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Membrane and DNA damaging/protective effects of eugenol, eucalyptol, terpinen-4-ol, and camphor at various concentrations on parental and drug-resistant H1299 cells

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Abstract: Eugenol, eucalyptol, terpinen-4-ol, and camphor, used as flavor agents in cosmetic and food products, have both prooxidant and antioxidant activities. To clarify the mechanisms of their cytotoxic effect and the factors affecting their antioxidant/prooxidant activities, we investigated cell membrane and DNA damage/protective effects induced by eugenol, eucalyptol, terpinen-4-ol, and camphor in parental and drug-resistant human lung cancer cell lines. The parental cells were found approximately 3, 6, and 8 times more sensitive to camphor cytotoxicity than drug-resistant cells at 24, 48, and 72 h respectively. The cytotoxic activity for both cell types was found to be in the order of camphor, eugenol, terpinen–4-ol, and eucalyptol, depending on their concentrations. Malondialdehyde and 8-hydroxy deoxyguanosine levels were also increased as a result of membrane and DNA damage in both cell lines exposed to the highest concentration of these test components. On the other hand, eugenol, eucalyptol, terpinen-4-ol, and camphor protected the cells against H_2O_2-induced cytotoxicity, membrane damage, and DNA damage when the cells were incubated with these test components at lower concentrations (< IC_{50}) before H_2O_2 treatment. These findings suggest that eugenol, eucalyptol, terpinen-4-ol, and camphor exhibit membrane and DNA protective/damaging effects changing in a manner dependent upon concentration, cell type, and time.

Key words: Monoterpenes, DNA, membrane, anticancer, antioxidant

1. Introduction

A wide variety of active phytochemicals, including terpenoids, have been identified in different herbs. Monoterpenes such as eugenol, eucalyptol, terpinen-4-ol, and camphor are highly hydrophobic substances and exert a wide spectrum of biological actions of great importance in many different areas (1,2). The hydrophobic nature of eugenol, eucalyptol, terpinen-4-ol, and camphor enables them to react with the lipids of the cell membrane and mitochondria, rendering them permeable and leading to leakage of cell components (3). They are considered useful agents for the prevention of diseases (4–9). Many studies have shown that phytochemicals in plant essential oils display antioxidant activity as a result of their capacity to scavenge free radicals (10–12). On the other hand, the effects of antioxidant concentrations on oxidation reactions depend on many factors such as the structure of the antioxidant, oxidation conditions, and changing of the oxidized structure. Antioxidants lose their antioxidant effects at higher concentrations and gain prooxidant structure. They can either protect DNA and membranes against oxidants as an antioxidant at lower concentrations, or damage DNA and membranes as a prooxidant at higher concentrations. Recent studies reveal that anti-/prooxidant and toxic properties of these molecules change depending on their concentration, and so they are not safe for humans (13).

Tumors are heterogeneous in many respects, including chemotherapeutic susceptibility (14). Resistance to chemotherapeutic agents is a major problem in the treatment of patients with small cell (SCLC) and nonsmall cell lung cancer (NSCLC). Acquired multidrug resistance is the main obstacle for the cure of SCLC. A group of drug-resistant cells can develop in tumors during the chemotherapy. In one study, the activities of NADPH-CYP reductase (2-fold), GST (11-fold), Se-dependent and independent-GSH-Px (7- to 11-fold), and GST-pi were found higher in epirubicin-resistant H1299 cells than parental cells (15). Lung tumor cells selected for acquired resistance to epirubicin in cultures have concurrently developed a tolerance to superoxide and hydrogen peroxide, most likely because of elevated activities of enzymatic defenses against oxyradicals. In another study, CYP3A4 microsome enzyme in drug-resistant cells used eucalyptol as a substrate (16,17).

Many phenolic components show various protective/damaging effects in different biological systems depending on the experimental conditions. We therefore tried to

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demonstrate the ability of eugenol, eucalyptol, terpinen-4-ol, and camphor to prevent cytotoxicity and membrane and DNA damage induced by strong oxidative agent H_2O_2 in parental and drug-resistant H1299 cells. We also evaluated their cytotoxicity and membrane or DNA damaging effects in the 2 cell lines.

2. Materials and methods

2.1. Cancer cell culture and chemicals

Eugenol (99%), eucalyptol (99%), terpinen-4-ol (98.5%), and camphor (98.5%) were purchased from Sigma-Aldrich (Germany).

