

## Influence of Some Citrus Essential Oils on Cell Viability, Glutathione-S-Transferase and Lipid Peroxidation in *Ehrlich ascites Carcinoma Cells*

Amal A. Mohamed \*<sup>1</sup>, Gehan A. El-Emary<sup>2</sup>, Hanaa F. Ali<sup>3</sup>

<sup>1</sup>Plant Biochemistry Department, National Research Centre, Dokki, Cairo- Egypt

<sup>2</sup>Institute of Productive Efficiency, Zagazig University, Egypt

<sup>3</sup>Biochemistry Department, Faculty of Agriculture, Cairo University, Egypt

\*Corresponding author: amin\_amal@yahoo.com

**Abstract:** Essential oils are the volatile fraction of aromatic and medicinal plants after extraction by steam or water distillation. They have been used for their pharmaceutical potential since early times, and even now are still subject to a great deal of attention. In this study citrus essential oils isolated from mandarin (*C. reticulata*), orange (*C. aurantium*), lemon (*C. limon*), and tangerine (*C. aurantium*) species were analyzed by gas chromatography-mass spectrometry (GC-MS). Main constituents separated in mandarin oil were dl-limonene (20.88%), neo-dihydrocaveol (4.96%), and allo-ocimene (4.78%). In orange oil, the principal compounds were linalool (10.5%),  $\alpha$ -terpinolene (7.06%), and nonyl-aldehyde (4.79%). In lemon oil, camphene (19.31%),  $\alpha$ -citral (17.13%), citronellal (13.64%), and limonene (6.55%) were among the principal components. Major constituents presented in tangerine oil were limonene (14.08%), citronellal (9.56%), and  $\alpha$ -terpinene (4.68%). The chemical compositions of citrus essential oils were highly different which may be due to the difference in their genetic make up. The effect of different concentrations (25-150 $\mu$ l/ml) of citrus essential oils on the viability of *Ehrlich ascites* carcinoma cells (EACC) was tested *in vitro*. Generally, it was found that incubation of tumor cells with different concentrations of essential oils reduced the viability of these cells. The activity of glutathione-S-transferase (GST), glutathione content (GSH), and lipid peroxidation (LPO) were studied in EACC tumor cells treated by essential oils. The essential oils treatments increased the activities of GST, increased the cellular GSH level and inhibited lipid peroxidation. These findings support the hypothesis that citrus essential oils may possess significant antitumor and antioxidant effects on EACC cell lines. [Journal of American Science 2010;6(10):820-826]. (ISSN: 1545-1003).

**Keywords:** Essential oil; glutathione; GC/MS; limonene; lipid peroxidation

### 1. Introduction

Essential oils (EOs), also called volatile oils are aromatic oily liquids obtained from different plant parts and widely used as food flavors. The constituents of the essential oils are mainly monoterpenes and sesquiterpenes which are hydrocarbons with the general formula (C<sub>5</sub>H<sub>8</sub>)<sub>n</sub>. Oxygenated compounds derived from these hydrocarbons include alcohols, aldehydes, esters, ethers, ketones, phenols and oxides. Volatile oils have been reported to exhibit various antibacterial, antifungal, antiviral and antioxidant properties (Prabuseenivasan et al., 2006). Citrus is one of the most important commercial fruit crops grown in the world. The genus citrus includes various species of oranges, mandarins, limes, tangerines, lemons and grapefruit. In current citrus industry, citrus fruits are marketed fresh or as processed juices which provide multitude health benefits not only from vitamin-C but also from other compounds. These compounds include the bitter limonoids, carotenoids (especially  $\beta$ -carotene), flavonoids, folic acid and dietary fiber and

