

## Research Article

# Rosmarinic Acid-Rich Extracts of Summer Savory (*Satureja hortensis* L.) Protect Jurkat T Cells against Oxidative Stress

Irakli Chkhikvishvili,<sup>1</sup> Tamar Sanikidze,<sup>1</sup> Nunu Gogia,<sup>1</sup> Tamar Mchedlishvili,<sup>1</sup> Maia Enukidze,<sup>1</sup> Marine Machavariani,<sup>1</sup> Yakov Vinokur,<sup>2</sup> and Victor Rodov<sup>2</sup>

<sup>1</sup> Institute of Medical Biotechnology, Tbilisi State Medical University, 33 Vazha Pshavela Ave. 0177, Tbilisi, Georgia

<sup>2</sup> Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, The Volcani Center, P.O. Box 6, 50250 Bet Dagan, Israel

Correspondence should be addressed to Victor Rodov; [vrodov@agri.gov.il](mailto:vrodov@agri.gov.il)

Received 5 July 2013; Revised 13 October 2013; Accepted 17 October 2013

Academic Editor: David Vauzour

Copyright © 2013 Irakli Chkhikvishvili et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Summer savory (*Satureja hortensis* L., *Lamiaceae*) is used in several regions of the world as a spice and folk medicine. Anti-inflammatory and cytoprotective effects of *S. hortensis* and of its rosmarinic acid-rich phenolic fraction have been demonstrated in animal trials. However, previous studies of rosmarinic acid in cell models have yielded controversial results. In this study, we investigated the effects of summer savory extracts on H<sub>2</sub>O<sub>2</sub>-challenged human lymphoblastoid Jurkat T cells. LC-MS analysis confirmed the presence of rosmarinic acid and flavonoids such as hesperidin and naringin in the phenolic fraction. Adding 25 or 50 μM of H<sub>2</sub>O<sub>2</sub> to the cell culture caused oxidative stress, manifested as generation of superoxide and peroxy radicals, reduced cell viability, G<sub>0</sub>/G<sub>1</sub> arrest, and enhanced apoptosis. This stress was significantly alleviated by the ethanolic and aqueous extracts of *S. hortensis* and by the partially purified rosmarinic acid fraction. The application of an aqueous *S. hortensis* extract doubled the activity of catalase and superoxide dismutase in the cells. The production of IL-2 and IL-10 interleukins was stimulated by H<sub>2</sub>O<sub>2</sub> and was further enhanced by the addition of the *S. hortensis* extract or rosmarinic acid fraction. The H<sub>2</sub>O<sub>2</sub>-challenged Jurkat cells may serve as a model for investigating cellular mechanisms of cytoprotective phytonutrient effects.

## 1. Introduction

Summer savory (*Satureja hortensis* L.) is an herb of the *Lamiaceae* family that is used in cooking and folk medicine in several regions of the world [1]. In Georgia, dried and ground summer savory (local name *kondari*) is one of the most popular spices, used either on its own or as an ingredient in spice blends. In addition, from ancient times, it has been known locally as an antimicrobial folk remedy for gastrointestinal problems [2]. Indigenous landraces of summer savory are cultivated in Georgia [3].

The leaves of summer savory are rich in phenolic compounds, particularly rosmarinic acid and flavonoids, which account for the high antioxidant capacity of these leaves [4, 5]. In our previous study of Georgian spices, we found

that *kondari* had one of the highest total phenolic content levels and one of the highest hydrophilic antioxidant capacity levels [6]. Rosmarinic ( $\alpha$ -O-caffeoyl-3,4-dihydroxy-phenyl lactic) acid was found to be the major compound in ethanolic extracts of summer savory and some other *Lamiaceae* herbs [4]. Rosmarinic acid is a phenylpropanoid derivative that is the second most common ester of caffeic acid in the plant kingdom.

Animal studies have revealed anti-inflammatory activity of *S. hortensis* extract and its polyphenolic fraction, in particular [7, 8]. This activity might be associated, at least partially, with rosmarinic acid, whose antiinflammatory and antiallergic properties have been demonstrated in animal and human trials [9, 10]. Osakabe et al. [10] suggested that the antiallergic effect of rosmarinic acid might be due to two independent

mechanisms: the scavenging of reactive oxygen species and the modulation of the inflammatory response. For example, the nephroprotective effect of rosmarinic acid was associated with improved antioxidant potency, including enhanced glutathione content and activity of antioxidant enzymes [11].

However, the cellular mechanisms by which rosmarinic acid exerts its anti-inflammatory effects are not fully understood and demand further investigation. The human lymphoblastoid T-cell Jurkat line, a constitutive producer of the potent T-cell growth factor interleukin 2 (IL-2), is a popular model for the study of immune signaling [12]. Jurkat cells can imitate both healthy and inflammatory T-cells in their response to oxidative metabolites, such as hydrogen peroxide [13]. Therefore, investigating the effect of *S. hortensis* extract on the proliferation and activity of T-cells may contribute to our understanding of the mechanism(s) of its anti-inflammatory and cytoprotective effects. Although  $H_2O_2$  plays an important role in antigen-dependent lymphocyte activation [14], excessive production of  $H_2O_2$  induces oxidative stress and impairs T-cell activity, leading to chronic inflammation and cell death.