The H1299 cell line was purchased from American Type Culture Collection (Rockville, MD, USA). Cells were routinely cultured in RPMI 1640 medium supplemented with 10% fetal calf serum and 1% antibiotic-antimycotic solution (penicillin, streptomycin, and amphotericin) in a humidified atmosphere containing 5% CO_2 at 37 °C. For subculturing, cells were harvested after trypsin/EDTA treatment at 37 °C. Cells were used when monolayer confluence had reached 75%. The drug-resistant (epirubicin-resistant) H1299 tumor cells were derived from the parental line by stepwise selection in increasing concentrations of epirubicin until the cells were capable of propagating in 220 ng/mL drug, as described previously (15,18).

2.2. Cell viability assay

The cancer cells (10,000 cells/well, monolayer) were plated in a 96-well plate. The next day the cells were treated with different concentrations of eugenol, eucalyptol, terpinen-4-ol, and camphor in the medium for 24, 48, and 72 h. At the end of the incubation periods, the cytotoxicity of these monoterpenes on cancer cells was determined by the CellTiter-Blue-Cell Viability Assay. The assay is based on the ability of living cells to convert a redox dye (resazurin) into a fluorescent end product (resorufin). Nonviable cells rapidly lose metabolic capacity and thus do not generate a fluorescent signal (19). Following cellular reduction, fluorescence is recorded at 560 nm (excitation) and 590 nm (emission) spectrofluorometrically (PerkinElmer LS 55). The data were expressed as average values obtained from 8 wells for each concentration. The IC_{10}, IC_{50}, and IC_{70} value were calculated using linear functions (the equation of a straight line). H_2O_2 cytotoxicity on cancer cells was measured following the same protocol. For measuring the antioxidant effect of eugenol, eucalyptol, terpinen-4-ol, and camphor against H_2O_2 cytotoxicity, the cells were preincubated with the test components at different concentrations (< IC_{50}) for 1 h, before hydrogen peroxide treatment for 24 h.

2.3 Determination of malondialdehyde level

The cells were plated at a density of (5–10) ´ 10^5 cells/100 mm dishes and incubated with different concentrations (IC_{10}, IC_{50}, and IC_{70}) of eugenol, eucalyptol, terpinen-4-ol, and camphor for 24 h. Cells were scraped off the culture plates with culture medium and were centrifuged at 400 × *g* for 10 min. The cell pellets were washed with PBS and then sonicated (3 × 15 s) in 50 mM potassium phosphate, pH 7.2, containing 1 mM PMSF (Sigma) and 1 µg/mL of leupeptin (Sigma) and centrifuged at 150,000 × *g* for 1 h. The supernatant was used for the determination of malondialdehyde (MDA) level. For measuring the membrane protective effect, the cells were preincubated with the maximum cytoprotective concentrations of the different test components for 1 h, before hydrogen peroxide treatment (IC_{10}, IC_{50}, and IC_{70}) for 24 h.

MDA levels in the cells were assayed as described by Wasowicz et al. (20). This fluorometric method for measuring thiobarbituric acid-reactive substances in supernatant is based on the reaction between MDA and thiobarbituric acid. The product of this reaction was extracted into butanol and measured by fluorescence spectrometer (525 nm excitation, 547 nm emission).

The concentration of proteins was determined by the Bradford method (21) with bovine serum albumin as a standard.

2.4. Determination of 8-hydroxy deoxyguanosine level

The cells were plated at a density of (5–10) × 10^5 cells/100 mm dishes and incubated with different concentrations (IC_{10}, IC_{50}, and IC_{70}) of eugenol, eucalyptol, terpinen-4-ol, and camphor for 24 h. After DNA purification by manufacturer's instructions from the cultured cells (Genomic DNA Mini Kit, Invitrogen, Carlsbad, CA, USA), the genomic DNA samples were used to determine the amount of 8-hydroxy deoxyguanosine (8-OHdG) with a competitive ELISA kit (Highly Sensitive 8-OHdG Check, Japan Institute for Control of Aging, Fukuroi, Shizuoka, Japan). Microtiter ELISA plates were precoated with 8-OHdG. Fifty microliters of the sample and the primary antibody were added to each well and they were incubated at 4 °C overnight. The wells were washed 3 times, and then 100 µL of secondary antibody was added to each well and incubated for 1 h at room temperature. The wells were again washed 3 times. After that, enzyme substrate solution was added and the wells were incubated at room temperature for 15 min. The reaction was stopped by adding the terminating solution. The absorbance was read at a wavelength of 450 nm (22,23). For measuring DNA protective effect, the cells were preincubated with the maximum cytoprotective concentrations of the different test components for 1 h, before hydrogen peroxide treatment (IC_{10}, IC_{50}, and IC_{70}) for 24 h.

