TÜBİTAK

Turkish Journal of Biology

http://journals.tubitak.gov.tr/biology/

Research Article

Turk J Biol
(2014) 38: 253-259
© TÜBİTAK
doi:10.3906/biy-1309-55

In vitro cytotoxic, genotoxic, and oxidative effects of acyclic sesquiterpene farnesene

Kübra ÇELİK[1], Başak TOĞAR[2,*], Hasan TÜRKEZ[3], Numan TAŞPINAR[4]

[1]Department of Physiology, Faculty of Medicine, Recep Tayyip Erdoğan University, Rize, Turkey
[2]Department of Biology, Faculty of Science, Atatürk University, Erzurum, Turkey
[3]Department of Molecular Biology and Genetics, Faculty of Sciences, Erzurum Technical University, Erzurum, Turkey
[4]Department of Medical Pharmacology, Medical Faculty, Atatürk University, Erzurum, Turkey

Received: 23.09.2013 • **Accepted:** 22.11.2013 • **Published Online:** 28.03.2014 • **Printed:** 28.04.2014

Abstract: Farnesene (FNS) is an acyclic sesquiterpene. It has a wide range of important biological effects such as antioxidant, antimicrobial, and antifungal properties, although its cytotoxic, cytogenetic, and oxidative effects have not been investigated in human blood tissue yet. To this aim, both MTT and lactate dehydrogenase (LDH) assays were carried out to evaluate cell viability and cytotoxicity. Total antioxidant capacity (TAC) and total oxidative stress (TOS) parameters were used to assess oxidative alterations. In addition, micronucleus and chromosomal aberration tests were used for mutagenic and genotoxic studies. The results revealed that FNS reduced cell viability at concentrations of higher than 100 µg/mL. All tested concentrations of FNS were found to be nongenotoxic. In addition, the in vitro treatments with FNS led to increases of TAC levels in cultured blood cells without changing TOS levels as compared to the control group. Our results demonstrate that FNS could be used as an antioxidant compound resource that may have applications in the food and drug industries.

Key words: Farnesene, cytotoxicity, human blood, genotoxicity, oxidative status

1. Introduction

Essential oils are complex volatile natural compounds formed by aromatic plants as secondary metabolites. They are an important source of potential therapeutic compounds (Sherry et al., 2013). Essential oil constituents from aromatic herbs and dietary plants include monoterpenes, sesquiterpenes, oxygenated monoterpenes, oxygenated sesquiterpenes, and phenolics, among others (Bhalla et al., 2013).

Sesquiterpenes, which are one of the most common terpenes, are a class of natural products with a diverse range of attractive industrial properties (Wang et al., 2011; Scalcinati et al., 2012). They are compounds containing 3 isoprene units, which have 15 carbons and 24 hydrogens per molecule ($C_{15}H_{24}$). There are more than 10,000 kinds of sesquiterpenes (Davis and Croteau, 2000). They have long been investigated for biological activities including anticarcinogenic (Afoulous et al., 2013), antimicrobial (Wang et al., 2013), antifungal (Kundu et al., 2013), antiinflammatory (Wang et al., 2013), and, more recently, antioxidant effects (Abolaji et al., 2013).

Farnesene (FNS) is a naturally occurring 15-carbon sesquiterpene, and it may be endogenously generated in the cells by enzymatic dephosphorylation of farnesyl pyrophosphate (Kunert et al., 2010; Bazemore et al., 2012). It is odoriferous, and terpene-like substances occur in other natural sources: the trail markers of red fire ants (Van der Meer et al., 1982), alarm pheromones of aphids (Sun et al., 2012), and even a defense substance of wild potato plants against aphids (Bruce et al., 2005). The limited number of recent investigations has revealed that FNS possesses a wide range of important biological activities such as antibacterial activity (Chehregani et al., 2010), antifungal activity (Al-Maskri et al., 2011), and free radical-scavenging activity (Sarikurkcu et al., 2013). Furthermore, a recent study showed that FNS possesses anticarcinogenic activity (Afoulous et al., 2013). However, information regarding the cytotoxic, cytogenetic, and oxidative effects of FNS in human blood cells remains unknown. Hence, the aim of the present study was to evaluate the cytotoxic/antiproliferative [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay], cytogenetic [micronucleus (MN) and chromosomal aberration (CA) assays], and oxidative [total antioxidant capacity (TAC) and total oxidative stress (TOS) analysis] effects of FNS on human peripheral blood cultures for its possible use in complementary and alternative medicine practices.

