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Cytotoxic, anti-carcinogenic and antioxidant properties of the most frequent plant volatiles

Minireview

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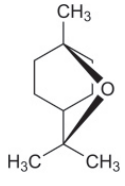
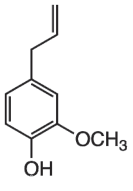
Flowers, berries, leaves, barks and roots of different plants have been used through the ages as a source of flavor in food and perfume preparations. The volatiles responsible for the flavor of botanicals can be extracted from the plant material as "essential oils" (EOs), called also volatile oils or ethereal oils. The term essential is intended to indicate that the oil is the fragrant essence of the plant from which it is extracted. EOs are constituted by hydrocarbons (monoterpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols and phenol ethers). Of the numerous groups of naturally occurring compounds examined so far terpenes are known as fragrances and flavoring agents. The data reported in this review including the data obtained in our laboratory show that many of EOs exhibit a range of biological activities inclusive of antioxidative, anti-mutagenic and anti-carcinogenic activities. Most of them belong to phytochemicals with chemopreventive potential. On the other hand some herbal products can cause serious adverse effects. A complex research of toxic, genotoxic, anti-mutagenic and anti-carcinogenic effects of EOs is therefore very important.

Key words: essential oils, antioxidant effects, DNA-protective effects, cytotoxicity

Characteristics of the five components of essential oils used in our experiments: For a more detailed study of cytotoxicity, genotoxicity and DNA protection in human cells we selected five substances out of a huge amount of EOs and their components. They occur very frequently in essential oils isolated from different plants. Their cytotoxicity against human cells decreases in the following order: carvacrol > thymol > eugenol > borneol > eucalyptol.

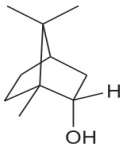


Eucalyptol (1,8-Cineole; $C_{10}H_{18}O$), a cyclic ether and a monoterpene, represents one of the components of EOs obtained from some species of eucalyptus (e.g. *Eucalyptus polybractea*). It is present also in EOs isolated from bay leaves, mugwort, sweet basil, wormwood, rosemary, sage and other aromatic plant foliage. This colorless liquid with a fresh camphor-like spicy smell has been proven to be capable of reducing inflammation and pain. The existing data suggest that eucalyptol belongs to volatile EO components which are metabolized in the human or animal organism [1, 2, 3]. The eucalyptol acts as a unique transport agent which delivers the active bio-affecting agent across the stratum corneum to the target area. Systemically effective therapeutic agents can be effectively delivered through the skin to the desired site, i.e. to the underlying tissues of the epidermis and dermis or to the general circulation.

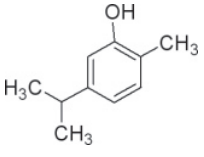


Eugenol ($C_{10}H_{12}O_2$) is an allyl chain-substituted guaiacol, a member of the allylbenzene class of chemical compounds. It is a clear to pale yellow oily liquid and a major component of EO isolated from *Eugenia caryophyllata* (Myrtaceae). It can be extracted also from clove oil, nutmeg, cinnamon, and bay leaf. Eugenol and its derivatives have a pleasant, spicy, clove-like odor and are used in medicine as a local antiseptic and anesthetic but also for the

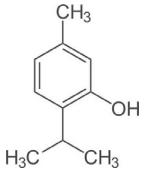
production of isoeugenol in the manufacture of vanillin. When mixed with zinc oxide eugenol forms cement used in dentistry. Despite its small molecular size eugenol has been reported to manifest a range of biological activities. Results of Ou et al. [4] suggested that eugenol may protect endothelial cells against dysfunction induced by oxidized LDL (low-density lipoprotein). An important negative effect of eugenol was described by Yao et al. [5], who found that this EO fully suppressed conductance of Cl^- channel TMEM16A activated by Ca^{2+} which is involved in epithelial fluid secretion. Suppression of conductance of TMEM16A, caused by eugenol, induced strong inhibition of intestinal smooth muscle contraction in mouse ileal segments. Antiproliferative effects of eugenol, contained in honey but also in honey as such, against Ehrlich Ascites and Solid Carcinoma has recently been described by Jaganathan et al. [6].



Borneol ($C_{10}H_{18}O$) represents a bicyclic organic terpene.Copyright The hydroxyl group in this compound is located in an endo position. Borneol exists as two enantiomers which have two different CAS (Chemical Abstracts Service) numbers. Naturally occurring d-(+)-borneol can be found in several species of Artemisia and Dipterocarpaceae. Borneol helps to promote mental alertness required in meditative practice and is widely used in traditional Japanese and Chinese medicine. Wu et al. [7] investigated the promoting effect of borneol on the penetration of puerarin eye drops and timolol maleate eye drops through the cornea and suggested that borneol had the potential to be used as an ophthalmic penetration enhancer. Many studies have reported that it enhances the absorption, distribution, permeability and efficacy of other drugs [8]. Study of immunomodulatory effects of borneol [9] showed that borneol was significantly able to suppress the pro-inflammatory cytokine mRNA expression in colonic inflammation in mice. Very interesting and important are the antihypertensive effects of borneol against N-nitro-L-arginine methyl ester hydrochloride (L-NAME) – induced hypertension in rats. These were manifested by significantly attenuated systolic and diastolic blood pressure. Borneol significantly reduced lipid peroxidation and increased activities and levels of enzymatic and non-enzymatic antioxidants in hypersensitive rats [10].