2.5. Data analysis

The results of the replicates were pooled and expressed as mean ± standard error. Analysis of variance was carried out. Significance was accepted at $P \leq 0.05$ (24).

3. Results and discussion

3.1. Cytotoxic and cytoprotective effects of eugenol, eucalyptol, terpinen-4-ol, and camphor on H1299 cells

Eugenol, eucalyptol, terpinen-4-ol, and camphor showed cytotoxic effects on parental and drug-resistant H1299 cells (Table 1). After 24-, 48-, and 72-h incubation periods, the cytotoxicity of camphor was found higher than that of the other test components in both parental and drug-resistant cells. The cytotoxic activity was found to follow the order of camphor > eugenol > terpinen-4-ol > eucalyptol for both cell types depending on their concentrations and incubation times. The toxic and convulsant properties of camphor are well known (25–27). The antiproliferative effects of alpha-terpineol, linalyl acetate, and camphor when applied alone or in combination on human colon cancer cells HCT-116 were demonstrated (28). The essential oils from some medicinal plants having camphor as a major component showed antiproliferative effects on THP-1 cells (29) and antimetastatic and apoptotic effects on highly metastatic HT-1080 human fibrosarcoma tumor cells (30). On the other hand, eucalyptol had less cytotoxic effects than carvacrol and thymol on K562 cells (31). In a human submandibular cell line, the cytotoxicity of eugenol was 1 order of magnitude lower than that of isoeugenol (32). Similarly, Fabian et al. (33) reported that eugenol had a very low detrimental cytotoxic effect on intestinal cells. However, in human VH10 fibroblasts, Caco-2 colon cells, and Hep G2, the cytotoxicity of eugenol was significantly higher than that of borneol (34). The cytotoxicity of eugenol to human HFF fibroblasts and human HepG2 hepatoma cells was increased somewhat in the presence of a hepatic S-9 microsomal fraction from Aroclor-induced rats or hamsters (35). On the other hand, terpinen-4-ol did not cause any cytotoxic effect on SK-MEL-28, MDA-MB-231, MCF7, 5637, and PC-3 human tumor cells but had detrimental effects on Hs578T cells (36,37). The IC_{50} concentration of this component was found to be higher than 100 µmol for A549 and DLD-1 cell lines (38).

In our study, drug-resistant cells were found less sensitive to eucalyptol and camphor than parental cells. Investigation of human metabolism of 1,8-cineole (eucalyptol) in vitro and in vivo showed that 2 metabolites, 2α-hydroxy-1,8-cineole and 3α-hydroxy-1,8-cineole, formed in human microsomes. The existing data suggest that 1,8-cineole (eucalyptol) is a substrate for CYP3A enzymes in rat and human liver microsomes (16,17). The activities of enzymes involved in detoxification and antioxidant mechanisms were also found higher in epirubicin-resistant H1299 cells than in parental cells (15). Our result supports this existing data.

We also measured the cytoprotective (antioxidant) effects of eugenol, eucalyptol, terpinen-4-ol, and camphor against the strong oxidant H_2O_2 in parental and drug-resistant H1299 cells. The cytotoxic effect of H_2O_2 is presented in the Figure. Table 2 shows the levels of H_2O_2-induced cytotoxicity in H1299 cells preincubated with different concentrations of eugenol, eucalyptol, terpinen-4-ol, and camphor. The cytoprotective effect varied depending on the concentration. While eucalyptol and camphor at their IC_{30}, eugenol at its IC_{20}, and terpinen-4-ol at its IC_{10} had the maximum cytoprotective effect on parental cells, camphor and eucalyptol at their IC_{10} and eugenol and terpinen-4-ol at their IC_{30} showed maximum cytoprotective effect in resistant cells against IC_{10}, IC_{50}, and IC_{70} H_2O_2 cytotoxicity.

Table 1. Cytotoxic effects of eugenol, eucalyptol, terpinen-4-ol, and camphor on parental and drug-resistant H1299 cells after 24, 48 and 72 h of incubation.