* Correspondence: basaktogar@gmail.com

2. Materials and methods

2.1. Experimental design

Heparinized blood samples were collected from 5 healthy women aged from 20 to 25 years old, who were nonsmoking, nonalcoholic, not under drug therapy, and with no recent history of exposure to mutagens. Human peripheral blood cultures were set up according to a slight modification of the protocol described by Evans and O'Riordan (1975). The heparinized blood samples (0.5 mL) were cultured in 6 mL of culture medium (Chromosome Medium B, Biochrom, Berlin, Germany) with 5 mg/mL of phytohemagglutinin (Biochrom). Farnesene ($C_{15}H_{24}$, Sigma-Aldrich, Steinheim, Germany) was dissolved in ethanol, and the ethanol was evaporated to dryness at ambient temperature. Concentrations of 10, 25, 50, 75 100, 200, and 400 µg/mL were added to the cultures just before incubation. All individual lymphocyte cultures without FNS were studied as a control group. The concentrations were selected according to the work of Türkez et al. (2013). Ascorbic acid (10 µM, Sigma-Aldrich) and hydrogen peroxide (25 µM, Sigma-Aldrich) were also used as the positive controls in TAC and TOS analysis, respectively. Mitomycin C (MMC; 10^{-7} M, Sigma-Aldrich) was used as the positive control in MTT, lactate dehydrogenase (LDH), CA, and MN assays.

2.2. MTT assay

The whole blood samples were seeded in 96-well plates. Cells were incubated at 37 °C and treated with FNS at different concentrations for 24 h. Viability of cells was assessed by spectrophotometrically measuring the formation of formazan from MTT (MTT Cell Proliferation Kit, Cayman Chemical Company, Ann Arbor, MI, USA). At the end of the experiment, the human blood cultures were incubated with 0.7 mg/mL MTT for 30 min at 37 °C. After incubation, the medium was removed using the spinning technique. The formazan was dissolved in DMSO, and the optical density was measured at 570 nm using an ELISA reader (Sigma-Aldrich) (Teyeb et al., 2012).

2.3. LDH assay

LDH assay was carried out with a LDH-cytotoxicity assay kit (Cayman Chemical Company) according to the manufacturer's protocol. In brief, 10^4 to 10^5 cells/well were seeded in 96-well plates and exposed to different concentrations (0–400 µg/mL) of FNS for 24 h. At the end of exposure, the 96-well plate was centrifuged at 400 × *g* for 5 min to settle the FNS present in the solution. Next, 100 µL of supernatant was transferred to a well of a 96-well plate that already contained 100 µL of reaction mixture from a Bio-Vision kit and was incubated for 30 min at room temperature. After incubation, the absorbance of the solution was measured at 490 nm using a microplate reader (Synergy-HT, BioTek, Winooski, VT, USA). LDH levels in the medium versus the cells were quantified and compared with the control values according to the instructions of the kit.

2.4. MN assay

Human lymphocytes were stimulated by FNS and cultured for 72 h; after 44 h of FNS stimulation, cytochalasin B (Sigma-Aldrich; final concentration of 6 µg/mL) was added. Cells were harvested by centrifugation and treated with a hypotonic solution (0.075 M KCl, 37.4 °C). The cells were then centrifuged again and a solution of fixation (methanol/acetic acid, 3/1) was added 3 times, and the resulting cells were suspended and dropped onto cold, clean slides. To prepare slides, 3–5 drops of the fixed cell suspension were dropped onto a clean slide and air-dried. The slides were stained with Giemsa stain in phosphate buffer (pH 6.8) and analyzed using a light microscope. MNs were scored in 1000 binucleated cells and the frequency of cells with MNs was determined (Fenech, 1993).