Carvacrol (cymophenol; $C_6H_3CH_3(OH)(C_3H_7)$ – 5-isopropyl-2-methyl phenol) is a constituent of the essential oil of *Origanum hirtum* or oil of thyme, oil obtained from pepperwort, wild bergamot and several other EOs. It can be extracted from Origanum oil or prepared synthetically. Carvacrol represents thick oil liquid with melting point of 0°C and boiling point 236-237°C. Oxidation with ferric chloride converts it into dicarvacrol. Carvacrol minimizes oxidation of the lipid components in foods and can serve as a natural replacement of synthetic antioxidative food additives [11]. EOs and aqueous tea infusions obtained from oregano, thyme and wild thyme prevent oxidation of LDL that transport dangerous cholesterol and triglycerides from the liver to peripheral tissues [12]. Jukic et al. [13] found that thymol, carvacrol and their derivatives exhibited a strong inhibitory effect on acetylcholinesterase (AChE), an enzyme that degrades the neurotransmitter acetylcholine. Inhibition of AChE represents one of the therapeutic strategies developed in Alzheimer's disease treatment. Lee et al. [14] proved that carvacrol activated the human collagen type I promoter activity and the synthesis of human type I procollagen preventing skin aging and wrinkle formation.



Thymol (a monoterpene phenol derivative of cymene; $C_{10}H_{13}OH$) is found in thyme oil. It is isomeric with carvacrol and can be extracted as a white crystalline substance with a pleasant aromatic scent and strong antiseptic properties. Thymol is described as large, colorless, translucent crystals of the hexagonal system that have an aromatic, thyme-like odor, and a pungent, aromatic taste. It is used as an antiseptic, local anesthetic, cooling agent, a food preservative but also in liniments, lip balms, toothpaste and mouthwash. Thymol acts as a local irritant and anesthetic to the skin and mucous membrane but can be harmful if swallowed, inhaled, or absorbed through the skin in a greater amount. Ocana and Reglero [15] studied the effects of thyme extracts isolated from three different species (*Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis*) on human macrophages derived from THP-1 monocytes and activated by oxidized LDL. The authors found that thyme extracts significantly reduce production and gene expression of the proinflammatory mediators which means that these extracts could have anti-inflammatory effects.

The World Health Organization (WHO) has stated that thymol and carvacrol residues in food are no danger to the consumer as long as they do not exceed 50 mg/kg [16]. Thus they could be applied as post harvest treatment for controlling fruit decay.

Cytotoxic effects. Owing to the lipophilic nature plant volatiles appear to accumulate in the microbe cell membranes and increase their permeability resulting in leakage of enzymes and metabolites. In addition to this antibacterial activity many essential oils and their ingredients exhibit a range of further biological activities including insecticidal and antifungal activity. List of the main EOs and their components which exhibit cytotoxicity against bacteria, fungi, arthropoda and protozoa summarizes Table 1.

Antimicrobial effects. EOs exhibit inhibiting activity against a wide spectrum of bacteria [17] and are very useful mainly in the clinical practice and food industry. *In vitro* studies of Friedman et al. [18] demonstrated bactericidal activity of different EOs at levels between 0.2 and 10 µl/ml against *Campylobacter jejuni*, *Escherichia coli O157:H7*, *Listeria monocytogenes*, *Salmonella enterica*, *Salmonella typhimurium*, *Shigella dysenteria*, *Bacillus cereus* and *Staphylococcus aureus*.

Gram-negative organisms are slightly less susceptible than gram-positive bacteria [11]. A very useful finding was that the plant-derived antimicrobials carvacrol, cinnamaldehyde and thymol significantly inhibited the kinetics of growth of activated spores of four *Bacillus cereus* strains and served as natural food preservatives in tyndallized carrot broth. They manifested a synergistic bactericidal effect [19]. Carvacrol, cinnamaldehyde, thymol and oregano oil inhibit significantly the growth of *Clostridium perfringens* in commercial meat products [20, 21]. The utility of selected aroma-compounds (citral, trans-cinnamaldehyde, (-)-perillaldehyde, (-)-citronellal, eugenol and carvacrol) for the reduction of bacteria in a room was investigated by Sato et al. [22]. They found that eugenol manifested the lowest and (-)-perillaldehyde the highest antimictobial activity. Sokovic et al. [23] determined chemical composition and antibacterial activity of EOs obtained from ten commonly used herbs (*Citrus aurantium*, *Citrus limon*, *Lavandula angustifolia*, *Matricaria chamomilla*, *Mentha piperita*, *Mentha spicata*, *Ocimum basilicum*, *Origanum vulgare*, *Thymus vulgaris* and *Salvia officinalis*) against the human pathogenic bacteria (*Bacillus subtilis*, *Enterobacter cloacae*, *Escherichia coli O157:H7*, *Micrococcus flavus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *S. epidermidis*, *S. typhimurium* and *Staphylococcus aureus*). Camphor, carvacrol, 1,8-cineole, linalool, linalyl acetate, limonene, menthol, a-pinene, b-pinene, and thymol were the main components of the oils studied. Carvacrol manifested the highest and the broadest antimicrobial activity of all these oils. Elaissi et al. [24] decribed the correlation between chemical composition and antibacterial activity of essential oils obtained from fifteen Eucalyptus species growing in the Korbous and Jbel Abderrahman arboreta (North East Tunisia). The main component of all oils tested was 1,8-cineole, followed by spathulenol, trans-pinocarveol, α-pinene, p-cymene, globulol, cryptone, β-phellandrene, viridiflorol, borneol, limonene and isospathulenol. The EOs were screened for their antibacterial activities using the agar disc diffusion method. The strongest activity of E. *platypus* oil was showed against *Enterococcus faecalis* whereas *E. amannii* oil showed the highest activity against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*. De Sousa et al. [25] investigated the effect of the combined application of carvacrol and 1,8-cineole against different bacteria present in minimally processed vegetables. The combination of carvacrol and 1,8-cineole at sub-inhibitory concentrations could constitute an interesting approach to sanitizing minimally processed vegetables.