	Eugenol (µM) P. cells - R. cells	Eucalyptol (mM) P. cells - R. cells	Terpinene-4-ol (µM) P. cells - R. cells	Camphor (µM) P. cells - R. cells
24 h				
IC_{10}	75.32 - 180	1.81 - 5	32.07 - 64	7.90 - 26
IC_{50}	410 - 1080	4.96 - 33	1800 - 5800	55.47 - 350
IC_{70}	1011 - 2350	19.39 - 70	3500 - 20,000	69.09 - 525
48 h				
IC_{10}	66 - 150	1.66 - 3.5	22.51 - 50	4.90 - 18
IC_{50}	300 - 787	3.94 - 17	1123 - 3100	39.79 - 235
IC_{70}	786 - 1800	14.89 - 50	2510 - 16,000	60.38 - 475
72 h				
IC_{10}	54 - 130	1.01 - 2	13.53 - 30	3.84 - 12
IC_{50}	211 - 598	2.95 - 10	683 - 2500	29.65 - 150
IC_{70}	488 - 1400	10.48 - 40	1775 - 11,500	58.70 - 420

P. cells = parental cells, R. cells = resistant cells.

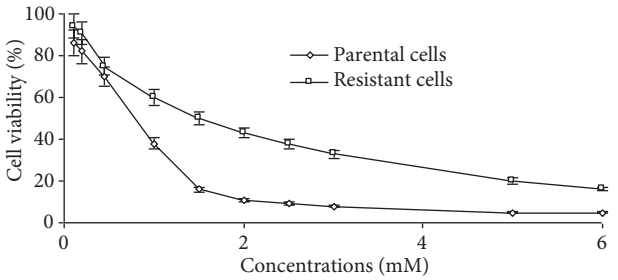


Figure. Cytotoxic effects of hydrogen peroxide on parental and drug-resistant H1299 cells.

3.2. Membrane and DNA protective/damaging effect of eugenol, eucalyptol, terpinen-4-ol, and camphor on H1299 cells

In this study, MDA and 8-OHdG levels were increased in cells exposed to eugenol, eucalyptol, terpinen-4-ol, and camphor (Table 3) as a result of the membrane and DNA damaging effects of these test components on the cells. Generally, the amount of MDA and 8-OHdG was found not to be statistically different from that of the control cells at IC_{10} concentrations ($P \leq 0.05$). The highest membrane damages were caused by camphor (IC_{70}) in parental cells and by eucalyptol (IC_{70}) in resistant cells. However, the highest DNA-damaging effect was observed after exposure to camphor (IC_{70}) in resistant cells and after treatment with eucalyptol (IC_{70}) in parental cells (Table 3; $P \leq 0.05$). The highest concentration (IC_{70}) of eugenol, eucalyptol, terpinen-4-ol, and camphor caused important membrane and DNA damaging effect on the cells ($P \leq 0.05$). They induced membrane and DNA damage and cytotoxicity in H1299 cells at relatively higher concentrations than those that mediate their anticancer activities. The induction of cytotoxic cell death can be accompanied by membrane and DNA damage (39). In our study, MDA and 8-OHdG

Table 2. Cytoprotective effects of camphor, eugenol, eucalyptol, and terpinen-4-ol ($<IC_{50}$) against H_2O_2 cytotoxicity on parental and drug-resistant H1299 cells.

Concentrations	IC_{10} H_2O_2 Cell viability (%) X ± SE	IC_{50} H_2O_2 Cell viability (%) X ± SE	IC_{70} H_2O_2 Cell viability (%) X ± SE
IC_{10} camphor (R)	85 ± 1.00 hi	79 ± 1.07 h	25 ± 0.09 bc
IC_{20} camphor (R)	79 ± 1.11 h	72 ± 1.09 gh	23 ± 0.13 bc
IC_{30} camphor (R)	73 ± 1.09 gh	47 ± 0.11 e	14 ± 0.06 ab
IC_{10} camphor (P)	91 ± 1.11 ij	56 ± 0.67 f	64 ± 0.78 fg
IC_{20} camphor (P)	92 ± 1.31 ij	89 ± 1.00 i	86 ± 1.09 i
IC_{30} camphor (P)	97.7 ± 1.09 j	90 ± 1.05 i	87 ± 1.00 i
IC_{10} eugenol (R)	64 ± 0.97 fg	24 ± 0.07 bc	19 ± 0.08 b
IC_{20} eugenol (R)	61 ± 0.81 fg	28 ± 0.22 c	13 ± 0.59 ab
IC_{30} eugenol (R)	81 ± 1.00 hi	32 ± 0.20 cd	22 ± 0.02 bc
IC_{10} eugenol (P)	96 ± 1.34 j	72 ±1.13 gh	60 ± 0.06 f
IC_{20} eugenol (P)	98 ± 1.11 j	78 ± 1.17 h	63 ± 0.13 fg
IC_{30} eugenol (P)	77 ± 1.55 h	45 ± 0.99 de	25 ± 0.10 bc
IC_{10} eucalyptol (R)	66 ± 0.63 g	63 ±1.01 fg	24 ± 0.08 bc
IC_{20} eucalyptol (R)	9 ± 0.37 a	9 ± 0.09 a	9 ± 0.03 a
IC_{30} eucalyptol (R)	15 ± 0.87 ab	14 ± 0.03 ab	11 ± 0.08 ab
IC_{10} eucalyptol (P)	90 ± 1.99 i	63 ± 0.65 fg	60 ± 0.14 f
IC_{20} eucalyptol (P)	92 ± 1.67 ij	69 ± 0.90 g	64 ± 0.50 fg
IC_{30} eucalyptol (P)	98 ± 1.78 j	72 ± 0.45 gh	78 ± 1.01 h
IC_{10} terpinen-4-ol (R)	83 ± 1.44 hi	80 ± 1.12 h	44 ± 0.22 de
IC_{20} terpinen-4-ol (R)	72 ± 1.33 gh	78 ± 0.56 h	36 ± 0.12 d
IC_{30} terpinen-4-ol (R)	98 ± 1.06 j	99 ± 1.11 j	45 ± 0.65 de
IC_{10} terpinen-4-ol (P)	98 ± 1.24 j	78 ± 1.01 h	57 ± 0.68 f
IC_{20} terpinen-4-ol (P)	85 ± 1.56 hi	52 ± 0.40 ef	48 ± 0.46 e
IC_{30} terpinen-4-ol (P)	75 ± 1.02 gh	47 ± 0.22 e	46 ± 0.22 e
Control	90 ± 1.19 i	50 ± 0.22 e	30 ± 0.28 c

