JPP Journal of Pharmacy and Pharmacology

JPP 2004, 56: 677–681

Received November 19, 2003
Accepted February 5, 2004
DOI 10.1211/0022357023321
ISSN 0022-3573

Melissa officinalis L. essential oil: antitumoral and antioxidant activities

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Acknowledgements and funding: The authors are grateful to Deise de A. Costa for technical assistance and to the Chromatography Laboratory of the Chemical Department of the Federal University of Sergipe for the availability of their GC/MS. This work was partially supported by grants from Conselho Nacional de Pesquisas (CNPq), Fundação Carlos Chagas de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ) and Fundação Universitária José Bonifácio (FUJB).

Abstract

Melissa officinalis L (lemon balm) is a traditional herbal medicine used widely as a mild sedative, spasmolytic and antibacterial agent. This paper focuses on the analysis of the chemical composition and the biological activities of *M. officinalis* essential oil obtained under controlled harvesting and drying conditions. An in-vitro cytotoxicity assay using MTT indicated that this oil was very effective against a series of human cancer cell lines (A549, MCF-7, Caco-2, HL-60, K562) and a mouse cell line (B16F10). This oil possessed antioxidant activity, as evidenced by reduction of 1,1-diphenyl-2-picryl-hydrazyl (DPPH). These results pointed to the potential use of *M. officinalis* essential oil as an antitumoral agent.

Introduction

Originally native to the Eastern Mediterranean region and Western Asia, *Melissa officinalis* L. (lemon balm) is also found in tropical countries (Brazil) where it is popularly known as erva-cidreira and melissa. Aqueous and alcoholic extracts of *M. officinalis* are traditionally used for their spasmolytic (Chakurski et al 1981), nervous sedative (Soulimani et al 1991), antiviral (Dimitrova et al 1993; Yamasaki et al 1998) and antioxidant (Lamiason et al 1991; Campos & Lissi 1995) activities. Much less is known about the properties of the essential oil from *M. officinalis* but it has been reported as having antibacterial, antifungal, antiparasitic and antispasmolytic activities (Dikshit & Husain 1984; Larrando et al 1995; Mikus et al 2000; Sadraei et al 2003).

Essential oils from a broad spectrum of plant species have been shown to have cytotoxic and/or antitumoral activity (Buhagiar et al 1999; Hayes & Markovic 2002; Moteki et al 2002). There has been accumulating evidence supporting a role of antioxidant mechanisms on the anti-cancer potential of dietary essential oils (Hansson et al 1994; Snklar 1998; Owen et al 2000; Dwivedi et al 2003). However, so far the presence of these activities in *M. officinalis* essential oil has not been demonstrated. This paper has evaluated the antioxidant activity of this oil, and has investigated its cytotoxicity on a series of human cancer cell lines A549 (lung), Caco-2 (colon), MCF-7 (breast), HL-60 and K562 (leukemia) and a mouse cancer cell line B16F10 (melanoma).

Materials and Methods

Essential oil

The culture of *M. officinalis* L was established at the Research Station "Campus Rural da UFS", Federal University of Sergipe, Brazil. Leaves were harvested at 0900 h, dried at 40 °C until complete dehydration had been achieved, and the essential oil was obtained by hydrodistillation in a Clevenger-type apparatus as described by Blank et al (2002). A Shimadzu QP5050A gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument was used to analyse the oil. The following conditions applied: column, DB5 (30 m long × 0.25 mm i.d. × 0.25-μm film thickness composed

of 5% phenylmethylpolysilo xane), connected to an ion trap detector operating in Electron Impact mode at 70V; carrier gas was He, flow rate 1 mL min^{-1}, split mode, with ratio of 1:5, and injection volume of 0.5 μL (in CH_2Cl_2); injector and ion-source temperatures were 250 and 280 °C, respectively. The oven temperature was programmed from 80 °C (isothermal for 2 min), with an increase of 3 °C min^{-1}, to 180 °C, then 10 °C min^{-1} to 300 °C, ending with a 10-min isothermal at 300 °C. The calculation of the retention indexes was made through co-injection with a n-alkane series (Van den Dool & Kratz 1963). Identification of the oil constituents were made based on the retention indexes (Adams 1995) and by comparison of mass spectra with computer search using NIST21 and NIST107 libraries. Compound concentrations were calculated from the GC peak areas and they were arranged in order of GC elution.