The response of Jurkat cells to  $H_2O_2$  is dose-dependent. Reversible oxidative changes that can be repaired by cellular antioxidant system occur at a  $H_2O_2$  concentration of  $20 \mu M$ , and the first signs of apoptosis are noted at  $50 \mu M H_2O_2$  [15]. Relatively high bolus doses of  $H_2O_2$  ( $150 \mu M$ ) induce apoptosis in Jurkat cells, but the continuous presence of a lower concentration of  $H_2O_2$  ( $2 \mu M$ ) inhibits the apoptotic process [16]. Both apoptosis and necrosis were observed in the Jurkat cells exposed to  $100 \mu M H_2O_2$  [17], while necrosis was far more common at  $500 \mu M H_2O_2$  [18]. Despite its well-documented cytoprotective activity in animal trials, concentrations of up to  $150 \mu M$  of rosmarinic acid failed to prevent the  $H_2O_2$ -mediated apoptosis of Jurkat cells and showed no antioxidant properties [19]. Moreover, even in the absence of exogenous hydrogen peroxide, rosmarinic acid was reported to induce the apoptosis of Jurkat cells [19, 20].

The discrepancy between the prooxidant behavior of rosmarinic acid toward Jurkat cells that has been observed in previous studies and its well-known antioxidant and anti-inflammatory properties hamper the use of Jurkat cells as a model for investigating the mode of action of this phytonutrient. In the present work, we reexamined the effects of summer savory extracts and their rosmarinic acid-rich phenolic fraction on  $H_2O_2$ -challenged Jurkat cells.

## 2. Materials and Methods

**2.1. Plant Material.** Plants of a local Georgian landrace of *S. hortensis* were grown in an experimental plot near Tbilisi from seeds purchased from commercial supplier. The plants were harvested at their vegetative state (55 days after seed germination), the phenological stage characterized by the highest phenolic compound content, highest flavonoid content, and greatest antioxidant activity (I. Chkhikvishvili, unpublished data). The collected plant material was air-dried in the shade at  $25\text{--}30^\circ C$ . The dried matter was stored in a closed glass container in a cool, dry place.

**2.2. Extraction and Purification.** The dried plant material (1 g samples) was sequentially extracted with chloroform, ethyl acetate, and ethanol at a 1:5 w/v ratio of plant material to solvent; the duration of each extraction step was 24 h. The residue was extracted with water by steeping for 20 min at  $90^\circ C$  and subsequent gradual cooling down to room temperature. Direct application of this water extraction technique to the dried plant material produced a "total aqueous extract." The solvents were removed by evaporation under vacuum at a temperature below  $40^\circ C$ , and the extracts were stored at  $-80^\circ C$  until use. For the purification of the phenolic fraction, the total aqueous extract was percolated through a polyamide column. The column was washed with water and the purified fraction was eluted with 96% ethanol.

**2.3. Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis.** The samples of purified phenolic fraction were dissolved in HPLC-grade methanol and filtered through a Millex-HV Durapore (PVDF) membrane ( $0.22 \mu m$ ) before being injected into the LC-MS instrument. Mass spectral analyses were carried out using the Ultraperformance LC-Quadruple Time of Flight (UPLC-QTOF) instrument (Waters Premier QTOF, Milford, MA, USA), with the UPLC column connected online to a PDA detector (Waters Acquity), and then to an MS detector equipped with an electrospray ion (ESI) source (used in ESI-negative mode). Separation was performed on a  $2.1 \times 50 \text{ mm i.d.}, 1.7 \mu m$  UPLC BEH C18 column (Waters Acquity).

The chromatographic and MS parameters were as follows: the mobile phase consisted of 0.1% formic acid in water (phase A) and 0.1% formic acid in acetonitrile (phase B). The linear gradient program was as follows: 100% to 95% A over 0.1 min, 95% to 5% A over 9.7 min, held at 5% A over 3.2 min, and then returned to the initial conditions (95% A) in 4.2 min. The flow rate was  $0.3 \text{ mL min}^{-1}$  and the column was kept at  $35^\circ C$ . Masses of the eluted compounds were detected with a QTOF Premier MS instrument. The UPLC-MS runs were carried out at the following settings: capillary voltage of 2.8 kV, cone voltage of 30 eV, and collision energy of 5 eV. Argon was used as the collision gas. The  $m/z$  range was 70 to 1,000 D. The MS system was calibrated using sodium formate and Leu-enkephalin was used as the lock mass. The MassLynx software version 4.1 (Waters) was used to control the instrument and calculate accurate masses.