Antifungal effects. Many EOs show a potent antifungal effect. Chami et al. [26] evaluated carvacrol and eugenol, the main components of EOs of some aromatic plants, for their therapeutic efficacy in the treatment of experimental oral candidiasis induced by *Candida albicans* in immunosuppressed rats. With the help of microbiological and histopathological techniques the authors confirmed the anticandidal activity of the compounds studied. This anticandidal activity was comparable with the anticandidal activity of the commercial preparation nystatin, used as a positive control. They suggested that carvacrol and eugenol are strong antifungal agents and proposed their use as therapeutic agents for the treatment of oral candidiasis. The antifungal activity of EOs isolated from the aerial parts of some of the Artemisia genus species were tested against 11 different types of fungi plant. Their effects were compared with those of a commercial antifungal reagent benomyl [27]. The results showed that all of the EOs had, at a very broad spectrum, potent antifungal effects against all of the fungi tested. However, pure camphor and 1,8-cineole, which are the major components of the oils, were able to show antifungal activity against only some of the fungal species. Pinto et al. [28] described similar results studying antifungal activity of the EO of *Thymus pulegioides* containing high amounts of carvacrol and thymol. The authors found that

Table 1. List of the main EOs and their components which exhibit cytotoxicity against bacteria, fungi, arthropoda and protozoa

Antibacterial effects	Antifungal effects	Anti-arthropodal and anti-protozoal effects
Carvacrol, cinnamaldehyde, thymol [19, 20, 21]	Carvacrol, eugenol [26]	EO of *Schizonepeta tenuifolia* containing pulegone, menthone and limonene [32]
(-)-perillaldehyde [22]	EOs isolated from the aerial parts of some of the Artemisia genus species [27]	Thymol, citronellal, eugenol and EO of rosemary [33]
Camphor, carvacrol, 1,8-cineole, linalool, linalyl acetate, limonene, menthol, a-pinene, b-pinene, thymol [23]	EO of *Thymus pulegioides* containing high amounts of carvacrol and thymol [28]	EOs isolated from *Origanum vulgare* L. and *Thymus vulgaris* L. [34]
1,8-cineole, spathulenol, trans-pinocarveol, α-pinene, p-cymene, globulol, cryptone, β-phellandrene, viridiflorol, borneol, limonene, isospathulenol [24]	EO obtained from *Thymus vulgaris* L. [29]	EOs isolated from traditional medicinal plants (*Lippia sidoides*, *Lippia origanoides*, *Chenopodium ambrosidoides*, *Ocidum gratissimum*, *Justicia pectorals* and *Vitex agnus-castus*) [35]
Combination of carvacrol and 1,8-cineole [25]	EOs obtained from aerial parts of aromatic plants such as oregano, thyme, lavender, rosemary, fennel and laurel containing carvacrol, borneol, camphor, anethole and 1,8-cineole [30]	EOs originated from genus Cymbopogon, Eucalyptus and Ocimum [36]
	Thymol, carvacrol and the mixture of both these pure EOs [31]	

the EO examined showed remarkable antifungal activity against clinically relevant fungi (e.g. Candida, Aspergillus) mainly due to lesion formation in the cytoplasmic membrane and a considerable reduction of the ergosterol content. EO obtained from another representative of the genus Thymus (*Thymus vulgaris* L.) manifested antifungal activity against different mould species isolated from damp dwellings (Aspergillus, Penicillium, Alternaria, Ulocladium, Absidia, Mucor, Cladosporium, Trichoderma, Rhizopus, Chaetomium and two strains of *Stachybotrys chartarum*) and could be used in low concentration for disinfection of mouldy walls in the dwellings [29]. Soylu et al. [30] tried to find an alternative to synthetic fungicides currently used to control the devastating oomycete pathogen *Phytophthora infestans*, the causal agent of late blight disease of tomato. Antifungal activities of EOs obtained from aerial parts of aromatic plants such as oregano, thyme, lavender, rosemary, fennel and laurel manifested antifungal dose-dependent effects against *Phytophthora infestans*. Major compounds found in EOs were carvacrol, borneol, camphor, anethole and 1,8-cineole. Antifungal efficiency of thymol, carvacrol and the mixture of both these pure EOs has been proven also against *Penicillium digitatum* and *Penicillium italicum* which are responsible for the decay of lemons [31]. The application of these EOs together with wax in citrus packing lines could be considered to be a good alternative to reducing the application of synthetic fungicides.