have been shown to prevent a variety of cancers and cardiovascular diseases. Nevertheless, citrus essential oils contain large amounts of terpenes, aliphatic sesquiterpene, oxygenated derivatives and aromatic hydrocarbons (Merle et al., 2004). The composition of the terpenic mix varies depending on the examined citrus species to which it owns. The mix of each species is in different proportion made of: limonene,  $\alpha$ -pinene,  $\beta$ -pinene, myrcene, linalool and terpinene. Monoterpenes are important constituents of citrus essential oil and other plants. A number of these monoterpenes have an antitumor activity. For example, d-limonene which comprises > 90% of the orange peel oil has chemopreventive activity against skin, liver, lung and forestomach cancers (Crowel, 1999) and has been reported to induce apoptosis on tumor cells (Hata et al., 2003). Similarly, perillyl alcohol, a hydroxylated limonene analog, exhibits chemopreventive activity against liver, mammary gland, pancreas and colon cancer in rodent (Reddy, 1997). It is presumed that a number of volatile oils could act as potential novel antiproliferative agents

(Dorman et al., 1995). A rapid enzyme assay for the screening of potential inhibitors of chemical carcinogenesis has been developed on the basis of induction of the detoxifying enzymes such as glutathione *S*-transferase (GST) and NAD(P)H quinone oxidoreductase (NQO1). Moreover; GST catalyzes a wide range of reactions involving the conjugation of glutathione (GSH) to electrophilic carcinogenic compounds to form less toxic conjugates for excretion. Cellular glutathione and related enzymes such as glutathione peroxidase and glutathione reductase are among the principal protective mechanisms against endogenous and exogenous toxic substances and free radical-mediated damage in liver tissue (Hayes and McLellan, 1999). Thus any compound that can induce an increase in the activity of these detoxifying enzymes may be considered as potential inhibitors or anticarcinogens (Lam et al., 1994). However, there is strong evidence that dietary supplementation with the citrus limonoids and limonin activates glutathione-*S*-transferase in the liver and small intestine of the rat (Lam and Hasegawa, 1989). From this viewpoint the present study was carried out to investigate the chemical composition of four citrus essential oils (mandarin, orange, lemon and tangerine) as well as the bioactivity of these oils as antitumor and antioxidant agents (GST activity, GSH level and the level of MDA) in order to evaluate their medicinal potential.

## 2. Material and Methods

### 2.1 Materials

Four citrus essential oils of mandarin (*C. reticulata*), orange (*C. aurantium*) lemon (*C. limon*), and tangerine (*C. aurantium*) was purchased from Katto Aromatic Co., Giza, Egypt (Producers of plant essential oils and aromatic substances); quality of the oils was ascertained to be more than 98% pure. These oils were stored at -20 °C for subsequent analysis.

### 2.2 Essential oil analysis

Samples of the essential oils (0.5 mg) were suspended in 1 ml of ethyl acetate (P.A., Merck, Germany) and 5 µl of this solution was analyzed by gas chromatography coupled with mass spectrometer (GC/MS, Hewlett- Packard GC-MS Model 5890 series II) equipped with a fused silica capillary column DB-5 (30 m x 0.25 mm x 0.25 µm). The electron impact technique (70 eV) was used and injector temperature was 240 °C and that of the detector was 230 °C. The carrier gas was helium at the working rate of 1.7 ml/min. The column temperature was initially 50 °C and then was gradually increased at the rate of 5 °C/min up to 180 °C and after that, the temperature was increased up to

240 °C at the rate of 8 °C. For detection of the oil components a flame ionization detector was used, set up at 230 °C. Components of the oils were identified by comparison of their mass spectra and retention indices with those published in the literature (Adams, 1995).

### 2.3 Viability of Ehrlich ascites carcinoma cells

Female Swiss albino mice, weighing 18-22 g, 8-10 week old were used. Animals were kept under environmental and nutritional conditions for 2 weeks then injected intraperitoneal by Ehrlich ascites carcinoma cells (EACC). The animals were used for tumor cell preparation (cell line).

