microsomal preparations to activate mutagens and carcinogens, Mutat. Res. 72 (1980) 257–264.
[7] M.D. Adams, S.E. Celniker, R.A. Holt, C.A. Evans, J.D. Gocayne, P.G. Amanatides, S.E. Scherer, et al., The genome sequence of *Drosophila melanogaster*, Science 287 (2000) 2185–2195.
[8] G.M. Rubin, M.D. Yandell, J.R. Wortman, G.L. Gabor Miklos, C.R. Nelson, I.K. Hariharan, et al., Comparative genomics of the eukaryotes, Science 287 (2000) 2204–2215.
[9] U. Graf, F.E. Würgler, A.K. Katz, H. Frei, H. Juon, B.C. Hall, P.G. Kale, Somatic mutation test in *Drosophila melanogaster*, Environ. Mutagen. 6 (1984) 153–188.
[10] E.W. Vogel, J.A. Zijlstra, Mechanistic and methodological aspects of chemically-induced somatic mutation and recombination in *Drosophila melanogaster*, Mutat. Res. 182 (1987) 243–264.
[11] U. Graf, N. Van Schaik, Improved high bioactivation cross for the wing mutation and recombination test in *Drosophila melanogaster*, Mutat. Res. 271 (1992) 59–67.
[12] D. Baudoux, Process, analysis and definition of essential oil, Aroma News, Nat. Aromatherap. Dev. 4 (1998) 1–4.
[13] I.B. Chinou, V. Roussis, D. Perdetzoglou, A. Loukis, Chemical and biological studies on two *Helichrysum* species of Greek origin, Planta Med. 62 (1996) 377–379.
[14] R.E. Pacella Norman, N. Van Schaik, U. Graf, New high bioactivation cross for the SMART wing assay, *Drosophila* Inf. Serv. 77 (1996) 152–153.
[15] A. Frolich, F.E. Würgler, New tester strains with improved bioactivation capacity for the *Drosophila* wing spot test, Mutat. Res. 216 (1989) 179–187.
[16] D.L. Lindsley, G.G. Zimm, The Genome of *Drosophila melanogaster*, Academic Press, San Diego, CA, 1992, p. 1133.
[17] M.B. Cohen, M.R. Berenbaum, M.A. Schuler, Induction of cytochrome P-450 mediated metabolism of xanthoxin in the black swallowtail, J. Chem. Ecol. 15 (1989) 2347–2355.
[18] L.C. Waters, A.C. Zelhof, B.J. Shaw, L.-Y. Chang, Possible involvement of the long terminal repeat of transposable element 17.6 in regulating expression of an insecticide resistance-associated P-450 gene in *Drosophila*, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 4855–4859.
[19] R. Feyreisen, J.F. Koener, D.E. Farnsworth, D.W. Nebert, Isolation and sequence of a cDNA encoding a cytochrome P-450 from insecticide-resistant strain of the house fly, *Musca domestica*, Proc. Natl. Acad. Sci. U.S.A. 86 (1989) 1465–1469.
[20] S.S. Sundseth, C.E. Nix, L.C. Waters, Isolation of insecticide resistance-related forms of cytochrome P-450 from *Drosophila melanogaster*, Biochem. J. 265 (1990) 213–217.
[21] I. Hällström, A. Blanck, Genetic regulation in the cytochrome P-450 and xenobiotic metabolism in *Drosophila melanogaster*, Biochem. Pharmacol. 33 (1984) 13–20.
[22] J.S. Prevec, N.D. Okoampah, R.A. Morton, Organophosphorus insecticide resistance in *Drosophila melanogaster* populations, J. Genet. 71 (1992) 121–134.
[23] M.R. Gomes-Carneiro, I. Felzenszwalb, F.J.R. Paumgartten, Mutagenicity testing of (±)-camphor, 1,8-cineole, citral, citronellal, (−)-menthol and terpineol with the *Salmonella*/microsome assay, Mutat. Res. 416 (1998) 129–136.