Anti-arthropodal and anti-protozoal effects. Currently the use of synthetic chemicals to retain the optimal numbers of insects and other arthropods raises several concerns related to environment and human health. An alternative to this is to use natural products that provide sufficient efficacy and are environmentally friendly. A large number of EOs extracted from different families of plants has shown to have a high toxic or repellent activity against arthropoda species. By using fumigation bioassay Park et al. [32] investigated EOs from 21 plants to find out about their insecticidal activities against larvae of *Lycoriella ingenua* (Diptera: Sciaridae). Oil of *Schizonepeta tenuifolia* (a very familiar herb used in traditional Chinese medicine for many centuries) showed the most potent insecticidal activity among the plant essential oils tested. At the level of 12.5 μg/ml of air concentration this oil caused 96.6% mortality of insects. An analysis utilizing gas chromatography-mass spectrometry led to identification of three major compounds found in *Schizonepeta tenuifolia* oil (pulegone, menthone and limonene), of which pulegone was the most toxic one. Waliwitiya et al. [33] investigated acute toxicities of three naturally occurring monoterpenoid components of EOs (thymol, citronellal, eugenol) and the EO of rosemary against late instars of *Agriotes obscurus* L. (Coleoptera: Elateridae). The authors determined both contact and volatile toxicities of thymol, eugenol and citronellal. Furthermore, these compounds manifested also an undesirable phytotoxicity on corn germination and seedling development. The best result was showed by rosemary oil, which had only minimal phytotoxic effects. Santoro et al. [34] investigated the effects of EOs, isolated from *Origanum vulgare* L. and *Thymus vulgaris* L., on growth and ultrastructure of diverse evolutive forms of *Trypanosoma cruzi*. With the help of transmission electron microscopy the authors proved a trypanocidal activity of the EOs studied showing a higher activity of thyme. They suggested thymol to be the main component responsible for this activity. EOs isolated from traditional medicinal plants (*Lippia sidoides*, *Lippia origanoides*, *Chenopodium ambrosidoides*, *Ocidum gratissimum*, *Justicia pectorals* and *Vitex agnus-castus*) also showed significant activities that can be very important in a search for an alternative treatment of Chagas disease, one of the main causes of mortality and morbidity in Latin America [35]. Well documented are also the repellent properties of EOs originated from genus Cymbopogon, Eucalyptus and Ocimum [36]. It is usually assumed that terpenic constituents are responsible for the hydrophobic feature of EOs which allows them to freely permeate the cell membranes and kill the parasites by affecting their cytoplasmatic metabolic pathways or organelles. However, due to the great number of constituents and the synergistic and antagonistic interactions existing between them it is likely that EOs reach other cellular targets besides the cellular membrane as well.

Cytotoxicity against mammalian cells; inhibiting effects on cancer cells. Plant volatiles are common components of the human diet. Therefore the increased human exposure to such compounds (a result of their application as crop protectants, food additives, etc.) requires a careful re-assessment of their toxicity and genotoxicity on the level of mammalian cells. One of the first attempts to do so was undertaken and reported by Stammati et al. [37] who investigated the toxicity of selected plant volatiles (cinnamaldehyde, carvacrol, thymol and S(+)-carvone) against human larynx carcinoma cells Hep-2 in short-term tests. All the compounds studied inhibited the viability and proliferation of Hep-2 cells in a dose-dependent manner. The value of IC_{50} (a median inhibitory concentration that causes 50% inhibition) ranged from 300 μM (cinnamaldehyde) to 700 μM (thymol) in the viability tests and from 200 μM (carvacrol) to 900 μM (carvone) in the proliferation test. Apoptosis, programmed cell death connected with morphological changes and fragmentation of DNA, can be triggered by a wide variety of stimuli and not all cells will necessarily die in response to the same stimulus. Moteki et al. [38] observed a suppression of growth and fragmentation of DNA in the leukemia cell lines Molt 4B and HL-60 cells caused by eucalyptol (1,8-cineole). The authors concluded that the observed changes in the leukemia cell lines resulted from the specific induction of apoptosis by this compound. They did not observe the same phenomenon in human stomach cancer KATO III cells though. Koparal and Zeytinoglu [39] found out that in human non-small-cell lung cancer (cell line A549) and adenocarcinoma large-cell carcinoma higher doses of carvacrol caused a decrease in cell number and apoptosis that is connected with degeneration of cell morphology and a decrease in total protein amount. The data obtained indicated that carvacrol, which is a very potent inhibitor of cell growth