### 2.4 Tumor cells (cell line)

A line of Ehrlich ascites carcinoma resistant to Endoxan has been used (El-Merzabani and Tawfik, 1976). The parent line was first supplied through the courtesy of Dr. G. Klein, Amsterdam, Holland. The tumor line is maintained in the National Cancer Institute, Cairo-Egypt, in female Swiss albino mice by weekly transplantation of 2 x 10<sup>6</sup> cells. The cells were taken from tumor transplanted animals after 7 days of transplantation. The cells were centrifuged at 1000 rpm for 5 min, washed with saline then the needed number of cells was prepared by suspending the cells in the appropriate volume of saline.

### 2.5 In vitro cytotoxicity

The viability percentage of tumor cells was measured by the modified cytotoxic trypan blue-exclusion method as described by Bennett et al., (1991). The culture medium used was prepared using RPMI 1640 media, 10% fetal bovine serum and L-glutamine (Gibco). Two ml of cells (containing 2x10<sup>6</sup> cells) were incubated over night, with the examined essential oils (essential oils as well as sterile saline solution as control) at five concentrations; 25, 50, 75, 100 and 150 µl/ml which transferred into a set of tubes. The tubes were incubated at 37 °C under 5% CO<sub>2</sub>. Thereafter, the tubes were centrifuged at 1000 rpm for 5 min and the separated cells were suspended in saline. For each examined extract and control, a new small test tube was used and 10 µl of cell suspension, 80 µl saline and 10 µl trypan blue were added and mixed. The number of living cells was calculated using a hemocytometer slide. Cells were counted in duplicates under light microscope at 100 x. Cells that showed signs of staining were considered to be dead, whereas those that excluded trypan blue were considered as viable.

Cell viability % = No of viable cells (unstained cells) / Total No of cells (stained and unstained) x 100

## 2.6 Determination of GST activity

GST activity was determined in treated (at 100 and 150  $\mu\text{l/ml}$  essential oils) and untreated tumor cells solution (2 ml containing  $2 \times 10^6$  cells) as described by Habig et al., (1974). Reaction mixture containing 50 mM phosphate buffer, pH 7.5, 1 mM of 1-chloro- 2, 4 dinitrobenzene (CDNB) and an appropriate volume of cell lines. The reaction was initiated by the addition of reduced glutathione (GSH) and formation of S-(2, 4-dinitro phenyl) glutathione (DNP-GS) was monitored as an increase in absorbance at 334 nm. The result was expressed as  $\mu\text{mol}$  of CDNB conjugation formed /mg protein /min.

## 2.7 Determination of GSH level

The level of total SH compound (GSH) was determined with Ellman's reagent according to Tukendorf and Rauser (1990). GSH was assayed by adding 2 ml of 0.5 Mm 5, 5' -dithio-bis -2-nitro benzoic acid, (DTNB) prepared in 0.2 M phosphate buffer, pH 8.0 to appropriate volume of treated cell lines solution. The GSH reacts with DTNB and forms a yellow colored complex with DTNB. The absorbance at 412 nm was read after 2 min ( $\Sigma=13.6 \text{ mM}^{-1}\text{cm}^{-1}$ ).

## 2.8 Lipid peroxidation (TBARS test)

The level of lipid peroxidation products was assayed through the formation of 2- thiobarbituric acid reactive substance (TBARS) according to Buege and Aust (1990), based on the reaction of malondialdehyde (MDA ) with thiobarbituric acid (TBA) at  $95^\circ\text{C}$ . The absorbance of the end product of lipid peroxidation (mainly malondialdehyde, MDA) was measured at 535 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The results were expressed as n mol MDA / mg protein.

## 2.9 Determination of total protein

Protein levels were determined spectrophotometrically at 595 nm, using comassie blue G 250 as a protein binding dye (Bradford, 1976). Bovine serum albumin (BSA) was used as a protein standard.

## Statistical analysis:

The Software COSTAT for Windows (version 10.0) was used for the statistical evaluation of the results. Statistical significance was determined by analysis of variance (ANOVA) and by the least significant differences (LSD) tests corrected for the number of comparisons. The probability level of 0.05 was used as the criterion for significance in all procedures according to Little and Hills (1992). All results were expressed as mean and standard deviation of the mean (SD).