2.5. CA assay

CA tests were performed not only to study the cytotoxicity of the material on cells, but also to determine the aberrations induced by the particular material on chromosomes of the human lymphocyte cell line. Human lymphocytes were stimulated by FNS and cultured for 72 h. Two hours prior to harvesting, 0.1 mL of colchicine (0.2 mg/mL, Sigma) was added to the culture flask. Cells were harvested by centrifugation and treated with a hypotonic solution (0.075 M KCl, 37.4 °C). Again, the cells were then centrifuged and a solution (methanol/acetic acid, 3/1) was added 3 times; the resulting cells were resuspended and dropped onto clean slides. To prepare slides, 3–5 drops of the fixed cell suspension were dropped on a cold, wet slide and air-dried. The slides were stained with Giemsa in phosphate buffer (pH 6.8). For each treatment, 30 well-spread metaphases were analyzed to detect the presence of chromosomal aberrations. Criteria to classify the different types of aberrations (chromatid or chromosome gap, and chromatid or chromosome break) were in accordance with the recommendation of Environmental Health Criteria 46 for environmental monitoring of human populations (IPCS, 1985).

2.6. TAC and TOS analysis

The major advantage of this test is that it measures the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of a single compound (Kusano and Ferrari, 2008). Since the measurement of different oxidant molecules separately is not practical and their oxidant effects are additive, the TOS of a sample is measured; this may be referred to as total peroxide, serum oxidation activity, reactive

oxygen metabolites, or another synonym (Erel, 2005). The automated Trolox equivalent TAC and TOS assays were carried out in plasma samples obtained from blood cultures for 24 h using commercially available kits (Rel Assay Diagnostics, Gaziantep, Turkey) (Erel, 2004, 2005).

2.7. Statistical analysis

Statistical analysis was performed using SPSS 18.0 (SPSS, Chicago, IL, USA). The statistical analysis of experimental values in the MTT, LDH, CA, MN, TAC, and TOS analyses was performed by using Duncan's test. Statistical decisions were made with a significance level of 0.05.

3. Results

The MTT and LDH release assays were used to quantify cell death in response to FNS on human lymphocyte cultures (Figures 1 and 2). The cultured human blood cells exposed to 10, 25, 50, and 75 µg/mL concentrations of FNS did not show any significant changes in cell viability over 24 h as determined by MTT and LDH assays. However, 100, 200, and 400 µg/mL concentrations of FNS exhibited a weak cytotoxic effect on human blood cultures.

Table 1 shows the biochemical data obtained with various concentrations of FNS on human peripheral blood cell cultures. As shown from the results presented in Table 1, 75, 100, 200, and 400 µg/mL concentrations of FNS treatments did not lead to any alterations in TAC levels, while 10, 25, and 50 µg/mL concentrations of FNS treatment caused significant increases of TAC levels. On the other hand, FNS did not change the TOS levels in cultured human peripheral blood cells at any concentration.

Results obtained from the analysis of MNs and CAs in human lymphocytes cultured with different

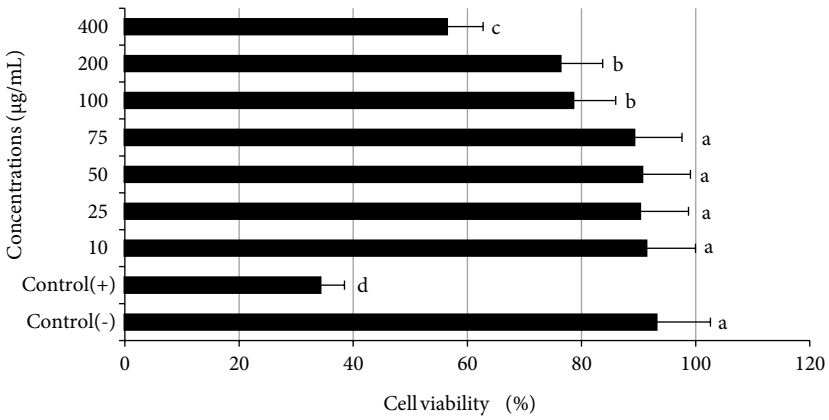

Figure 1. Viability of human blood cells after 24 h of exposure to 0–400 µg/mL FNS. The results are presented as percentage of the control group (n = 5). Data are expressed as mean ± SD. Values with the same superscript letters are not significantly different from each other at the level of $P < 0.05$. Control(-): negative control; Control(+) : positive control; MMC: 10^{-7} M.