**2.4. Cell Culture and Experimental Design.** The human T-cell leukemia lymphoblastoid Jurkat cells (DSMZ ACC 282) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). The cells were grown in suspension culture at  $37^\circ C$  under 5% humidified  $CO_2$  in bioactive medium RPMI 1640 (Gibco, Grand Island, NY, USA) containing inactivated embryonic bovine serum (Sigma, St. Louis, MO, USA), L-glutamine ( $4 \text{ mM}$ ), penicillin ( $100 \text{ U mL}^{-1}$ ), and streptomycin ( $100 \text{ U mL}^{-1}$ ). The experiments were carried out at cell densities of  $0.3$  to  $0.6 \times 10^6 \text{ cells mL}^{-1}$ . In order to imitate the oxidative stress conditions,  $H_2O_2$  (Sigma) was added to the Jurkat culture to reach the concentrations of 25

and 50  $\mu\text{M}$ , corresponding to low and intermediate stress severity, respectively [15]. In the unstressed control treatment, water was added to the samples instead of  $\text{H}_2\text{O}_2$ . The *S. hortensis* extracts were added to the cultures at a rate of 2  $\text{mg mL}^{-1}$  as the  $\text{H}_2\text{O}_2$  was added.

In a separate trial, the effect of cell pretreatment with *S. hortensis* extract on their response to subsequent  $\text{H}_2\text{O}_2$  oxidative stress was investigated. Cell suspensions ( $2 \times 10^6$  cells  $\text{mL}^{-1}$ ) were incubated with *S. hortensis* rosmarinic acid fraction as described above. After the incubation period, the cells were harvested by centrifugation at 1500 g for 5 minutes, washed, resuspended in fresh medium, and exposed to  $\text{H}_2\text{O}_2$ . Cellular responses to oxidative stress were evaluated by free radicals generation and cell viability as described below.

**2.5. Hydrogen Peroxide Scavenging Capacity.** The ability of *S. hortensis* extracts to scavenge hydrogen peroxide in the absence of cells was tested in order to check possible contribution of this abiotic  $\text{H}_2\text{O}_2$  decomposition to experimental results. The  $\text{H}_2\text{O}_2$ -scavenging capacity of extracts was tested as described by Ruch et al. [21]. A solution of hydrogen peroxide 50  $\mu\text{M}$  was prepared in phosphate buffer (pH 7.4). Phenolic extracts (2  $\text{mg mL}^{-1}$ ) in distilled water and 50  $\mu\text{M}$  hydrogen peroxide solution were added to incubation system comprising bioactive medium RPMI 1640 (GIBSO) with inactivated embryonic bovine serum (Sigma), L-glutamine (4  $\text{mM}$ ), penicillin (100  $\text{U mL}^{-1}$ ), and streptomycin (100  $\text{U mL}^{-1}$ ). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the incubation medium with hydrogen peroxide. The percentage of hydrogen peroxide scavenging by *S. hortensis* extracts was calculated. The trial revealed a 17% reduction of  $\text{H}_2\text{O}_2$  concentration due to the interaction with *S. hortensis* rosmarinic acid fraction.

**2.6. Electron Paramagnetic Resonance (EPR) Spectroscopy.** The effect of *S. hortensis* extracts on the generation of free radicals in  $\text{H}_2\text{O}_2$ -challenged and unchallenged cells was studied using the electron paramagnetic resonance (EPR) method. EPR spectra were registered on a radiospectrometer, RE 1307 (EPSI, Chernogolovka, Russia). Peroxyl radicals were detected with spin-trap  $\alpha$ -phenyl-tertbutylnitron (PBN; Sigma) (50  $\text{mM}$  on  $0.6 \times 10^6$  cells in 0.5 mL medium) at room temperature at microwave power (20 mV). Superoxide radicals were detected with a spin-trap 5,5 dimethyl-1-pyrrolyn-IV-oxide (DMPO) (Sigma) (50  $\text{mM}$  on  $0.6 \times 10^6$  cells in 0.5 mL medium) at room temperature at microwave power (20 mV).

**2.7. Cell Viability and Proliferation.** The viability of the cells was determined using the MTT cell proliferation assay. Cell suspensions ( $2 \times 10^6$  cells  $\text{mL}^{-1}$ ) were incubated with  $\text{H}_2\text{O}_2$  and *S. hortensis* preparations as described above. After the incubation period, the cells were harvested by centrifugation at 1500 g for 5 minutes, washed, and resuspended

in fresh medium. The 8  $\text{mg mL}^{-1}$  solution of 3-(4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium bromide (MTT) (Sigma) in buffer (140  $\text{mM}$  NaCl, 5  $\text{mM}$  HEPES, pH 7.4) was added to the cell suspension at a rate of 30  $\mu\text{L}$  per 100  $\mu\text{L}$  suspension and the mixture was incubated for 4 h at 37°C in a 5%  $\text{CO}_2$  atmosphere. After this incubation, the supernatant was carefully removed and the colored formazan crystals produced from the MTT were dissolved in 100  $\mu\text{L}$  of dimethyl sulfoxide (DMSO). The absorption values of the solutions were measured at 570 nm. The distribution of the Jurkat cells among the different cell-cycle phases was studied using flow cytometry. Mitochondrial transmembrane potential ( $\Delta\Psi$ ) in the cell culture was determined by flow cytometry using the lipophilic cation test 3,3'-dihexyloxycarbocyanine iodide ( $\text{DiOC}_6$ ) described by Zamzami et al. [22].