## 3. Results and Discussions

### 3.1 Chemical composition of essential oils

The analytical results of mandarin and orange essential oils are shown in table (1), the principal components of the mandarin oil were dl-limonene (20.88%), neo-dihydrocaveol (4.96%), allo-cimene (4.78%), camphene (4.47%), and linalool (3.52 %), which represented (38.61 %) of the total mandarin oil. For the orange oil, the linalool was detected at a level of (10.5%) as a major compound. Other important compounds were  $\alpha$ -terpinolene (7.06%) and nonyl-aldehyde (4.79 %), carvone (4.52%) and citronellol (3.97 %) which was found as a major compound in orange oil. On the other hand, camphene (19.31 %) was the major percentage of the lemon oil followed by  $\alpha$ -citral (17.13%) as shown in table (2). The major compounds identified in tangerine oil, were limonene (14.08%), citronellal (9.56%),  $\alpha$ -terpinene (4.68%) and trace levels (below 0.1%) of  $\alpha$ -pinene (0.07%) and globulol (0.08%) respectively.

Table 1. Major components (in %) of mandarin and orange oils separated by Gas Chromato-graphy-Mass spectroscopy.

Mandarin oil			Orange oil		
Compound name	Rt	%	Compound name	Rt	%
Alpha-Pinene	3.38	3.27	Beta-Phellandrene	3.24	2.82
dl-Limonene	5.03	20.88	Myrcene	3.58	1.03
Allo-ocimene	6.34	4.78	Alpha-terpinolene	5.2	7.06
Neo-dihydrocaveol	6.5	4.96	Nonyl-aldehyde	7.12	4.79
Cis-Limonene oxide	9.59	2.19	Cis-Limonene oxide	7.73	0.79
Linalool	11.99	3.52	Decanal	8.73	0.76
Camphene	12.04	4.47	Linalool	9.01	10.5
Linalyl acetate	12.12	0.08	Verbenol	10.3	2.87
Farnesene	13.87	0.7	Carvone	12.2	4.52
Sabinene	22.16	1.06	Mentha-triene	12.9	0.11
Alpha-Farnesene	22.82	0.07	Perillaldehyde	13.1	1.28
Borneol	25.05	3.03	Cis-Carveol	14.1	0.46
Limonene glycol	25.84	2.29	Caprylic acid	17.0	0.15
Undecanoic acid	27.3	2.01	Cinnamic-aldehyde	17.1	3.81
Methyl-anthranilate	29.69	0.09	Farnesene	17.9	1.04
Benzaldehyde	30.18	0.49	Citronellol	19.8	3.97
Carvone	31.9	1.5	Heptadecanol	20.4	0.08
Unknowns	21.7	4.5	Unknowns	16.8	9.8

Rt; Retention time (min)

These results are in accordance with the previous findings regarding GC separation of essential oils from sweet orange, tangerine, bergamote, and grapefruit peel. (Gancel et al., 2002; Hognadottir and Russell, 2003; Khanum et al., 2004). They found terpenes and oxygenated compounds such as limonene,  $\gamma$ -terpinene,  $\beta$ -pinene,  $\alpha$ -pinene, myrcene, valencene, linalool, octanal, decanal, and butylbutyrate as the major constituents through GC separation Steam-distilled volatile peel oil of Indian orange when analyzed through GC and GC-MS, limonene was found more dominant followed by myrcene,  $\alpha$ -terpinolene and  $\beta$ -pinene (Kirbaslar and Kirbaslar, 2003). Almost similar results were reported by Feger et al., (2003); Tu et al., (2003) while working on orange and tangerine essential oils.

Table 2. Major components (in %) of lemon and tangerine oils (%) separated by Gas Chromatography-Mass spectroscopy.