[24] E. Zeiger, B. Anderson, S. Haworth, T. Lawlor, K. Mortelmans, *Salmonella* mutagenicity tests. Part IV. Results from the testing of 300 chemicals, Environ. Mol. Mutagen. 11 (1988) 1–158.
[25] B. Kauderer, F Zamith, F.J. Paumgartten, G. Speit, Evaluation of the mutagenicity of beta-myrcene in mammalian cells in vitro, Environ. Mol. Mutagen. 18 (1991) 28–34.
[26] C.S. Ough, Ethylcarbamate in fermented beverages and foods. I. Naturally occurring ethylcarbamate, J. Agric. Food Chem. 24 (1976) 323–328.
[27] Z.U. Ahmed, G.W.R. Walker, The effects of urethane sodium monohydrogen arsenate and selenocystine on crossing over in *Drosophila melanogaster*, Can. J. Genet. Cytol. 17 (1975) 55–66.
[28] A.J. Bateman, The mutagenic action of urethane, Mutat. Res. 39 (1976) 75–96.

[29] M.L. Ribovich, J.A. Miller, E.C. Miller, L.G. Timmins, Labeled 1, *N*,6-ethenoadenosine and 3, *N*,4-ethenocytidine in hepatic RNA of mice given [ethyl-1,2-^{3}H] or [ethyl-1-^{14}C] ethyl carbamate (urethane), Carcinogenesis 3 (1982) 539–546.
[30] J.A. Miller, E.C. Miller, The metabolic activation and nucleic acid adducts of naturally-occurring carcinogens: recent results with ethyl carbamate and spice flavors safrole and estragole, Br. J. Cancer 48 (1983) 1–15.
[31] R. Gupta, H.M. Dani, In vitro formation of organ specific ultimate carcinogens of 4-dimethylaminoazobenzene and urethane by microsomes, Toxicol. Lett. 45 (1989) 49–53.
[32] P. Hubner, P.M. Groux, B. Weibel, C. Sengstag, J. Horlbeck, P.M. Leong-Morgenthaler, J. Luthy, Genotoxicity of ethyl carbamate (urethane) in *Salmonella* yeast and human lymphoblastoid cells, Mutat. Res. 390 (1997) 11–19.
[33] R. El Hamss, M. Analla, J. Campus-Sanchez, A. Alonso-Moraga, A. Muñoz-Serrano, M. Idaomar, A dose dependent antigenotoxic effect of turmeric, Mutat. Res. 446 (1999) 135–139.
[34] A. Frolich, F.E. Würgler, Genotoxicity of ethyl carbamate in the *Drosophila* wing spot test: dependence on genotype-controlled metabolic capacity, Mutat. Res. 244 (1990) 201–208.
[35] W.A. Russin, J.D. Hoesly, C.E. Elson, M.A. Tanner, M.N. Gould, Inhibition of rat mammary carcinogenesis by monoterpenoids, Carcinogenesis 10 (1989) 2161–2164.
[36] P.L. Crowell, Prevention and therapy of cancer by dietary monoterpenes, J. Nutr. 129 (1999) 775–778.
[37] C. Roscheisen, H. Zamith, F.J. 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.
[38] Y. Kuroda, A.K. Jain, H. Tezuka, T. Kada, Antimutagenicity in cultured mammalian cells, Mutat. Res. 267 (1992) 201–209.
[39] A.C. De-Oliveira, L.F. Ribeiro-Pinto, J.R. Paumgartten, In vitro inhibition of CYPB1 monooxygenase by beta-myrcene and other monoterpenoid compounds, Toxicol. Lett. 92 (1997) 39–46.
[40] J.S. Bertram, L.N. Kolonel, F.L. Meyskens, Rationale and strategies for chemoprevention of cancer in humans, Cancer Res. 47 (1987) 3012–3031.