Cell viability assay

Essential oil was solubilized in dimethylsulfoxide (DMSO; Sigma, St Louis, MO) then diluted in culture media for use. Cell lines (HL-60, K562, A549, Caco-2, MCF-7 and B16F10) were maintained in RPMI-1640 or Dulbecco's Minimum Essential Medium (DMEM), supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 2 mM L-glutamine at 37 °C/5% CO_2. Cells (10^4/well) were cultivated for 24 h before the test compounds were added. After this time different dilutions of the essential oil (1:2000, 1:10 000, 1:50 000 and 1:100 000 v/v) or of DMSO (at the same concentration carried by the oil) were added to triplicate wells and the cells were cultivated for a further 48 h. Cell viability was evaluated by (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) MTT (Mosmann 1983) and the percentage inhibition of cell viability was calculated using cells treated with DMSO as controls. Cisplatin (Technoplatin, Zodiac, SP, Brazil) and vincristine (Sigma, St Louis, MO) were used as positive controls of antitumoral activity for A549 and K562, respectively.

Antioxidant activity

The antioxidant activity was measured by the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH; Sigma, St Louis, MO) reduction assay as described by Ramos et al (2003). Samples of the oil in ethanol (ranging from 0.005 to 25.0 μL mL^{-1}) were mixed with a solution of DPPH (0.3 mM in ethanol) at room temperature and 30 min later the absorbance was measured at 515 nm. An ethanolic solution of the oil was used as a blank and the solution of DPPH in ethanol was used as a negative control. The IC50 values were calculated by linear regression of plots where the abscissa represented the volume of tested oil and the ordinate the average percent of antioxidant activity from three separate tests.

Statistical analysis

Analysis of variance was used to evaluate the inhibitory effect of the different dilutions of the oil on the cells. A post-hoc test (Tukey) was used to determine the effectiveness of each dilution relative to the control. A non-parametrical statistical test (Kruskal–Wallis) was used to analyse the effect of the different dilutions of the oil in each cell.

Results

Considering the great application of essential oils in industry, we searched for the best conditions to obtain an oil with a composition that would fit international demand. It was observed that harvesting at 0900 h and drying the leaves at 40 °C resulted in the best chemical composition (Blank et al 2002). The chemical analysis of the *M. officinalis* essential oil is shown in Table 1. The essential oil content and yield obtained were 0.97% and 18.030 L per hectare.

To investigate the tumoricidal activity of *M. officinalis* essential oil, we evaluated its effect on the viability of five human cancer cell lines and a mouse melanoma cell line. As shown in Figure 1, for the oil dilutions ranging from 1:50 000 to 1:2 000 ($0.02–0.5 \times 10^3$) the essential oil induced a dose-dependent inhibitory effect on all lines tested that was significantly different (Kruskal–Wallis and one-way analysis of variance, $P < 0.0001$). At the dilution of 1:100 000 (0.01×10^3) the effect of the oil was negligible. Thus, the inhibitory effect of oil on cell viability ranged from $93.9 \pm 0.6\%$ for K562 to $73.9 \pm 16.4\%$ for B16F10 at 1:2000 dilution (0.5×10^3) and from $95.2 \pm 1.2\%$ to $45.1 \pm 5.8\%$ for K562 and MCF-7, respectively, at 1:10 000 dilution (0.1×10^3). Two-way analysis of variance showed that the cells were also different ($P = 0.026$) at different oil

Table 1 Composition of the essential oil of *Melissa officinalis* leaves harvested at 0900 h and oven dried at 40 °C.

Retention time (min)	Component	(%)	RRI exp.*	RRI lit.**
4.891	6-Methyl-5-hepten-2-one	1.10	982	985
7.795	Linalool	0.83	1099	1098
9.227	–	0.49	1141	–
9.914	*Cis*-Chrysanthenol	1.81	1161	1162
10.563	Isomenthol	2.91	1180	1182
13.084	Neral	39.28	1241	1240
14.343	Geranial	47.32	1270	1270
16.066	Methyl geranate	0.50	1322	1323
16.622	–	0.70	1336	–
17.576	–	0.44	1356	–
18.105	–	1.00	1372	–
18.508	Geranyl acetate	1.51	1383	1383
20.160	(E)-Caryophyllene	0.94	1424	1418
26.707	Caryophyllene oxide	1.17	1584	1581

*RRI, relative retention index calculated applying Van den Dool & Kratz (1963). **Comparison of the relative retention time with those reported by Adams (1995).