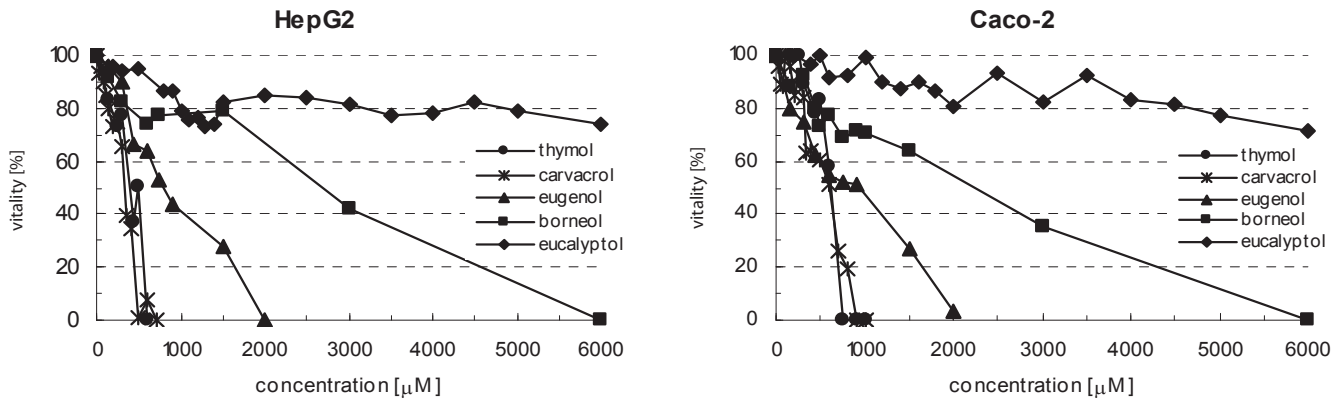


Figure 1. Effect of thymol, carvacrol, eugenol, borneol and eucalyptol on viability of HepG2 cells (left diagram) and Caco-2 cells (right diagram). Cells were treated with different concentrations of individual compounds for 24 hours and after the treatment their viability was measured by trypan blue exclusion assay. Results are mean of three independent experiments.

in A549 cell line, could have an anticarcinogenic effect. The effect of eugenol that is a major component of EO isolated from the *Eugenia caryophyllata* (Myrtaceae), in particular its anticancer effect upon HL-60 human promyelocytic leukemia cells, was investigated by Yoo et al. [40]. The authors found that EO – mediated apoptosis in HL-60 cells occurs via reactive oxidative species (ROS) generation that forms eugenol phenoxyl radicals. Eugenol – treated HL-60 cells displayed features of apoptosis including DNA fragmentation and formation of DNA ladders in agarose gel electrophoresis. The compounds capable of inducing apoptosis of human cancer cells have recently drawn a great deal of attention due to their potential utilization as anti-cancer agents. Many chemo-preventive agents indeed exert the anti-cancer effects by inducing apoptosis of the cancer cells. Pisano et al. [41] studied the anti-proliferative activity of eugenol along with six natural and synthetic eugenol – related compounds for their capability to inhibit cell growth of primary melanoma cell lines established from patient tissue samples. Melanoma cells are considered to be the most aggressive skin cancer cell types against which the chemotherapeutic agents available are not effective. Eugenol and isoeugenol monomers and their respective O-methylated forms did not inhibit melanoma cell proliferation. Conversely the eugenol-related biphenyl (S)-6,6'-dibromo-dehydrodieugenol elicited a specific anti-proliferative activity toward neuro-ectodermal tumor cells and triggered apoptosis. The activity of this compound should be further investigated using *in vivo* melanoma models in order to evaluate its real anticancer effectiveness on such a tumor type. EOs and their isolated constituents have been reported to be well tolerated by mammalian cells. Fabian et al. [42] evaluated simultaneously the cytotoxic effects of four EOs obtained from cinnamon, clove, oregano and thyme and their major components eugenol, carvacrol and thymol against enteroinvasive *Escherichia coli* bacteria and human cancer colonic cells Caco-2. Cytotoxic effects of EOs against colonic cells were assayed on the basis of viability staining followed by fluorescence microscopy. The most prospective EO showing a high antibacterial and a low cytotoxic effect against colonic cells seems to be oil of oregano particularly its component carvacrol, which slightly increased the incidence of apoptotic cell death but showed extensive antimicrobial activity even at lower concentrations.

Contribution of our laboratory to cytotoxic effect evaluation. We investigated cytotoxic effects of five frequently occurring compounds of EOs described above (eucalyptol, eugenol, borneol, thymol and carvacrol) versus two histopathologically different types of human cancer cells, namely human hepatoma cells HepG2 and human colonic cells Caco-2. Human hepatoma cells HepG2 retain many specialized liver functions and drug metabolizing enzyme activities comparable with human hepatocytes [43]. The Caco-2 cells, isolated from a human colon adenocarinoma, has been shown to have abilities to develop in culture enterocyte-like cell functions including the presence of brush border microvilli, vitamin uptake and ion transport, tight junctions, or forming brush border microvilli [44, 45]. Caco-2 cells cultured *in vitro* retained the phase I enzyme CYP1A1 and those of the phase II enzymes retained glutathione-S-transferases, glucuronidase and sulfotransferases [46]. Fig. 1 shows that in both HepG2 and Caco-2 cells the cytotoxic effect of the compounds studied decreased in the following order: carvacrol > thymol > eugenol > borneol > eucalyptol. IC_{50} of these 5 compounds represented approximately 340 μM (carvacrol), 540 μM (thymol), 1000 μM (eugenol), 2000 μM (borneol) and 7500 μM (eucalyptol) in HepG2 cells. In Caco-2 cells IC_{50} values represented approximately: 600 μM (carvacrol), 680 μM (thymol), 800 μM (eugenol), 2200 μM (borneol) and 9600 μM (eucalyptol) [47, 48, 49]. Bactericidal effects of EOs occurred at many times lower concentrations

Cytotoxic effects of EOs are attributed generally to their destructive effects on the cell membranes but other causes must also be considered. Liang and Lu [50] found that carvacrol

at a concentration of 400-1000 μM induced an elevation of intracellular calcium concentration [Ca^{2+}] in human glioblastoma cells and killed the cells in a concentration-dependent manner. The authors suggested that carvacrol – induced cell death might involve ROS – mediated apoptosis. Cha and Kim [51] examined the cell viability and induction of apoptosis in KB cells treated with *Cryptomeria japonica* essential oil for 12 h at several concentrations. KB cell line, frequently used for screening of antineoplastic agent, was derived from a human carcinoma of the nasopharynx. The results showed that EO induced the apoptosis in KB cells via mitochondrial stress and activation of caspases. The authors concluded that the EO from *C. japonica*, used in traditional Asiatic medicines, may have a great potential as a cancer chemopreventive and therapeutic agent.