**2.8. Antioxidant Enzymes.** Jurkat cell extract was prepared by centrifuging the cell suspensions at 500 g and then homogenizing the cellular precipitate in a lysis buffer (pH 7.9) that was comprised of 1.5  $\text{mM}$   $\text{MgCl}_2$ , 10  $\text{mM}$  KCl, 1  $\text{mM}$  dithiothreitol, 1  $\mu\text{g mL}^{-1}$  leupeptin, 1  $\mu\text{g mL}^{-1}$  aprotinin, and 10  $\text{mM}$  HEPES. The volume of the buffer was twice the volume of the precipitate. Lysis of the cells was performed by passing the suspension through a 27-gauge needle 10 times. The obtained homogenate was centrifuged for 20 min at 10,000 g. The supernatant was used to determine the levels of enzyme activity. Catalase (EC 1.11.1.6) activity was measured spectrophotometrically as the decomposition of  $\text{H}_2\text{O}_2$  at 240 nm [23]. One unit of catalase activity was defined as the amount of enzyme decomposing 1  $\mu\text{mol}$  of  $\text{H}_2\text{O}_2$  per minute. The superoxide dismutase (SOD; EC 1.15.1.1) was assayed using NADPH and phenazine methosulfate (PMS) reagents for the reduction of nitroblue tetrazolium salt (NBT) into blue-colored formazan measured spectrophotometrically at 560 nm [24]. One unit of SOD activity was defined as the amount of enzyme oxidizing 1 nmol NADPH per minute. The activity of both enzymes was expressed in terms of units per mg of protein. A total protein micro Lowry kit (Sigma) was used to determine the protein content.

**2.9. Interleukin Analysis.** Jurkat cells were prestimulated by incubation with 50  $\mu\text{g/mL}$  phytohemagglutinin (PHA) at 37°C for 5 min and cultured for 24 h with nonstimulated Jurkat cells (40% stimulated and 60% non-stimulated cells). The pro- and anti-inflammatory cytokines IL-2 and IL-10 were assayed using ELISA kits (Bender Medsystems, Vienna, Austria) and the Multiscan microplate reader (LabSystem, Helsinki, Finland).

**2.10. Statistics.** The trials were performed in five replications. The statistical analysis of the obtained results, including calculation of means and standard deviations, was conducted using the IBM SPSS Statistics program. The statistical significance of the differences between the means was analyzed by pair-wise comparison of treatment results with nontreated control using Student's *t*-test at  $P < 0.05$ .

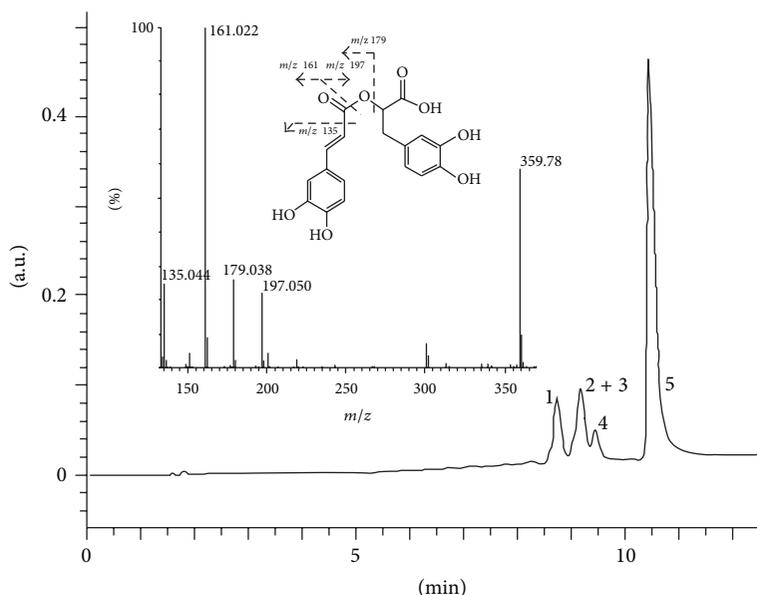


FIGURE 1: HPLC chromatogram of the *S. hortensis* rosmarinic acid fraction. The peak 5 represents rosmarinic acid and the peak 2 + 3 partially separated naringin and hesperidin. The peaks 1 and 4 were tentatively identified as rutin and apigenin-7-glucoside, respectively. Insert: mass spectrum of the rosmarinic acid and its fragmentation scheme.