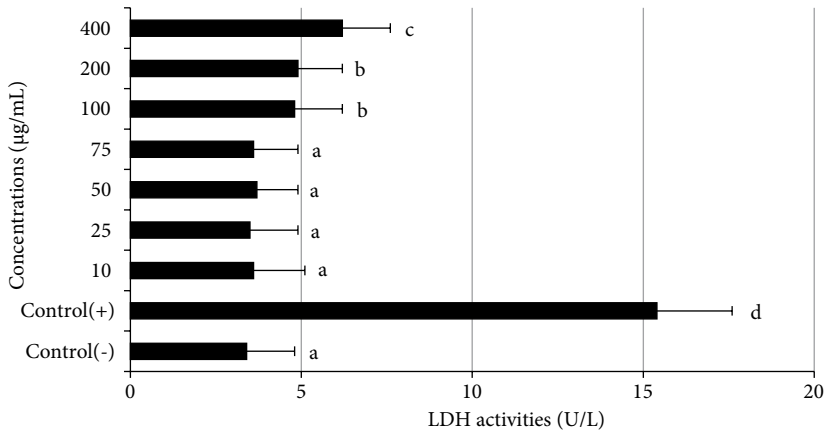

Figure 2. Extracellular level of LDH in cultured human blood cells maintained in the presence of FNS for 24 h. The abbreviations are as in Figure 1.

Table 1. TAC and TOS levels in cultured human peripheral blood cells exposed to FNS for 24 h.

Concentrations (μg/mL)	TAC (Trolox equiv/mmol L^{-1})	TOS (H_2O_2 equiv/μmol L^{-1})
Control(-)	6.4 ± 0.3[b]	11.2 ± 2.2[a]
Control(+)	12.8 ± 0.8[d]	38.2 ± 4.3[b]
10	8.9 ± 0.5[c]	11.4 ± 2.8[a]
25	9.6 ± 0.5[c]	11.5 ± 2.5[a]
50	9.4 ± 0.6[c]	11.4 ± 2.4[a]
75	6.2 ± 0.5[b]	11.2 ± 2.5[a]
100	6.1 ± 0.5[b]	11.3 ± 2.6[a]
200	4.6 ± 0.4[a]	11.4 ± 2.5[a]
400	4.1 ± 0.5[a]	11.5 ± 3.1[a]

Control(-) : negative control; Control(+) : positive control; ascorbic acid: 10 μM for TAC assay; hydrogen peroxide: 25 μM for TOS assay. Values with the same superscript letters are not significantly different from each other at the level of $P < 0.05$.

Table 2. Chromosome aberration (CA) and micronucleus (MN) rates in human lymphocytes after treatment with FNS in vitro.

Concentrations (μg/mL)	MNs/1000 cells	CAs/cell
Control(-)	3.24 ± 0.32[a]	0.21 ± 0.03[a]
Control(+)	8.14 ± 0.97[b]	0.72 ± 0.08[b]
10	3.31 ± 0.32[a]	0.22 ± 0.02[a]
25	3.24 ± 0.36[a]	0.23 ± 0.02[a]
50	3.36 ± 0.33[a]	0.21 ± 0.03[a]
75	3.28 ± 0.38[a]	0.24 ± 0.02[a]
100	3.39 ± 0.41[a]	0.22 ± 0.03[a]
200	3.41 ± 0.39[a]	0.24 ± 0.03[a]
400	3.44 ± 0.41[a]	0.23 ± 0.02[a]

Control(-) : negative control; Control(+) : positive control; MMC: 10^{-7} M. Values with the same superscript letters are not significantly different from each other at the level of $P < 0.05$.

FNS concentrations are shown in Table 2. FNS at tested concentrations did not induce significant ($P < 0.05$) numbers of MNs or CAs. However, the MMC-applied culture (as a positive control) showed approximately 3-fold increases of both parameters as compared to the control group.