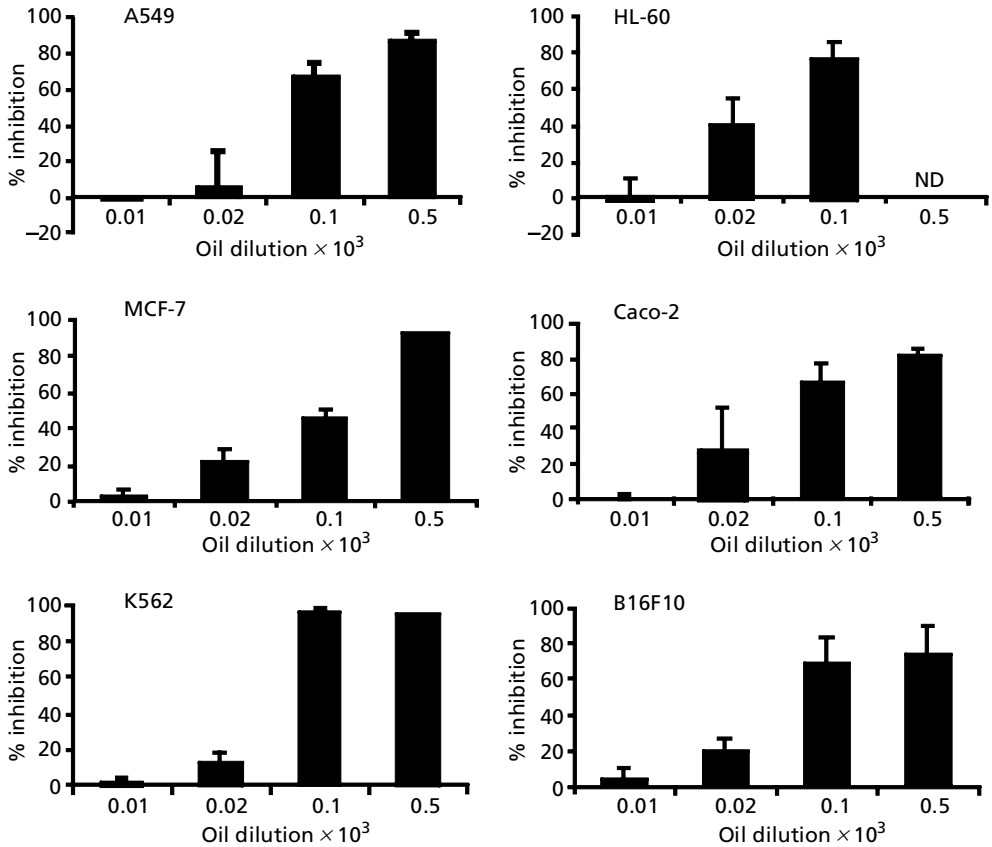

Figure 1 Cytotoxic activity of *M. officinalis* essential oil on cancer cell lines. Cell viability was assessed by MTT. Data were expressed as mean ± s.d. of at least three different experiments performed in triplicate. ND, not determined.

concentrations ($P < 0.0001$) that was suggestive of a specific effect of the oil. Treatment of A549 with 50 μg mL^{-1} cisplatin and of K562 with 60 nM vincristine reduced the viability of the cells by 98.0% and 95.1 ± 3.1%, respectively.

Evaluation of the antioxidant activity of *M. officinalis* essential oil by DPPH reduction assay (Figure 2) gave an EC50 value of 2.0 μL.

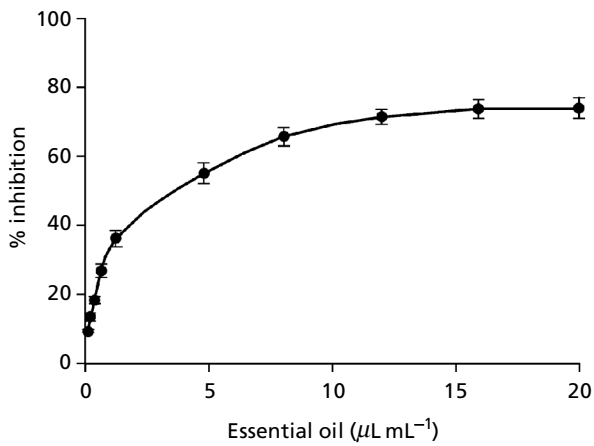

Figure 2 Antioxidant activity of *M. officinalis* essential oil. Antioxidant activity was measured by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) reduction. Data were expressed as mean ± s.d. of at least three different experiments.