Results are means of 5 different experiments. Values that are followed by different letters within each column are significantly different ($P \leq 0.05$). $df_1 = 2$, $df_2 = 95$, F = 11.96, SE = standard error, P = parental cells, R = resistant cells.

Table 3. Membrane and DNA damaging effects of camphor, eugenol, eucalyptol, and terpinen-4-ol on parental and drug-resistant H1299 cells.

Concentrations	MDA (nmol/mg protein) X ± SE	8-OHdG (ng/mL) X ± SE
IC_{10} camphor (P)	0.58 ± 0.03 ab	0.08 ± 0.02 a
IC_{50} camphor (P)	1.30 ± 0.05 b	0.09 ± 0.02 a
IC_{70} camphor (P)	3.10 ± 0.08 d	0.13 ± 0.03 a
IC_{10} camphor (R)	0.30 ± 0.36 a	0.09 ± 0.03 a
IC_{50} camphor (R)	0.50 ± 0.04 a	0.10 ± 0.02 a
IC_{70} camphor (R)	1.14 ± 0.03 l b	0.17 ± 0.03 a
IC_{10} eugenol (P)	0.96 ± 0.02 ab	0.08 ± 0.02 a
IC_{50} eugenol (P)	1.10 ± 0.03 b	0.08 ± 0.03 a
IC_{70} eugenol (P)	1.78 ± 0.04 bc	0.10 ± 0.04 a
IC_{10} eugenol (R)	0.94 ± 0.02 ab	0.08 ± 0.02 a
IC_{50} eugenol (R)	1.60 ± 0.02 bc	0.09 ± 0.03 a
IC_{70} eugenol (R)	2.30 ± 0.09 c	0.14 ± 0.03 a
IC_{10} eucalyptol (P)	0.60 ± 0.02 ab	0.09 ± 0.02 a
IC_{50} eucalyptol (P)	0.70 ± 0.02 ab	0.12 ± 0.01 a
IC_{70} eucalyptol (P)	0.75 ± 0.01 ab	0.16 ± 0.03 a
IC_{10} eucalyptol (R)	0.90 ± 0.02 ab	0.08 ± 0.03 a
IC_{50} eucalyptol (R)	1.90 ± 0.02 bc	0.09 ± 0.02 a
IC_{70} eucalyptol (R)	2.80 ± 0.06 cd	0.11 ± 0.03 a
IC_{10} terpinen-4-ol (P)	0.47 ± 0.03 a	0.09 ± 0.02 a
IC_{50} terpinen-4-ol (P)	0.52 ± 0.01 a	0.11 ± 0.05 ab
IC_{70} terpinen-4-ol (P)	0.87 ± 0.02 ab	0.14 ± 0.06 ab
IC_{10} terpinen-4-ol (R)	0.36 ± 0.02 a	0.09 ± 0.01 a
IC_{50} terpinen-4-ol (R)	0.60 ± 0.03 ab	0.11 ± 0.01 ab
IC_{70} terpinen-4-ol (R	1.50 ± 0.04 bc	0.14 ± 0.03 ab
Control (P)	0.30 ± 0.02 a	0.08 ± 0.01 a
Control (R)	0.35 ± 0.03 a	0.08 ± 0.01 a
0.5% DMSO	0.33 ± 0.02 a	0.08 ± 0.01 a