4. Discussion

In the present study, we evaluated the cell viability and cytotoxicity of FNS on cultured human blood cells via MTT and LDH assays. The MTT assay is a nonradioactive, fast, and economical assay widely used to quantify cell viability and proliferation. The LDH assay is a colorimetric

method for determining the number of dead cells in proliferation (Türkez et al., 2011). In this study, the cytotoxic effects of FNS on cultured human peripheral blood cells were demonstrated by its weak inhibition of cell viability and low LDH leakage. Our results are in accordance with recent studies, which have revealed that several sesquiterpenes such as britannin (in HepG2, MCF-7, MDBK, and A-549 cells), artesunate (in rat hepatic stellate cells), gossypol (in human retinoblastoma cells), dihydroartemisinin (in HepG2 human hepatoma cells), and deoxynivalenol (in human lymphocyte cells) exhibited cytotoxic effects in a concentration-dependent manner (Meky et al., 2001; Hsiao et al., 2012; Moghadam et al., 2012; Wang et al., 2012). In addition, Walia et al. (2012) evaluated the cytotoxic effect of the essential oil of leaves of *Malus domestica* (containing α-farnesene, 9.6%) in several cancer cell lines. They found that the essential oil could be cytotoxic for C-6 glioma cell lines. Furthermore, Königs et al. (2008) studied the effect of deoxynivalenol in human primary hepatocytes and compared these data to the effects in the HepG2 cell line. They found that deoxynivalenol has a distinct cytotoxic effect on human primary hepatocytes. Our results reveal that FNS, like other sesquiterpenes, showed a concentration-dependent cytotoxic effect. This study is the first paper in the literature to explore the cytotoxic activity of FNS.

Recently, studies have shown that sesquiterpenes have antioxidant/prooxidant properties at different concentrations. For assessing the antioxidant/oxidant effects of FNS, TAC and TOS assays were performed. The TAC and TOS assays are rapid and reliable automated colorimetric assays that are frequently used to determine oxidative alterations (Kusano and Ferrari, 2008). The results in Table 1 show that FNS increased TAC levels of the cells without any alterations in TOS levels as compared to untreated cultures. Sarikurkcu et al. (2013) reported that essential oil of *Phlomis bourgaei* Boiss (containing FNS sesquiterpene) showed antioxidant activity in 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and beta-carotene/linoleic acid assay. In addition, it was found that farnesol effectively suppressed 1,2-dimethylhydrazine (DMH)-induced colonic mucosal damage by ameliorating oxidative stress and inflammatory and apoptotic responses (Khan and Sultana, 2011). Likewise, Hwang et al. (1996) reported that atractylon showed antioxidant activity by using DPPH radical scavenging assay in rat hepatocytes.

In this study, we have evaluated the genetic effects of FNS on human lymphocyte cultures by using MN and CA assays. The data presented in this study suggest that FNS is neither cytotoxic nor genotoxic in vitro. To our knowledge, there is no information on the genotoxic effect of FNS on cell culture systems. Therefore, we have discussed its genotoxicity potential as compared with other sesquiterpenes. Al-Zubairi et al. (2010) reported that zerumbon (a sesquiterpene phytochemical from a type of edible ginger) did not induce genotoxicity in cultured human peripheral blood lymphocytes. In addition, Di Sotto et al. (2010) reported that beta-caryophyllene exhibited antigenotoxic activity in human peripheral blood lymphocyte cells against the toxicity of ethyl methanesulfonate and colcemid. On the other hand, Gil da Costa et al. (2012) found that ptaquiloside (a norsesquiterpene) was genotoxic in cultured human lymphocytes by using the sister chromatid exchange (SCE) test. In addition, gossypol was also found to be genotoxic in human lymphocyte cells **by using** SCE (Best and McKenzie, 1988). Moreover, artesunate showed weak genotoxic effects at low doses and clastogenic effects at high doses in bone marrow cells from male Swiss mice (Aquino et al., 2011).

In light of the findings obtained in the present study, it is suggested that FNS had no mutagenic effects on human lymphocyte cultures at the dose studied. Moreover, FNS exhibited antioxidant properties at the concentration added to the cultures. Consequently, FNS could be used as an antioxidant compound resource that may find applications in the food and drug industries.

Acknowledgment
The authors are grateful to all volunteers for the blood samples.

References

Abolaji AO, Eteng MU, Omonua O, Adenrele Y (2013). Influence of coadministration of artemether and lumefantrine on selected plasma biochemical and erythrocyte oxidative stress indices in female Wistar rats. Hum Exp Toxicol 32: 206–215.

Afoulous S, Ferhout H, Raoelison EG, Valentin A, Moukarzel B, Couderc F, Bouajila J (2013). Chemical composition and anticancer, antiinflammatory, antioxidant and antimalarial activities of leaves essential oil of *Cedrelopsis grevei*. Food Chem Toxicol 56: 352–362.