Mutagenicity, anti-mutagenicity and DNA-protective effects. The increasing interest in the use of EOs and their components in food preservation, which was in recent years amplified by a negative consumer perception of synthetic preservatives, raises the need to evaluate such compounds for both mutagenic and antimutagenic properties. The research aimed at the evaluation of potential mutagenic/antimutagenic activities of phytochemicals performed on different prokaryotic and eukaryotic model systems is very important for nutrition and clinical practice [52]. Such research can either exclude or include EO to be tested as a reliable food additive. Azizan and Blevins [53] attempted to find a correlation between pungent properties of six components of EOs (capsaicin, thymol, borneol, allyl isothiocyanate, eugenol and cinnamaldehyde) and their mutagenicity using Ames bacterial tests carried out with and without S9 metabolic activation. The authors failed to find any relation between the investigated mutagenic activity and the pungent properties of the chemicals used in the test. Only capsaicin manifested a mutagenic effect on TA100 bacteria in the presence of S9 metabolic activation. Stammati et al. [37] used several short-term microbial *in vitro* assays to evaluate genotoxicity of cinnamaldehyde, carvacrol, thymol and S(+)-carvone. At non-toxic doses and regardless of metabolic activation carvacrol and thymol increased the number of revertants by 1.5-1.7-times in the Ames test. None of the four plant volatiles caused DNA damage at non-toxic doses in the SOS-chromotest. A marked dose-dependent differential toxicity was observed with carvone and, to a lesser extent, with cinnamaldehyde in the DNA repair test while with thymol and carvacrol this effect was less pronounced. A set of cytogenetic analyses of human blood peripheral lymphocytes treated with carvacrol were carried out by Ipek et al. [54] using the *in vitro* sister chromatid exchange (SCE) assay. According to the data obtained carvacrol did not increase the formation of SCE, however it inhibited the rate of SCE induced by mitomycin C and acted as an antigenotoxic agent. Lima et al. [55] studied the viability of freshly isolated rat hepatocytes exposed to an oxidative compound (*tert*-butyl hydroperoxide; *t*-BHP) in the presence and in the absence of essential oil isolated from *Salvia officinalis* L. The authors found that in the range of the concentrations tested the EO did not show protective effects against *t*-BHP – induced toxicity evaluated on the basis of the LDH assay, lipid peroxidation and glutathione status. On the other hand, Vukovic-Gacic et al. [56] discovered that the same EO from sage and its monoterpenes thujone, 1,8-cineole and camphor, whose toxicity differed, reduced the number of UVC – induced revertants in bacteria *Salmonella typhimurium*, *Escherichia coli WP2* and *Escherichia coli K* in a concentration-dependent manner reaching 50-70% of inhibition at the maximum non-toxic concentrations. The metabolic activation had no effect on anti-mutagenic potential of the EOs tested. The data obtained appoint sage monoterpenes for further chemoprevention studies. The commonly used EOs from *Melaleuca alternifolia* (tea – tree oil) and *Lavandula angustifolia* (lavender oil) show antimicrobial activity and are able to treat minor health problems. Evandri et al. [57] investigated their mutagenic and antimutagenic activities using the bacterial reverse mutation assay in *Salmonella typhimurium TA98* and *TA100* strains and in *Escherichia coli WP2 uvrA* strain. They did so with and without an extrinsic metabolic activation system. None of the EOs manifested mutagenic activity on the bacteria tested, whereas lavender oil exerted strong anti-mutagenic activity reducing mutant colonies in the *TA98* strain exposed to the direct mutagens 2-nitrofluorene and 1-nitropyrene. The anti-mutagenic property of lavender oil makes it a promising candidate for new applications in human healthcare. By using the comet assay technique Aydin et al. [58] tested the modulating effects of thyme and its major components against the oxidative DNA damage induced in human lymphocytes by hydrogen peroxide (H_2O_2). The same group of authors investigated the influence of thyme against the heterocyclic amine 2-amino-3-methylimidazo[4,5-f]-quinoline (IQ) and mitomycin C [59]. The authors found out that the phenolic compounds thymol and carvacrol at concentrations below 200 and 100 μM, respectively significantly reduced oxidative DNA damage.