### 3. Results

**3.1. Analysis of *S. hortensis* Extracts.** HPLC analysis revealed a number of phenolic compounds in the ethanolic extract of *S. hortensis*, rosmarinic and ferulic acids being the major compounds. In addition, a number of phenolic acids (caffeic, *p*-coumaric), flavonoid aglycones (catechin, epicatechin, luteolin, apigenin), and glycosides (rutin, hesperidin, apigenin-7-glucoside) were tentatively identified in the ethanolic extract. Partial purification of the rosmarinic acid provided a fraction comprising four major peaks. The tentative identification of the rosmarinic acid as the most abundant component of the fraction was based on its UV absorption spectrum and retention time as compared with those of the authentic standard sample. The identity was confirmed by LC-MS based on the presence of a deprotonated molecular ion  $[M-H]^-$  at  $m/z$  359 and characteristic fragment ions at  $m/z$  123,  $m/z$  135,  $m/z$  161,  $m/z$  179, and  $m/z$  197, in accordance with data in the literature [25, 26] and fragmentation scheme (Figure 1). Two flavonoid glycosides were identified by LC-MS through comparisons with standard samples as hesperidin based on a  $[M-H]^-$  at  $m/z$  609, a characteristic hesperetin fragment ion at  $m/z$  301, naringin based on  $[M-H]^-$  at  $m/z$  579, and a characteristic naringenin fragment ion at  $m/z$  271. In addition, two more flavonoid glycosides were tentatively identified in the fraction as rutin and apigenin-7-glucoside.

***H<sub>2</sub>O<sub>2</sub>-Induced Oxidative Stress as Affected by *S. hortensis* Extracts.*** The addition of 25 or 50  $\mu$ M of hydrogen peroxide caused oxidative stress in the Jurkat cells, which was manifested as the generation of superoxide and peroxy radicals that could be detected by EPR spectroscopy. The amount of

radicals formed depended on the concentration of  $H_2O_2$ ; no radicals were detected in the absence of hydrogen peroxide (Table 1).

Chloroform and ethyl acetate extracts of *S. hortensis* had only limited effects on the oxidative state of the cells, slightly reducing the amount of radicals detected at higher hydrogen peroxide concentrations. On the other hand, considerable alleviation of the oxidative stress and almost complete elimination of the radicals were observed in the presence of the ethanolic *E. hortensis* extract. Significant antioxidant effects were also associated with the aqueous extract and with the partially purified rosmarinic acid fraction, although the efficacy of the latter preparation was markedly lower than that of the crude ethanolic extract. In line with these findings, the total aqueous extract of *S. hortensis* doubled the activity of the antioxidant enzymes catalase and superoxide dismutase in the Jurkat cells, even in the absence of exogenous hydrogen peroxide (Figure 2).

**3.2. Effects on Jurkat Cell Viability.** In the absence of any exogenous  $H_2O_2$  challenge, adding ethanolic *S. hortensis* extract or the purified phenolic fraction to Jurkat cells slightly improved their viability. Other *S. hortensis* extracts had no significant effects on the viability of unstressed Jurkat cells, as measured by the MTT test (Table 2).

Hydrogen peroxide-induced oxidative stress reduced the viability of Jurkat cells in a dose-dependent manner. This hydrogen peroxide effect was alleviated by the application of ethanolic and aqueous extracts of *S. hortensis* and by the phenolic fraction. The aqueous *S. hortensis* extract was the most effective for restoring cell viability to the level observed in the unstressed control culture (Table 2).

TABLE 1: Effects of *S. hortensis* extracts and of the partially purified rosmarinic-acid fraction on the generation of superoxide ( $O_2^-$ ) and peroxy ( $LOO^*$ ) radicals in Jurkat cells subjected to hydrogen peroxide-induced oxidative stress.

	Hydrogen peroxide concentration, $\mu M$					
	0		25		50	
	$O_2^-$	$LOO^*$	$O_2^-$	$LOO^*$	$O_2^-$	$LOO^*$
No additives (control)	0	0	$2.4 \pm 0.2$	$3.0 \pm 0.2$	$3.1 \pm 0.1$	$3.8 \pm 0.3$
Chloroform extract	0	0	$2.0 \pm 0.2$	$3.2 \pm 0.2$	$2.1 \pm 0.2^*$	$2.8 \pm 0.3^*$
Ethyl acetate extract	0	0	$2.0 \pm 0.2$	$3.2 \pm 0.2$	$2.1 \pm 0.2^*$	$2.8 \pm 0.3^*$
Ethanol extract	0	0	0*	0*	$0.1 \pm 0.1^*$	0*
Aqueous extract	0	0	0*	$0.3 \pm 0.1^*$	$0.1 \pm 0.1^*$	$0.5 \pm 0.1^*$
Rosmarinic acid fraction	0	0	$1.0 \pm 0.2^*$	$2.2 \pm 0.2^*$	$1.1 \pm 0.2^*$	$1.8 \pm 0.3^*$

Values represent averages of five replications  $\pm$  standard deviations. Values marked with the asterisk are significantly different from the control in the same column at  $P \leq 0.05$ , according to Student's *t*-test.