Al-Maskri AY, Hanif MA, Al-Maskari MY, Abraham AS, Al-Sabahi JN, Al-Mantheri O (2011). Essential oil from *Ocimum basilicum* (Omani Basil): a desert crop. Nat Prod Commun 6: 1487–1490.

Al-Zubairi AS, Abdul AB, Syam MM (2010). Evaluation of the genotoxicity of zerumbone in cultured human peripheral blood lymphocytes. Toxicol In Vitro 24: 707–712.

Aquino I, Perazzo FF, Maistro EL (2011). Genotoxicity assessment of the antimalarial compound artesunate in somatic cells of mice. Food Chem Toxicol 49: 1335–1339.

Bazemore RA, Feng J, Cseke L, Podila GK (2012). Biomedically important pathogenic fungi detection with volatile biomarkers. J Breath Res 6: 016002.

Bhalla Y, Gupta VK, Jaitak V (2013). Anticancer activity of essential oils: a review. J Sci Food Agr 93: 3643–3653.

Bruce TJ, Birkett MA, Blande J, Hooper AM, Martin JL, Khambay B, Prosser I, Smart LE, Wadhams LJ (2005). Response of economically important aphids to components of *Hemizygia petiolata* essential oil. Pest Manag Sci 61: 1115–1121.

Chehregani A, Mohsenzadeh F, Mirazi N, Hajisadeghian S, Baghali Z (2010). Chemical composition and antibacterial activity of essential oils of *Tripleurospermum disciforme* in three developmental stages. Pharm Biol 48: 1280–1284.

Davis ME, Croteau R (2000). Cyclization enzymes in the biosynthesis of monoterpenes, sesquiterpenes, and diterpenes. Top Curr Chem 209: 54–92.

Di Sotto A, Mazzanti G, Carbone F, Hrelia P, Maffei F (2010). Inhibition by beta-caryophyllene of ethyl methanesulfonate-induced clastogenicity in cultured human lymphocytes. Mutat Res 699: 23–28.

Erel O (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 37: 277–285.

Erel O (2005). A new automated colorimetric method for measuring total oxidant status. Clin Biochem 38: 1103–1111.

Evans HJ, O'Riordan ML (1975). Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. Mutat Res 31: 135–148.

Gil da Costa RM, Oliveira PA, Bastos MM, Lopes CC, Lopes C (2012). Ptaquiloside-induced early-stage urothelial lesions show increased cell proliferation and intact β-catenin and e-cadherin expression. Environ Toxicol doi: 10.1002/tox.21803 (Epub ahead of print).

Hsiao WT, Tsai MD, Jow GM, Tien LT, Lee YJ (2012). Involvement of Smac, p53, and caspase pathways in induction of apoptosis by gossypol in human retinoblastoma cells. Mol Vis 18: 2033–2042.

Hwang JM, Tseng TH, Hsieh YS, Chou FP, Wang CJ, Chu CY (1996). Inhibitory effect of atractylon on tert-butyl hydroperoxide induced DNA damage and hepatic toxicity in rat hepatocytes. Arch Toxicol 70: 640–644.

IPCS (1985). International Program on Chemical Safety Environmental Health Criteria 46. Guidelines for the Study of Genetic Effects in Human Populations. Geneva, Switzerland: WHO, pp. 45–54.

Khan R, Sultana S (2011). Farnesol attenuates 1,2-dimethylhydrazine induced oxidative stress, inflammation and apoptotic responses in the colon of Wistar rats. Chem Biol Interact 192: 193–200.

Königs M, Schwerdt G, Gekle M, Humpf HU (2008). Effects of the mycotoxin deoxynivalenol on human primary hepatocytes. Mol Nutr Food Res 52: 830–839.

Kundu A, Saha S, Walia S, Shakil NA, Kumar J, Annapurna K (2013). Cadinene sesquiterpenes from *Eupatorium adenophorum* and their antifungal activity. J Environ Sci Health B 48: 516–522.

Kunert G, Reinhold C, Gershenzon J (2010). Constitutive emission of the aphid alarm pheromone, (E)-β-farnesene, from plants does not serve as a direct defense against aphids. BMC Ecology 10: 23.

Kusano C, Ferrari B (2008). Total antioxidant capacity: a biomarker in biomedical and nutritional studies. J Mol Cell Biol 7: 1–15.