Contribution of our laboratory to genotoxic and DNA-protective effects evaluation. We evaluated DNA-damaging (genotoxic) and DNA-protective effects of 24-h treatment of HepG2 and Caco-2 cells with five different compounds of essential oils: eucalyptol (0-5000 μM), eugenol (0-600 μM), borneol (0-1500 μM), carvacrol (0-300 μM) and thymol (0-200 μM). The level of DNA lesions was measured by standard alkaline single cell gel electrophoresis (SCGE; comet assay) suggested by Singh et al. [60] and modified by Slamenova et al. [61] and Gabelova et al. [62]. At concentrations $\leq IC_{50}$ the compounds studied did not induce DNA strand breaks either in human HepG2 or in Caco-2 cells, however, incubation of human cells in the presence of the monoterpens thymol and carvacrol led to a significant protection of cellular DNA against a strong oxidative agent – hydrogen peroxide [47]. We also found out [63] that *in vivo* applied synthetic carvacrol exhibited primarily a strong hepato-protective activity against oxidative damage to DNA. Incubation of the cells with eucalyptol and eugenol did not manifest any protective effects. Borneol acted

protectively against H_2O_2 only in human hepatoma HepG2 cells, which retain many specialized liver functions and drug metabolizing enzyme activities, but not in the metabolically poorer colonic cells Caco-2. The distinctions found in the two human cell lines pre-treated with borneol are at present unclear, but they could be connected either with differences in the specific action of borneol on the cellular signal transduction system [32] or with a different inhibition of glucuronidation with borneol [64]. We conclude that the protective efficiency of carvacrol and thymol against H_2O_2 – induced DNA lesions testify to their antioxidant properties. Sinha et al. [65] proved the anti-genotoxic effects of palmarosa and citronel EOs (extensively used in ancient Indian and South-east Asian traditional medicines) on human lymphocytes damaged by genotoxic compounds methylmethane sulphonate and hydrogen peroxide. In addition, a significant dose-dependent antioxidant activity of both EOs studied was observed and they may be considered for a natural source of a new and safe antioxidant. Anticancer activity of *Salvia officinalis* EO against the squamous human carcinoma cell line of the oral cavity (UMSCC1) described lately Sertel et al. [66]. The authors proved that sage EO reduced the viability of UMSSC1 to a minimum at concentrations $>IC_{50}$ (135 μg/ml).

Antioxidant effects. Free radicals are unavoidable byproducts of normal cellular oxygen metabolism in mammalian organisms. In addition to their purposeful roles as regulating or signaling molecules, they can alter the structure of cellular macromolecules such as lipids, proteins and DNA and contribute to the pathogenesis of a number of human diseases. The oxidation of biomolecules by free radicals limits also the shelf life of raw and processed foodstuffs. Adverse effects of free radicals can be diminished by synthetic or natural antioxidants, i.e. compounds that can bind to reactive oxygen species. However, the use of synthetic antioxidants has caused some problems due to their highly volatile nature, instability at high temperatures, and strict law restrictions. Recently, there has been a growing interest of modern society in use of fewer synthetic additives and more natural plant products appropriate for the prevention of oxidation, controlling pathogens and/or toxin-producing microorganisms in foods, cosmetics and pharmaceuticals. EOs are known to possess potential as natural agents for food preservation; in fact their effectiveness against a wide range of microorganisms is related to their hydrophobicity [11]. Besides this activity many EOs and their components have been qualified as natural antioxidants and proposed as potential substitutes of synthetic antioxidants in specific sectors of food preservation [67]. As early as in 1994, Aeschbach et al. [68] observed that thymol, carvacrol, 6-gingerol and hydroxytyrosol were good scavengers of peroxyl radicals and decreased peroxidation of phospholipid liposomes in the presence of iron(III) and ascorbate. From this time further, several research groups reported antiradical activity of total EOs of different plant species as well as their different fractions or pure constituents, e.g. carvacrol or thymol. Various techniques were used: 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay [63, 69, 70, 71, 72], β-carotene bleaching test [68, 70, 72], thiobarbituric acid reactive species (TBARS) assay [67, 69, 73], ferric ion reducing antioxidant power (FRAP) assay [72], oxygen radical absorbance capacity assay [74] and method measuring the formation of hydroperoxydienes from linoleic acid [67]. With help of electron spin resonance (ESR) spectroscopy Fujisawa et al. [75] found that eugenol and eugenol-related synthetic compounds possessed radical scavenging activity comparable with the activity of conventional antioxidants. Jirovetz et al. [76] proved the scavenging activity of a commercial rectified clove leaf (*Eugenia caryophyllus*) EO and its main constituent eugenol against the DPPH radical. Radical scavenging activity of eugenol was confirmed also by Slamenova et al. [49] and Mastelic et al. [70]. Rectified clove leaf (*Eugenia caryophyllus*) EO manifested a significant inhibitory effect against hydroxyl radicals and acted as an iron chelator. With respect to lipid peroxidation, the inhibitory activity of clove oil, determined using a linoleic acid emulsion system, indicated a higher antioxidant activity than that of the standard butylated hydroxytoluene (BHT). Antimicrobial and antifungal activities of the EO and various extracts from herbal parts and callus cultures of *Origanum acutidens* (carvacrol being the main component) were accompanied by a moderate antioxidant capacity proved by DPPH and β-carotene/linoleic acid assays [77]. EOs isolated from the aerial parts of different species of Artemisia contain mainly camphor, 1,8-cineole and chamazulene but also lower amounts of nuciferol propionate and nuciferol butanoate, caryophyllene oxide, borneol, α-terpineol and other compounds. All of the EOs studied manifested DPPH radical scavenging ability but camphor, 1,8-cineole [27] and borneol [49] did not. *Rosmarinus officinalis* L. EO manifested antioxidant activities in DPPH radical scavenging [78, 79, 80] and β-carotene bleaching assays [79] and inhibited lipid peroxidation [80]. These properties were concentration-dependant and greater than that of main components 1,8-cineole, α-pinene and β-pinene [79]. It is very difficult to attribute the antioxidant effect of a total essential oil to one or a few active components, because an EO always contains a mixture of different chemical compounds. In addition to the major compounds, also minor compounds may make a significant contribution to the oil's antioxidant activity [79].