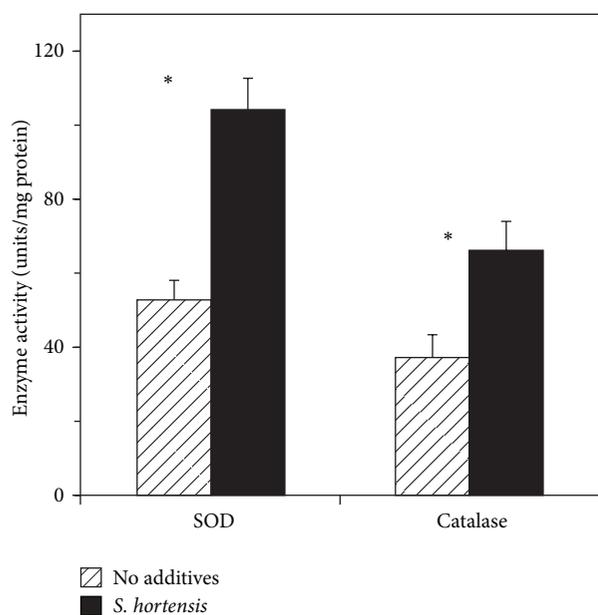


FIGURE 2: Effect of the total aqueous *S. hortensis* extract on the activities of superoxide dismutase (SOD) and catalase in Jurkat cells. Error bars represent standard deviations of five replications. Bars marked with an asterisk are significantly different from the control at  $P \leq 0.05$ , according to Student's *t*-test.

3.3. *Effect of Pretreatment of Jurkat Cells with S. hortensis Extract on Subsequent Cellular Sensitivity to Oxidative Stress.* The data presented in Table 3 demonstrate that pretreatment of Jurkat cells with the rosmarinic acid fraction significantly alleviated the oxidative stress incurred to cells by subsequent exposure to hydrogen peroxide, as expressed by free radical generation and decline in cell viability. This alleviation could not be attributed to the peroxide-scavenging activity of the extracts because no direct contact of the extracts with the peroxide took place in that case. In addition, the direct peroxide-scavenging capacity of the rosmarinic acid fraction did not exceed 17%, so that its contribution to the cell protection was rather limited.

3.4. *Effects on the Cell Cycle.* Oxidative stress changed the cell-cycle phase distribution of the Jurkat cells, restricting cell proliferation and increasing the relative proportions of G0/G1 cells (the G0/G1 arrest) and apoptotic cells among the total cell population. These trends were alleviated by the addition of the ethanolic *S. hortensis* extract, so that the amount of apoptotic cells in that treatment was not significantly different from that observed in the unstressed control (Table 4). Adding the *S. hortensis* extract alone, without hydrogen peroxide, had no significant effect on the cell-cycle phase distribution of the Jurkat cells (data not shown). The alleviation of  $H_2O_2$ -induced apoptosis by the ethanolic *S. hortensis* extract and by the partially purified rosmarinic acid fraction was also evident from the index of mitochondrial transmembrane potential determined by flow cytometry (Table 5).

3.5. *Interleukin Production.* The production of both IL-2 and IL-10 interleukins by Jurkat cells was stimulated by hydrogen peroxide and further enhanced by the addition of the *S. hortensis* extract and its phenolic fraction (Table 6).

## 4. Discussion

Our study has confirmed that rosmarinic acid is an abundant phenylpropanoid compound in summer savory. To the best of our knowledge, hesperidin and naringin have not been previously reported in *S. hortensis*, but they have been found in other *Satureja* species [27] and in other genera of this family, such as *Mentha* [25].

The present research has demonstrated for the first time that *S. hortensis* and its rosmarinic acid-rich fraction can protect Jurkat cells from oxidative stress caused by hydrogen peroxide. These findings are in line with the antioxidant, cytoprotective, and anti-inflammatory activities of *S. hortensis* [7] and rosmarinic acid [9, 10] that have been observed *in vivo* in animals and humans. Similar protective antioxidant properties were exhibited by *S. hortensis* extracts when applied to  $H_2O_2$ -stressed lymphocytes isolated from blood taken from healthy rats [28]. In cell cultures, rosmarinic acid protected

TABLE 2: Effects of *S. hortensis* extracts on the viability of Jurkat cells in the presence or absence of hydrogen peroxide.

	Hydrogen peroxide concentration, $\mu\text{M}$		
	0	25	50
	MTT test results, $A_{570}$		
No additives (control)	0.69 $\pm$ 0.02	0.36 $\pm$ 0.01	0.22 $\pm$ 0.01
Chloroform extract	0.63 $\pm$ 0.02	0.37 $\pm$ 0.01	0.24 $\pm$ 0.03
Ethyl acetate extract	0.58 $\pm$ 0.05	0.47 $\pm$ 0.03	0.42 $\pm$ 0.03*
Ethanol extract	0.74 $\pm$ 0.03*	0.56 $\pm$ 0.05*	0.46 $\pm$ 0.03*
Aqueous extract	0.61 $\pm$ 0.01	0.68 $\pm$ 0.04*	0.67 $\pm$ 0.02*
Rosmarinic acid fraction	0.75 $\pm$ 0.04*	0.62 $\pm$ 0.03*	0.42 $\pm$ 0.04*

Values represent averages of five replications  $\pm$  standard deviations. Values marked with the asterisk are significantly different from the control in the same column at  $P \leq 0.05$ , according to Student's *t*-test.