Ma W, Miao Z, Novotny MV (1999). Induction of estrus in grouped female mice (*Mus domesticus*) by synthetic analogues of preputial gland constituents. Chem Senses 24: 289–293.

Meky FA, Hardie LJ, Evans SW, Wild CP (2001). Deoxynivalenol-induced immunomodulation of human lymphocyte proliferation and cytokine production. Food Chem Toxicol 39: 827–836.

Moghadam MH, Hajimehdipoor H, Saeidnia S, Atoofi A, Shahrestani R, Read RW, Mosaddegh M (2012). Anti-proliferative activity and apoptotic potential of britannin, a sesquiterpene lactone from *Inula aucheriana*. Nat Prod Commun 7: 979–980.

Sarikurkcu C, Sabih Ozer M, Cakir A, Eskici M, Mete E (2013). GC/MS evaluation and in vitro antioxidant activity of essential oil and solvent extracts of an endemic plant used as folk remedy in Turkey: *Phlomis bourgaei* Boiss. Evid Based Complement Alternat Med 2013: 293080.

Scalcinati G, Partow S, Siewers V, Schalk M, Daviet L, Nielsen J (2012). Combined metabolic engineering of precursor and co-factor supply to increase α-santalene production by *Saccharomyces cerevisiae*. Microb Cell Fact 11: 117.

Sherry M, Charcosset C, Fessi H, Greige-Gerges H (2013). Essential oils encapsulated in liposomes: a review. J Liposome Res 23: 268–275.

Sun YF, De Biasio F, Qiao HL, Iovinella I, Yang SX, Ling Y, Riviello L, Battaglia D, Falabella P, Yang XL et al. (2012). Two odorant-binding proteins mediate the behavioural response of aphids to the alarm pheromone (E)-β-farnesene and structural analogues. PLoS One 7: e32759.

Teyeb H, Zanina N, Neffati M, Douki W, Najjar MF (2012). Cytotoxic and antibacterial activities of leaf extracts of *Astragalus gombiformis* Pomel (Fabaceae) growing wild in Tunisia. Turk J Biol 36: 53–58.

Togar B (2013). The cytological, biochemical and genetic effects of selected sesquiterpenes on healthy neuron and N2a neuroblastoma cell cultures. PhD, Atatürk University Graduate School of Natural and Applied Sciences, Erzurum, Turkey.

Türkez H, Çelik K, Toğar B (2013). Effects of copaene, a tricyclic sesquiterpene, on human lymphocytes cells in vitro. Cytotechnology DOI: 10.1007/s10616-013-9611-1 (Epub ahead of print).

Türkez H, Geyikoğlu H, Çolak S (2011). The protective effect of boric acid on aluminum-induced hepatotoxicity and genotoxicity in rats. Turk J Biol 35: 293–301.

Van der Meer RK, Williams FD, Lofgren CS (1982). Hydrocarbon components of the trail pheromone of the red imported fire ant, *Solenopsis invicta*. Tetrahedron Lett 22: 1651–1654.

Walia M, Mann TS, Kumar D, Agnihotri VK, Singh B (2012). Chemical composition and in vitro cytotoxic activity of essential oil of leaves of *Malus domestica* growing in Western Himalaya (India). Evid Based Complement Alternat Med 2012: 649727.

Wang C, Li CJ, Yang JZ, Ma J, Chen XG, Hou Q, Zhang DM (2013). Anti-inflammatory sesquiterpene derivatives from the leaves of *Tripterygium wilfordii*. J Nat Prod 76: 85–90.

Wang C, Yoon SH, Jang HJ, Chung YR, Kim JY, Choi ES, Kim SW (2011). Metabolic engineering of *Escherichia coli* for α-farnesene production. Metab Eng 13: 648–655.

Wang H, Wang Y, Liu P, Wang W, Fan Y, Zhu W (2013). Purpurides B and C, two new sesquiterpene esters from the aciduric fungus *Penicillium purpurogenum* JS03-21. Chem Biodivers 10: 1185–1192.

Wang Y, Fang BW, Peng LX (2012). Impact of artesunate on the expression and secretion of transforming growth factor-b1 of primary rat hepatic stellate cells. Zhonghua Gan Zang Bing Za Zhi 20: 294–299.