Discussion

The ingestion of plant extracts with medicinal properties represents an alternative for the treatment of different pathological states in economically unprivileged countries. However, in the absence of a scientific basis, such practices may generate serious adverse effects. The analysis of the pharmacological activity of plant extracts may therefore make possible the design of less expensive therapies to be used in developing regions.

Essential oil from *M. officinalis* has a great economical importance due to its use in industry, especially the pharmaceutical industry. The effects of harvesting time and drying on the production of the essential oil obtained from a culture established in the northeastern region of Brazil was investigated by Blank et al (2002). Hollá et al (1997) analysed a commercially available essential oil of *M. officinalis* cultivated in the Slovak Republic and

obtained 33.60% geranial, 22.18% neral and 0.00% *cis*-geraniol and *trans*-geraniol. Under the conditions used in this experiment, harvesting at 0900 h and drying the leaves at 40 °C, the proportion of the main components of the oil, geranial (47.32%) and neral (39.28%), was improved in relation to the oil produced by Hollá et al (1997) and no geraniol was encountered.

Our data demonstrated that *M. officinalis* essential oil inhibited the viability of several tumour cell lines in a concentration-dependent manner. At oil dilutions ranging from 1:50 000 to 1:2000 a significant effect was observed for each cell. A non-specific toxic effect of the oil was ruled out by the use of a two-way analysis of variance in as much as it showed differences between different cells and different dilutions of the oil. The capacity to kill cancer cell lines is not a property particular to *M. officinalis* essential oil. In-vitro cytotoxic activity (Setzer et al 1999; Buhagiar et al 1999; Angelopoulou et al 2001; Hayes & Markovic 2002; Moteki et al 2002) and in-vivo tumoricidal activity (Shwaireb 1993) of essential oils had been described already. In some cases, this activity was attributed to specific components of the oil. Geraniol, a component of some essential oils, has been shown to inhibit the proliferation of human colon cancer cells in-vitro (Carnesecchi et al 2001) and the growth of pancreatic (Burke et al 1997), hepatic and skin (Yu et al 1995) tumours in-vitro and in-vivo. Since the oil used in our study did not contain geraniol (Table 1), we need to identify which of its components was responsible for the observed antitumoral activity.

Antioxidant activity of Melissa tea and hydro-alcoholic extracts have been described (Lamiason et al 1991; Lugasi et al 1996). We demonstrated that this activity was present in *M. officinalis* essential oil. The antitumour properties of several antioxidants have been described, such as *Panax ginseng* (Keum et al 2000), glycolic acid (Hong et al 2001; Ahn et al 2002), resveratrol (Surh et al 1999; Kozuki et al 2001; Kapadia et al 2002) and of the ethanolic extract of the flowers of *Prunus persica* (Heo et al 2001). Thus our data indicated that the antioxidant properties of *M. officinalis* essential oil might contribute to its antitumoral activity.

Conclusion

In addition to establishing the best conditions to obtain essential oil from *Melissa officinalis*, the results demonstrated that this oil has antioxidant and tumoricidal activity indicative of its potential use for cancer treatment and/or prevention. Experiments are necessary to identify which of the oil components are responsible for these activities.

References

Adams, R. P. (1995) Identification of essential oil components by gas chromatography/mass spectroscopy. Illinois: Allured Publishing Corporation, p. 469

Ahn, K. S., Park, K. S., Jung, K. M., Jung, H. K., Lee, S. H., Chung, S. Y., Yang, K. H., Yun, Y. P., Pyo, H. B., Park, Y. K., Yun, Y. W., Kim, D. J., Park, S. M., Hong, J. T. (2002) Inhibitory effect of glycolic acid on ultraviolet B-induced c-fos expression, AP-1 activation and p53-p21 response in a human keratinocyte cell line. *Cancer Lett.* **186**: 125–135

Angelopoulou, D., Demetzos, C., Dimas, C., Perdetzoglou, D., Loukis, A. (2001) Essential oils and hexane extracts from leaves and fruits of *Cistus monspeliensis*. Cytotoxic activity of ent-13-epi-manoyl oxide and its isomers. *Planta Med.* **67**: 168–171