Results are means of 5 different experiments. Values that are followed by different letters within each column are significantly different ($P \leq 0.05$). $df_1 = 2$, $df_2 = 95$, $F = 11.96$, SE = standard error, P = parental cells, R = resistant cells.

levels were increased in cells exposed to eugenol, eucalyptol, terpinen-4-ol, and camphor as a consequence of the membrane and DNA damaging effects of these test components on the cells, as showed in the results. In one study, eugenol was found to act as a very potent inhibitor of cell growth in the human HFF fibroblasts and human HepG2 (35). The cytotoxicity of eugenol is likely to be mediated by phenoxyl radicals and/or eugenol quinone methide (32). As a result, these molecules can cause lipid peroxidation in membrane and 8-OHdG formation in DNA. In another study, eugenol showed DNA-damaging effect in human VH10 fibroblasts and to a lower degree in Caco-2 colon cells, but not in metabolically active HepG2 hepatoma cells (34).

Recently, studies have shown that phenolic components have antioxidant/prooxidant properties at different concentrations. Eugenol, eucalyptol, terpinen-4-ol, and camphor significantly decreased membrane and DNA damage in H_2O_2-treated H1299 cells (Table 4). The selected protective concentrations are the concentrations that showed the highest cytoprotective effect against H_2O_2 cytotoxicity. Table 2 shows that camphor and eucalyptol at IC_{20} and IC_{30} and eugenol and terpinen-4-ol at IC_{10} and IC_{20} concentrations have the maximum cytoprotective effects against H_2O_2 cytotoxicity in parental cells, while camphor at IC_{10} and IC_{20} and the other test components at IC_{10} and IC_{30} show the maximum effects in resistant cells. At these cytoprotective concentrations, eugenol, eucalyptol,

Table 4. Protective effects of camphor, eugenol, eucalyptol, and terpinen-4-ol against membrane and DNA damaging effects of H_2O_2 on parental H1299 cells.

Concentrations	MDA (nmol/mg protein) X ± SE Parental cells	8-OHdG (ng/mL) X ± SE Parental cells
IC_{20} camphor + IC_{10} H_2O_2	0.81 ± 0.04 ab	0.20 ± 0.01 a
IC_{20} camphor + IC_{50} H_2O_2	0.89 ± 0.03 ab	1.27 ± 0.03 b
IC_{20} camphor + IC_{70} H_2O_2	0.98 ± 0.02 ab	2.30 ± 0.09 c
IC_{30} camphor + IC_{10} H_2O_2	0.88 ± 0.02 ab	0.25 ± 0.01 a
IC_{30} camphor + IC_{50} H_2O_2	0.97 ± 0.03 ab	1.46 ± 0.02 b
IC_{30} camphor + IC_{70} H_2O_2	1.56 ± 0.04 bc	2.50 ± 0.04 c
IC_{10} eugenol + IC_{10} H_2O_2	0.93 ± 0.03 ab	2.69 ± 0.04 cd
IC_{10} eugenol + IC_{50} H_2O_2	1.05 ± 0.05 b	8.90 ± 0.05 ij
IC_{10} eugenol + IC_{70} H_2O_2	1.47 ± 0.04 b	11.60 ± 0.03 lm
IC_{20} eugenol + IC_{10} H_2O_2	0.80 ± 0.03 ab	2.80 ± 0.04 cd
IC_{20} eugenol + IC_{50} H_2O_2	0.86 ± 0.04 ab	9.00 ± 0.09 ij
IC_{20} eugenol + IC_{70} H_2O_2	0.94 ± 0.05 ab	11.95 ± 0.06 lm
IC_{20} eucalyptol + IC_{10} H_2O_2	0.20 ± 0.01 a	0.28 ± 0.01 a
IC_{20} eucalyptol + IC_{50} H_2O_2	0.30 ± 0.02 a	1.45 ± 0.03 b
IC_{20} eucalyptol + IC_{70} H_2O_2	0.43 ± 0.03 a	2.43 ± 0.09 c
IC_{30} eucalyptol + IC_{50} H_2O_2	0.47 ± 0.04 a	0.20 ± 0.01 a
IC_{30} eucalyptol + IC_{50} H_2O_2	0.50 ± 0.04 a	1.28 ± 0.01 b
IC_{30} eucalyptol + IC_{70} H_2O_2	0.53 ± 0.04 ab	2.18 ± 0.08 c
IC_{10} terpinene-4-ol + IC_{10} H_2O_2	0.99 ± 0.06 ab	0.17 ± 0.02 a
IC_{10} terpinene-4-ol + IC_{50} H_2O_2	1.13 ± 0.09 b	1.21 ± 0.02 b
IC_{10} terpinene-4-ol + IC_{70} H_2O_2	1.20 ± 0.03 b	2.26 ± 0.02 c
IC_{20} terpinene-4-ol + IC_{10} H_2O_2	0.82 ± 0.03 ab	0.20 ± 0.01 a
IC_{20} terpinene-4-ol + IC_{50} H_2O_2	0.87 ± 0.05 ab	1.34 ± 0.02 b
IC_{20} terpinene-4-ol + IC_{70} H_2O_2	0.93 ± 0.04 ab	2.45 ± 0.03 c
IC_{10} H_2O_2 (control)	1.30 ± 0.03 b	2.62 ± 0.03 cd
IC_{50} H_2O_2 (control)	1.80 ± 0.04 bc	7.79 ± 0.07 hi
IC_{70} H_2O_2 (control P)	2.40 ± 0.07 c	11.97 ± 0.11 lm
Control	0.30 ± 0.01 a	0.10 ± 0.01 a
0.5% DMSO	0.31 ± 0.01 a	0.10 ± 0.01 a