TABLE 3: Effect of pretreatment of the Jurkat cells with partially purified *S. hortensis* rosmarinic acid fraction on the cellular response to subsequent hydrogen peroxide-induced oxidative stress.

	Hydrogen peroxide concentration, $\mu\text{M}$		
	0	25	50
	Peroxy radicals generation, EPR signal intensity (arbitrary units)		
Nontreated control	0	3.0 $\pm$ 0.2	3.8 $\pm$ 0.3
Rosmarinic acid fraction	0	1.9 $\pm$ 0.2*	2.1 $\pm$ 0.3*
	Cell viability (MTT test results, $A_{570}$ )		
Nontreated control	0.69 $\pm$ 0.02	0.36 $\pm$ 0.01	0.22 $\pm$ 0.01
Rosmarinic acid fraction	0.75 $\pm$ 0.04*	0.59 $\pm$ 0.04*	0.37 $\pm$ 0.03*

Values represent averages of five replications  $\pm$  standard deviations. Values marked with the asterisk are significantly different from the control in the same column at  $P \leq 0.05$ , according to Student's *t*-test.

TABLE 4: Effects of hydrogen peroxide and of the ethanolic *S. hortensis* extract on the cell-cycle phase distribution of Jurkat cells.

	Cell-cycle phases, %			
	G0/G1	S	G2/M	G0/Apoptosis
No additives (control)	23.8 $\pm$ 3.4	54.5 $\pm$ 3.3	19.0 $\pm$ 2.9	2.7 $\pm$ 3.6
$\text{H}_2\text{O}_2$ 25 $\mu\text{M}$	42.3 $\pm$ 3.3*	36.7 $\pm$ 3.4*	12.5 $\pm$ 1.7*	8.5 $\pm$ 1.9*
$\text{H}_2\text{O}_2$ 25 $\mu\text{M}$ + <i>S. hortensis</i> (ethanolic extract)	37.5 $\pm$ 2.5*	43.0 $\pm$ 3.3*	16.0 $\pm$ 3.4	3.5 $\pm$ 1.3

Values represent averages of five replications  $\pm$  standard deviations. Values marked with the asterisk are significantly different from the control in the same column at  $P \leq 0.05$ , according to Student's *t*-test.

human neuronal cells against hydrogen peroxide-induced apoptosis [29] and inhibited in a dose-dependent manner the formation of reactive oxygen and nitrogen species in RAW264.7 macrophages stimulated with lipopolysaccharide or phorbol 12-myristate 13-acetate [30].

On the other hand, in a previous study, rosmarinic acid failed to protect Jurkat cells from  $\text{H}_2\text{O}_2$ -mediated oxidative damage and actually induced their apoptosis [19, 20]. Such prooxidant cytotoxic reactions in cell cultures are associated with the generation of  $\text{H}_2\text{O}_2$  through the interaction of phenolic compounds with culture media ingredients (e.g., transient metals) and can, therefore, be considered artifacts [31, 32]. Inclusion of catalase or metmyoglobin in the growth medium negates these reactions and allows the realization of the cytoprotective antioxidant potential of phenolic compounds [31].

One possible explanation for the apparent discrepancy between our results and those of Kolettas et al. [19] might be that the high dose of antioxidant materials used in our study could overcome the influence of  $\text{H}_2\text{O}_2$ , either added exogenously or generated in cell cultures with participation of transient metals. Indeed, in a metal-catalyst system, most phenolic compounds exhibited pro-oxidant effects at low doses and shifted to antioxidant activity at higher concentrations [33]. Furthermore, it was shown recently that high doses (2–3 mM) of caffeic acid and other phenylpropanoids protected Jurkat cells from  $\text{H}_2\text{O}_2$ -induced DNA damage by chelating intracellular labile iron [34]. The presence of the potent flavonoid antioxidants in the phenolic fraction, in addition to rosmarinic acid, might further strengthen its antioxidant capacity. Enhancement of the activity of the antioxidant enzymes by *S. hortensis* (Figure 2) might

TABLE 5: Effects of ethanolic *S. hortensis* extract and of the partially purified rosmarinic-acid fraction on the incidence of apoptosis in Jurkat cells in the presence of hydrogen peroxide.

	Cell counts		K ratio*
	Healthy	Apoptotic	
No additives (control)	212	8	26.5
H <sub>2</sub> O <sub>2</sub> 25 μM	268	3519	0.08
H <sub>2</sub> O <sub>2</sub> 25 μM + <i>S. hortensis</i> (ethanolic extract)	2090	539	3.9
H <sub>2</sub> O <sub>2</sub> 25 μM + rosmarinic acid fraction	1211	108	11.2

\*K-ratio of healthy to apoptotic Jurkat cells.