Blank, A. F., Alves, P. B., Fonte, S. M., Santos, M. F., Dantas, I. B., Silva, P. A., Mendonça, M. C., Arrigoni-Blank, M. F., Costa, A. G., Silva-Mann, R. (2002) Effect of harvest time and drying on the essential oil content and chemical composition of melissa (*Melissa officinalis* L.). In: XVII Symposium of Medicinal Plants of Brazil, Abstracts of the Symposium, Cuiabá, Brazil (Abstract, AG-40)

Buhagiar, J. A., Podesta, M. T., Wilson, A. P., Micallef, M. J., Ali, S. (1999) The induction of apoptosis in human melanoma, breast and ovarian cancer cell lines using an essential oil extract from the conifer *Tetraclinis articulata*. *Anticancer Res.* **19**: 5435–5443

Burke, Y. D., Stark, M. J., Roach, S. L., Sen, S. E., Crowell, P. L. (1997) Inhibition of pancreatic cancer growth by the dietary isoprenoids farnesol and geraniol. *Lipids* **32**: 151–156

Campos, A. M., Lissi, E. A. (1995) Evaluation of the antioxidant capacity of herbal teas by a procedure based on the bleaching of ABTS radical cations. *Bol. Soc. Chil. Quim.* **40**: 375–381

Carnesecchi, S., Schneider, Y., Ceraline, J., Duranton, B., Gosse, F., Seiler, N., Raul, F. (2001) Geraniol, a component of plant essential oils, inhibits growth and polyamine biosynthesis in human colon cancer cells. *J. Pharmacol. Exp. Ther.* **298**: 197–200

Chakurski, I., Matev, M., Koichev, A., Angelova, I., Stefanov, G. (1981) Treatment of chronic colitis with an herbal combination of *Taraxacum officinale, Hipericum perforatum, Melissa officinalis, Calendula officinalis* and *Foeniculum vulgare*. *Vutr. Boles.* **20:** 51–54

Dikshit, A., Husain, A. (1984) Antifungal action of some essential oils against animal pathogens. *Fitoterapia* **55**: 171–176

Dimitrova, Z., Dimov, B., Manolova, N., Pancheva, S., Ilieva, D., Shishkov, S. (1993) Antiherpes effect of *Melissa officinalis* L. extracts. *Acta Microbiol. Bulg.* **29**: 65–72

Dwivedi, C., Muller, L. A., Goetz-Parten, D. E., Kasperson, K., Mistry, V. V. (2003) Chemopreventive effects of dietary mustard oil on colon tumor development. *Cancer Lett.* **196**: 29–34

Hansson, L. E., Nyren, O., Bergstrom, R., Wolk, A., Lindgren, A., Baron, J., Adami, H. O. (1994) Nutrients and gastric cancer risk. A population-based case-control study in Sweden. *Int. J. Cancer* **57**: 638–644

Hayes, A. J., Markovic, B. (2002) Toxicity of Australian essential oil *Backhousia citriodora* (Lemon myrtle). Part 1. Antimicrobial activity and in vitro cytotoxicity. *Food Chem. Toxicol.* **40**: 535–543

Heo, M. Y., Kim, S. H., Yang, H. E., Lee, S. H., Jo, B. K., Kim, H. P. (2001) Protection against ultraviolet B- and C-induced DNA damage and skin carcinogenesis by the flowers of *Prunus persica* extracts. *Mutat. Res.* **496**: 47–59

Hollá, M., Svajdlenka, E., Tekek, J., Vaverkova, S., Havranek, E. (1997) Composition of the essential oil from *Melissa officinalis* L. cultivated in Slovak Republic. *J. Essent. Oil Res.* **9**: 481–484

Hong, J. T., Kim, E. J., Ahn, K. S., Jung, K. M., Yun, Y. P., Park, Y. K., Lee, S. H. (2001) Inhibitory effect of glycocolic acid on the ultraviolet-induced skin tumorigenesis in SKH-1

hairless mice and its mechanism of action. *Mol. Carcinogen.* **31**: 152–160

Kapadia, G. J., Azuine, M. A., Tokuda, H., Takasaki, M., Mukainaka, T., Konoshima, T., Nishino, H. (2002) Chemopreventive effect of resveratrol, sesamol, sesame oil and sunflower oil in the Epstein-Barr virus early antigen activation assay and the mouse skin two-stage carcinogenesis. *Pharmacol. Res.* **45**: 499–505