Results are means of 5 different experiments. Values that are followed by different letters within each column are significantly different ($P \leq 0.05$). $df_1 = 2$, $df_2 = 95$, F = 11.96, SE = standard error, P = parental cells, R = resistant cells.

terpinen-4-ol, and camphor showed different membrane and DNA protective effects against H_2O_2 oxidation (Table 4 and 5). The most effective membrane protective effect was found with eucalyptol for both parental and drug-resistant cells. On the other hand, the highest DNA protective effect was observed with terpinen-4-ol for parental cells and camphor for drug-resistant cells. If we compare the protective effect of test components in parental and drug-resistant cells at the highest concentration (IC_{70}), drug-resistant cells showed a very strong resistance to H_2O_2-induced DNA damaging effect. This means that resistant cells have more DNA protective ability than parental cells.

In one study, neither DNA damaging nor DNA protective effect was observed following eucalyptol pretreatment of K562 cells (31). Epigallocatechin-3-gallate (1 μM), a polyphenol abundant in tea, was shown to significantly reduce MDA production after H_2O_2/Fe^{2+} exposure, indicating cell protection against oxidative stress (40). The MDA level increased in H_2O_2-exposed (IC_{50} and IC_{70}) hepatoma G2 cells, while this decreased when the cells were preincubated with carvacrol and thymol before H_2O_2 exposure (2).

Table 5. Protective effects of camphor, eugenol, eucalyptol, and terpinen-4-ol against membrane and DNA damaging effects of H_2O_2 on drug-resistant H1299 cells.