Mandarin oil			Orange oil		
Compound name	Rt	%	Compound name	Rt	%
Alpha-Pinene	3.01	2.31	Alpha-Pinene	3.17	0.07
Alpha-Fenchene	3.27	1.17	Limonene	4.77	14.08
Cyclohexane	4.77	4.9	Citronellal	7.66	9.56
Limonene	6.14	6.55	Aloxiprin	8.31	0.11
Citronellal	9.41	13.64	Alpha-Terpinene	9.65	4.68
Cis-Carveol	9.9	0.41	Linalool	10.63	0.18
Alpha-Fenchene	12.13	4.87	Heptadiene	10.98	0.25
Camphene	13.62	19.31	Trans-menthadiene	12.05	2.76
Alpha-Citral	14.51	17.13	Tarns-Ocimene	13.32	1.72
Carvacol	16.88	0.95	Cis-Limonene oxide	14.23	1.95
Terpniol	15.22	1.2	Trans-Carveol	15.07	2.19
Thymol	20.83	1.54	Methyl-heptadiene	16.05	5.0
Carvacrol	21.21	0.23	Limonene dioxide	17.91	0.74
Heptanal	22.19	0.32	Perillyl alcohol	18.05	0.53
Citral	29.07	0.53	Cyclo-octanone	19.92	0.43
Dihydroisopimaric	30.88	0.65	Ledol	21.12	0.15
Dihydro-abitec	35.28	0.1	Trans-Decalone	22.26	1.8
Unknowns	19.9	13.13	Globulol	22.48	0.08
			Benzyl-dicarboxylic	22.93	0.94
			Unknowns	19.1	9.88

Rt; Retention time (min)

### 3.2 Antitumor activity of citrus oils

Antitumor activity of citrus essential oils was evaluated on the basis of "Trypan blue exclusion assay". The viability of Ehrlich ascites carcinoma cells (EACC) after incubation for 2 hr with different concentration of essential oils was evaluated and the obtained data are presented in table (3). Data showed that, the incubation of tumor cells with different concentration of essential oils affected the percent of cell viability. All concentration ranges showed decrease in cell viability as compared to that of control treatment. Among all tested oils, by mandarin and orange oils showed the best activity on the tumor cells at the concentration of 150  $\mu$ l/ml (the value of dead cells was 81 and 85% respectively). These both essential oils contained much dl-limonene (20.88%) and linalool (10.5%) respectively, and the same trend was found in case of lemon oil. However, samples treated by tangerine oil showed its best activity at the concentration of 100  $\mu$ l/ml (the value of dead cells was 62 %), this oil contained much limonene (14.08%) and citronellal (9.56%). In case of mandarin oil, the survival rate of the normal cells (control) was 3% of viable cells. The killing effect of citrus essential oils may be due to the role of camphore and limonene which were able to increase the cytoplasm membrane permeability. Also, probably because their capability of dissolving into the phospholipids bilayer aligning between the fatty acid chains and causing a distortion of the membrane physical structure in various tumor cell lines (Monajemi et al., 2005). Based on these finding it has been expected that essential oils with a higher percentage of limonene, show greater cytotoxicity. However, other mechanism for limonene action have been suggested, including the induction of carcinogen metabolizing enzymes, growth factor receptor expression, and inhibition of 3-hydroxy-3-methyl glutaryl CoA reductase (Poon et al., 1996). Additionally, d-limonene oxygenated derivatives, e.g. perillyl alcohol, carveol, carvone, geraniol and menthol, have shown biological activity *in vivo* against certain types of malignant tumors (Crowell, 1999). Hence, it can be concluded that these components may have great cytotoxic effects.