Keum, Y. S., Park, K. K., Lee, J. M., Chun, K. S., Park, J. H., Lee, S. K., Kwon, H., Surh, Y. J. (2000) Antioxidant and anti-tumor promoting activities of the methanol extract of heat processed ginseng. *Cancer Lett.* **150**: 41–48

Kozuki, Y., Miura, Y., Yagasaki, K. (2001) Resveratrol suppresses hepatoma cell invasion independently of its anti-proliferative action. *Cancer Lett.* **167**: 151–156

Lamiason, J. L., Petitjean Freytet, C., Carnat, A. (1991) Medicinal Lamiaceae with antioxidant properties, a potential source of rosmarinic acid. *Pharm. Acta Helv.* **66**: 185–188

Larrando, J. V., Agut, M., Calvo Torras, M. A. (1995) Antimicrobial activity of essences from labiates. *Microbios* **82**: 171–172

Lugasi, A., Dworschak, E., Hovari, J. (1996) Antioxidant property of polyphenolic compounds of culinary herbs and medicinal plants. *Spec. Publ. – R. Soc. Chem.* **179**: 372–375

Mikus, J., Harkenthal, M., Steverding, D., Reichling, J. (2000) In vitro effect of essential oils and isolated mono- and sesquiterpenes on *Leishmania major* and *Trypanosoma brucei*. *Planta Med.* **66**: 366–368

Mosmann, T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **65**: 55–63

Moteki, H., Hibasami, H., Yamada, Y., Katsuzaki, H., Imai, K., Komiya, T. (2002) Specific induction of apoptosis by 1,8-cineole in two human leukemia cell lines, but not in a human stomach cancer cell line. *Oncol. Rep.* **9**: 757–760

Owen, R. W., Giacosa, A., Hull, W. E., Haubner, R., Wurtele, G., Spiegelhalder, B., Bartsch, H. (2000) Olive-oil consumption and health: the possible role of antioxidants. *Lancet Oncol.* **1**: 107–112

Ramos, A., Visozo, A., Piloto, J., Garcia, A., Rodriguez, A., Rivero, R. (2003) Screening of antimutagenicity via antioxidant activity in Cuban Medicinal Plants. *J. EthnoPharmacol.* **87**: 241–246

Sadraei, H., Ghannadi, A., Malekshahi, K. (2003) Relaxant effect of essential oil of *Melissa officinalis* and citral on rat ileum contractions. *Fitoterapia* **74**: 445–452

Setzer, W. N., Setzer, M. C., Moriarity, D. M., Bates, R. B., Haber, W. A. (1999) Biological activity of the essential oil of *Myrcianthes* sp. nov. "black fruit" from Monteverde, Costa Rica. *Planta Med.* **65**: 468–469

Shwaireb, M. H. (1993) Caraway oil inhibits skin tumors in female BALB/c mice. *Nutr. Cancer* **19**: 321–325

Snklar, G. (1998) Mechanisms of cancer inhibition by anti-oxidant nutrients. *Oral Oncol.* **34**: 24–29

Soulimani, R., Fleurentin, J., Mortier, F., Misslin, R., Derrieu, G., Pelt, J. M. (1991) Neurotropic action of the hydroalcoholic extract of *Melissa officinalis* in the mouse. *Planta Med.* **57**: 105–109

Surh, Y. J., Hurh, Y. J., Kang, J. Y., Lee, E., Kong, G., Lee, S. J. (1999) Resveratrol, an antioxidant present in red wine, induces apoptosis in human promyelocytic leukemia (HL-60) cells. *Cancer Lett.* **140**: 1–10

Yamasaki, K., Nakano, M., Kawahata, T., Mori, H., Otake, T., Ueba, N., Oishi, I., Inami, R., Yamane, M., Nakamura, M., Murata, H., Nakanishi, T. (1998) Anti HIV1 activity of herbs in labiatae. *Biol. Pharm. Bull.* **21**: 829–833

Yu, S. G., Hildebrandt, L. A., Elson, C. E. (1995) Geraniol, an inhibitor of mevalonate biosynthesis, suppresses the growth of hepatomas and melanomas transplanted to rats and mice. *J. Nutr.* **125**: 2763–2767

Van den Dool, H., Kratz, P. D. (1963) A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogr.* **11**: 463–471