Concentrations	MDA (nmol/mg protein) X ± SE Drug-resistant cells	8-OHdG (ng/mL) X ± SE Drug-resistant cells
IC_{10} camphor + IC_{10} H_2O_2	0.93 ± 0.0 3 ab	0.23 ± 0.01 a
IC_{10} camphor + IC_{50} H_2O_2	1.04 ± 0.04 b	1.17 ± 0.01 b
IC_{10} camphor + IC_{70} H_2O_2	1.60 ± 0.02 bc	1.52 ± 0.01 bc
IC_{20} camphor + IC_{10} H_2O_2	0.89 ± 0.02 ab	0.33 ± 0.01 a
IC_{20} camphor + IC_{50} H_2O_2	0.94 ± 0.03 ab	1.37 ± 0.01 b
IC_{20} camphor + IC_{70} H_2O_2	1.02 ± 0.05 b	1.90 ± 0.02 bc
IC_{10} eugenol + IC_{10} H_2O_2	0.97 ± 0.06 ab	3.30 ± 0.03 d
IC_{10} eugenol + IC_{50} H_2O_2	1.40 ± 0.02 b	7.40 ± 0.05 h
IC_{10} eugenol + IC_{70} H_2O_2	1.90 ± 0.07 bc	10.41 ± 0.07 k
IC_{30} eugenol + IC_{10} H_2O_2	0.84 ± 0.03 ab	3.10 ± 0.02 d
IC_{30} eugenol + IC_{50} H_2O_2	0.95 ± 0.02 ab	7.09 ± 0.03 h
IC_{30} eugenol + IC_{70} H_2O_2	1.40 ± 0.02 b	9.87 ± 0.05 jk
IC_{10} eucalyptol + IC_{10} H_2O_2	0.37 ± 0.02 a	0.48 ± 0.01 a
IC_{10} eucalyptol l+ IC_{50} H_2O_2	0.39 ± 0.01 a	1.00 ± 0.09 ab
IC_{10} eucalyptol + IC_{70} H_2O_2	0.44 ± 0.02 a	1.30 ± 0.02 b
IC_{30} eucalyptol + IC_{50} H_2O_2	0.26 ± 0.01 a	0.55 ± 0.01 ab
IC_{30} eucalyptol + IC_{50} H_2O_2	0.30 ± 0.01 a	1.29 ± 0.01 b
IC_{30} eucalyptol + IC_{70} H_2O_2	0.41 ± 0.01 a	1.50 ± 0.02 b
IC_{10} terpinene-4-ol + IC_{10} H_2O_2	0.84 ± 0.02 ab	2.15 ± 0.04 c
IC_{10} terpinene-4-ol + IC_{50} H_2O_2	1.05 ± 0.01 b	5.52 ± 0.09 fg
IC_{10} terpinene-4-ol + IC_{70} H_2O_2	1.15 ± 0.04 b	9.51 ± 0.03 jk
IC_{30} terpinene-4-ol + IC_{10} H_2O_2	0.45 ± 0.03 a	0.30 ± 0.11 a
IC_{30} terpinene-4-ol + IC_{50} H_2O_2	0.54 ± 0.04 ab	1.35 ± 0.02 b
IC_{30} terpinene-4-ol + IC_{70} H_2O_2	1.15 ± 0.09 b	1.82 ± 0.04 bc
IC_{10} H_2O_2 (control)	1.20 ± 0.02 b	2.51 ± 0.04 cd
IC_{50} H_2O_2 (control)	1.60 ± 0.04 bc	6.65 ± 0.01 gh
IC_{70} H_2O_2 (control P)	2.10 ± 0.05 c	10.55 ± 0.09 kl
Control	0.32 ± 0.01 a	0.10 ± 0.01 a
0.5% DMSO	0.33 ± 0.01 a	0.11 ± 0.01 a

Results are means of 5 different experiments. Values that are followed by different letters within each column are significantly different ($P \leq 0.05$). $df_1 = 2$, $df_2 = 95$, F = 11.96, SE = standard error, P = parental cells, R = resistant cells.

In many studies, essential oil components had dose-dependent antiproliferative effects on cancer cells, which makes them potentially interesting for adjuvant experimental cancer treatments. Some of them induced membrane and DNA damage and cytotoxicity in cancer cells at relatively higher concentrations than those that mediate their anticancer properties. Our results are supported by all of the studies mentioned above.

Further understanding of the underlying mechanism of eugenol, eucalyptol, terpinen-4-ol, and camphor protection against H_2O_2 insult through reduction of intracellular oxygen radicals in H1299 cells may lead to the development of new therapeutic treatments for cancer. Their protective effects against H_2O_2 toxicity in lung cells might be of importance and may contribute in part to their clinical efficacy in the treatment of lung carcinoma. These results suggest that eugenol, eucalyptol, terpinen-4-ol, and camphor may potentially be a valuable source of natural therapeutic agents. Indeed, it is becoming increasingly evident that certain phytochemicals, particularly those included in our daily diet, have important chemopreventive properties. In the present study, eugenol, eucalyptol, terpinen-4-ol, and camphor induced DNA and membrane damage and cytotoxicity in H1299 cells at concentrations

higher than those beneficial for anticancer protection. These findings suggest that eugenol, eucalyptol, terpinen-4-ol, and camphor exhibit anticancer/antioxidant effects in a concentration- and time-dependent manner.

In our study, the protective or damaging effect of eugenol, eucalyptol, terpinen-4-ol, and camphor has been evaluated in vitro on parental and drug-resistant cells at different concentrations and treatment durations. These test components had cytotoxic effects at high concentrations and cytoprotective (antioxidant) effects against strong oxidant H_2O_2 cytotoxicity at lower concentrations on both parental and drug-resistant cells. The membrane and DNA damaging/protective capacity against H_2O_2 damaging also varied depending on eugenol, eucalyptol, terpinen-4-ol, and camphor concentrations in parental and drug-resistant H1299 cells.

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