### 3.3 Effect of citrus oils on antioxidant compounds

The effect of essential oils at the selected concentration (100 and 150  $\mu$ l/ml) on the glutathione S-transferase (GST), glutathione content (GSH), and lipid peroxidation (LPO) in Ehrlich ascites carcinoma cells was given in table (4). Generally, activity of the anti-oxidative enzyme GST was enhanced by essential oils treatment compared to control group. Mandarin oil treatment at 150  $\mu$ l/ml

had a higher GST activity (the value was 3.2  $\mu\text{mol}/\text{mg protein}/\text{min}$ ) than in orange, lemon and tangerine oils (2.4, 1.99, and 2.1  $\mu\text{mol}/\text{mg protein}/\text{min}$ ) respectively. The increasing % of GST in EACC cells treated by 150  $\mu\text{l}/\text{ml}$  mandarin, orange,

lemon, and tangerine oils were 336.8, 252.6, 209.5 and 221.1 % respectively, (control values 100%).

### 3.2 Antitumor activity of citrus oils

Table 3. Effect of different concentrations of essential oils on the viability of Ehrlich ascites

Tumor cells + Essential oils ( $\mu\text{l}/\text{ml}$ )	Essential oils							
	Mandarin oil		Orange oil		Lemon oil		Tangerine oil	
	% of viable cells	% of dead cells						
25	71 $\pm$ 0.2	29 $\pm$ 0.1	75 $\pm$ 0.5	25 $\pm$ 0.3	82 $\pm$ 0.2	18 $\pm$ 0.1	80 $\pm$ 1.1	20 $\pm$ 0.5
50	55 $\pm$ 0.4	45 $\pm$ 0.7	66 $\pm$ 0.2	34 $\pm$ 0.5	78 $\pm$ 0.5	22 $\pm$ 1.2	73 $\pm$ 0.6	27 $\pm$ 1.2
75	38 $\pm$ 0.2	62 $\pm$ 0.4	52 $\pm$ 0.7	48 $\pm$ 0.7	71 $\pm$ 0.6	29 $\pm$ 0.6	59 $\pm$ 1.1	41 $\pm$ 0.6
100	30 $\pm$ 0.5	70 $\pm$ 1.2	34 $\pm$ 1.5	66 $\pm$ 0.1	62 $\pm$ 1.2	38 $\pm$ 0.6	38 $\pm$ 0.9	62 $\pm$ 0.4
150	19 $\pm$ 0.1	81 $\pm$ 1.0	15 $\pm$ 1.1	85 $\pm$ 1.7	53 $\pm$ 0.8	47 $\pm$ 0.2	45 $\pm$ 0.4	55 $\pm$ 0.3
Control	97 $\pm$ 0.8	3 $\pm$ 0.5	98 $\pm$ 0.9	2 $\pm$ 0.5	98 $\pm$ 0.9	2 $\pm$ 0.3	99 $\pm$ 1.0	1 $\pm$ 0.1

Control: Tumor cells + saline solution  $\pm$ SE refers to standard error (n=6)

Glutathione (GSH) content was higher in treated cells compared to control treatment. GSH content was gradually increased by increasing of oil concentration. At 150  $\mu\text{l}/\text{ml}$ , the level of GSH was increased by 242.8, 142.8, 133.3 and 152.4% of the control 100% in mandarin, orange, lemon, and tangerine respectively. However, GSH may play a protective role in scavenging of single oxygen, peroxides and hydroxyl radicals (Sander, 2003). The active ingredients of essential oils may disturb the metabolic behavior of tumor cells in special GSH/GSSG Redox System. This phenomenon was previously illustrated by Gupta et al., (2004) who studied the relationship between antitumor activity and antioxidant role in Ehrlich ascites. The increase in the GST activity in general, used as indication for the antitumor activity of the tested materials in both normal and tumor transplanted animals. Therefore, this enzyme has been used as antitumor factor (Oude-Ophuis et al., 1998). In the tumor cells, the increase of cellular enzymes that regulate the cell oxidative stress such as SOD and GST and antioxidants such as GSH induced cancer regression and stimulated large number of tumor necrosis factor- $\alpha$  (TNF). Tumor necrosis factor (TNF) is one of the most important growth modulatory cytokines produced by almost all cell types of the immune system. This factor is related to GSH level in cancer cells and the sensitivity of these cells to TNF depends on GSH content and their rate of proliferation (Obrador et al., 1997). ROS formed in cells tissues results in lipid peroxidation and subsequently to increase in malondialdehyde (MDA) level. Table (4) depicts the