In addition to *in vitro* studies, also some *ex vivo* animal bioassays described antioxidant status of plant extracts and EOs and their positive effects on animals. Lima et al. [55] proved (by quantification of plasma transaminase activity and liver glutathione-S-transferase – GST and glutathione reductase – GR activities) the antioxidant potential of a traditional water infusion (tea) of common sage (*Salvia officinalis* L.). The replacement of water by sage tea for 14 days in the diet of rodents, which did not affect either body weight, food consumption or liver toxicity, increased significantly liver GST activity in rats and mice and improved the antioxidant status of hepatocytes. Of great importance is the finding of Sasaki et al. [81] that dietary or oral administration of thyme (0.5%

or 2.0%), thymol (50-200 mg/kg) or carvacrol (50-200 mg/kg) once a day for 7 successive days caused in mice a slight but significant 1.1-1.4-fold increase of the activities of xenobiotic metabolizing enzymes, i.e. phase I enzymes, such as 7-ethoxycoumarin-O-deethylase (ECOD) and phase II enzymes, such as GST and quinone reductase (QR). Study of Aristatile et al. [82] was designed to investigate the hepatoprotective and antioxidant properties of carvacrol on d-galactosamine (D-GalN) – induced hepatotoxicity and oxidative damage in male albino Wistar rats. D-GalN hepatotoxic rats exhibited elevation in the activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transpeptidase and lipidperoxidative markers such as thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides. Activities of enzymatic antioxidants (superoxide dismutase, catalase and glutathione peroxidase) and the levels of non-enzymatic antioxidants (vitamin C, vitamin E and reduced glutathione) in the plasma, erythrocytes, liver and kidney were decreased in the hepatotoxic rats. Oral administration of carvacrol for 21 days brought these parameters towards normal. These results suggest that carvacrol could afford a significant hepatoprotective and antioxidant effect against D-GalN – induced rats. After 7 day administration of borneol (17 or 34 mg/kg) in drinking water of rats concentration-dependant increase of total GSH in isolated hepatocytes was detected [83]. The stimulation of endogenous antioxidant defence systems – glutathione and related enzymes – by many plant compounds is a well-known phenomenon. Such mechanisms might entail the interaction of natural compounds with cell signaling and influence gene expression with the consequent modulation of specific enzymatic activities that drive the intracellular response against oxidative stress [84].

The antioxidant capacity of plants is believed to be responsible for the health promoting properties of fruits and vegetables. Plant antioxidants are composed of a broad variety of different substances like ascorbic acid and tocopherols, polyphenolic compounds or terpenoids, which perform several important functions in plants and humans. Monoterpenes and diterpenes, which are the main components of essential oils, act as allelopathic agents [85], attractants in plant-plant or plant-pathogen/herbivore interactions or repellants. When assessing the antioxidant activity of plant antioxidants, an important point is to consider their interaction with other antioxidants. They may work synergistically, antagonistically and additively [86]. Especially combinations of hydrophilic and lipophilic antioxidants may exert synergistic effects, as has been shown for rutin in combination with gamma-terpinene, lutein or lycopene [87].

Conclusions

Essential oils exhibit a range of beneficial biological activities and belong to the main representatives of phytochemicals with chemopreventive potential. All these properties of EOs are very valuable for clinical and industrial practice. However, the fact, that an EO is derived from a plant does not necessarily mean that it is fully harmless for humans or that it cannot kill a wide variety of other life. Research of toxic and genotoxic effects of EOs is therefore very important. Our research group tried to contribute to the knowledge about toxic and genotoxic effects of volatile plant oils on human cells. We investigated the viability, the level of DNA lesions and sensitivity of DNA against hydrogen peroxide and other genotoxins in cells pretreated with different compounds of EOs occurring frequently in different plants. We focused on eucalyptol (a cyclic ether and a monoterpene), eugenol (a member of the allylbenzene class of chemical compounds), borneol (a bicyclic monoterpene) and two isomers thymol and carvacrol (monoterpene phenol derivatives) with strong antiseptic properties. The amount of DNA lesions induced by hydrogen peroxide was in both cell types reduced by low concentrations of carvacrol and thymol, compounds of essential oil of thyme, origanum, etc., and in HepG2 cells also by borneol, which is present in essential oil of some species of the genus Artemisia. These compounds showed unambiguous DNA-protective effects. Further compounds of essential oils investigated (eugenol and eucalyptol) failed to show any DNA protective effect. To sum up, the mild cytotoxic effect of carvacrol and thymol ($IC\leq_{50}$) on hepatoma and colonic cells had no DNA-damaging effect and even reduced the level of DNA lesions induced in these cells by an oxidative agent, and thus acted as antioxidants. It is evident that at $IC\leq_{50}$ the antioxidative activity of carvacrol and thymol is not accompanied by pro-oxidative effects.

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