levels of MDA in carcinoma cells; the levels were lower in treated cells when compared with untreated cells. After treatment by 150  $\mu\text{l}/\text{ml}$  of essential oils, the level of lipid peroxidation in mandarin, orange, lemon, and tangerine oils were reduced by 59.2, 42.8, 38.8 and 75.5 %, respectively in comparison to control treatment (100%). Several researchers have tried to correlate GSH levels with tumors. It has been reported, for instance, that many tumors can be considered GSH-dependent and it is suspected that cancer cells use GSH for protection against oxidative damage. Also, increased levels of GSH in tumors are related to anti-chemotherapeutic effects and multidrug resistance (Shimura et al., 2000). Lipid peroxide formed in the primary site would be transferred through the circulation and provoke damage by propagating the process of lipid peroxidation MDA, the end product of lipid peroxidation was reported to be higher in tumor tissue than in non diseased organs (Yagi, 1991).

### 4 Conclusions

In conclusion, major aroma compounds found in essential oils of citrus; in particular limonene, linalool,  $\alpha$ -terpinolene, carvone, citronellal and camphene; exhibit potent antitumor and antioxidant activities. Furthermore, ingestion of these aroma compounds may help to prevent in vivo oxidation damage such as lipid peroxidation, which is associated with cancer, premature aging and diabetes. To confirm this conclusion investigation of cytotoxic effects of highly pure compounds of citrus oils is suggested.

Table 4. The activity of glutathione S-transferase (GST), reduced glutathione (GSH) and lipid peroxidation (LPO) in Ehrlich ascites carcinoma cells treated with volatile oils.

Tumor cells + different con. of essential oils	Essential oils	Treatments (µl/ml)	GST*		GSH**		LPO***	
			Sp. activity	Rel. content	Level	Rel. content	MDA	Rel. content
Mandarin	100	100	2.2 <sup>d</sup> ±0.1	231.5	4.4 <sup>c</sup> ±0.05	209.5	3.4 <sup>d</sup> ±0.02	69.4
	150		3.2 <sup>e</sup> ±1.09	336.8	5.1 <sup>d</sup> ±0d.19	242.8	2.9 <sup>c</sup> ±0.03	59.2
Orange	100	100	2.0 <sup>d</sup> ±0.08	210.5	2.4 <sup>a</sup> ±0.07	114.3	2.8 <sup>bc</sup> ±0.04	57.1
	150		2.4 <sup>d</sup> ±0.18	252.6	3.0 <sup>b</sup> ±0.17	142.8	2.1 <sup>a</sup> ±0.08	42.8
Lemon	100	100	1.2 <sup>b</sup> ±0.04	126.3	2.3 <sup>a</sup> ±0.11	109.5	2.6 <sup>b</sup> ±0.02	59.2
	150		1.99 <sup>a</sup> ±0.24	209.5	2.8 <sup>b</sup> ±0.41	133.3	1.9 <sup>a</sup> ±0.06	38.8
Tangerine	100	100	1.6 <sup>c</sup> ±0.29	168.4	2.9 <sup>b</sup> ±0.01	138.1	4.1 <sup>f</sup> ±0.09	83.7
	150		2.1 <sup>a</sup> ±0.29	221.1	3.2 <sup>b</sup> ±0.21	152.4	3.7 <sup>e</sup> ±0.07	75.5
Control			0.95 <sup>a</sup> ±0.14	100	2.1a±0.3	100	4.9 <sup>g</sup> ±0.02	100
LSD 0.05			0.245		0.3437		0.217	

\*Specific activity of GST: µmol /mg protein /min.

\*\*GSH level: µmol /mg protein

\*\*\*LPO: n mol MDA /mg protein

Rel. content: relative content

±SE refers to standard error (n=6)

MDA: Malondialdehyde

Control: Tumor cells + saline solution

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