TABLE 6: Effects of the ethanolic *S. hortensis* extract and of the partially purified rosmarinic-acid fraction on the production of interleukins by Jurkat cells in the presence of hydrogen peroxide.

	IL-2, pg mL <sup>-1</sup>	IL-10, pg mL <sup>-1</sup>	IL-2/IL-10
No additives (control)	0.90 ± 0.05	3.21 ± 0.04	0.28 ± 0.04
H <sub>2</sub> O <sub>2</sub> 25 μM	2.61 ± 0.04*	6.80 ± 0.05*	0.38 ± 0.01
H <sub>2</sub> O <sub>2</sub> 25 μM + <i>S. hortensis</i> (ethanolic extract)	15.30 ± 0.04*	20.01 ± 0.08*	0.76 ± 0.07
H <sub>2</sub> O <sub>2</sub> 25 μM + <i>S. hortensis</i> rosmarinic acid fraction	20.80 ± 0.07*	38.40 ± 0.06*	0.54 ± 0.07

Values represent averages of five replications ± standard deviations. Values marked with the asterisk are significantly different from the control in the same column at  $P \leq 0.05$ , according to Student's *t*-test.

also contribute to the neutralization of hydrogen peroxide. Catalase and SOD play important roles in the control of oxidative stress and apoptosis in Jurkat cells [35]. Similar to our findings, an aqueous extract of another rosmarinic acid-containing *Lamiaceae* herb, *Perilla frutescens*, was shown to upregulate the mRNA and protein expression of these antioxidant enzymes in cultured human vein endothelial cells [36].

Another noteworthy phenomenon observed in this work was a parallel increase in the levels of the IL-2 and IL-10 interleukins. Robust production of IL-2 is the major trait of the Jurkat cell line [12]. There is a synergistic interaction between these two interleukins during the immune response [37]. Anti-inflammatory factors such as IL-10 may be released in order to balance the dramatic increase in proinflammatory cytokines in stressful situations, and thereby control the magnitude and duration of the inflammatory response [38]. Interestingly, adding antioxidant-rich plant materials to the diets of animals enduring proinflammatory conditions has been shown to increase the level of IL-10 [39] or the levels of both IL-2 and IL-10 [40] in parallel with a decrease in the levels of pro-inflammatory factors, such as IL-6, TNF- $\alpha$ , and IL-1 $\beta$ . In addition, these dietary interventions preserved

normal antioxidant enzyme activity, inhibited lipid peroxidation, and increased the HDL levels in the treated animals, resulting in the alleviation of disorders and enhanced immunity. Rosmarinic acid increased the secretion of IL-10 in a lipopolysaccharide-stimulated macrophage model [41].

Addition of the *S. hortensis* extract or its phenolic fraction restored the viability and proliferation of H<sub>2</sub>O<sub>2</sub>-challenged Jurkat cells, alleviated the G0/G1 arrest, and controlled the apoptosis of these cells. Altogether, these phenomena were in line with the general scheme of cellular response to oxidative stress, implying that low doses of reactive oxygen species promote cell proliferation, intermediate doses result in growth arrest, and severe oxidative stress ultimately causes cell death via apoptotic or necrotic mechanisms [42]. Apparently, the addition of *S. hortensis* extracts alleviated the oxidative stress exerted on the cells by hydrogen peroxide. These effects may be attributed to the direct radical-scavenging activity of rosmarinic acid and other phenolic compounds, as well as to indirect mechanisms such as the enhancement of antioxidant enzymes and the release of anti-inflammatory signaling molecules, such as IL-10.

## 5. Conclusions

The present research has demonstrated that rosmarinic acid-rich extract of *S. hortensis* can protect Jurkat cells from oxidative stress caused by hydrogen peroxide. These findings are in line with the antioxidant, cytoprotective, and anti-inflammatory activities of rosmarinic acid that have been observed in animals and humans. Therefore, the H<sub>2</sub>O<sub>2</sub>-challenged Jurkat cells may serve a model for investigating cellular mechanisms of cytoprotective effects of phytonutrients. It should be kept in mind, however, that these results were achieved with a rather high concentration of rosmarinic acid that supposedly could overcome the culture-associated artifacts. Further research is needed, in order to optimize the experimental system.

## Acknowledgments

The authors are grateful to Dr. Mira Weissberg, Institute of Plant Sciences, The Volcani Center, ARO, for performing the LC-MS analysis. The Israeli partners acknowledge the financial support by the Center of Absorption in Science, Ministry of Immigration Absorption, Israel (KAMEA Program).

## References

- [1] S. Momtaz and M. Abdollahi, "An update on pharmacology of *Satureja* species; from antioxidant, antimicrobial, antidiabetes and anti-hyperlipidemic to reproductive stimulation," *International Journal of Pharmacology*, vol. 6, no. 4, pp. 454–461, 2010.
- [2] N. Gelovani, T. Tsintsadze, Kh. Tzikarishvili, and L. Targamadze L, "Savory (*Satureja hortensis* L.) in the Georgian historical chronicles," *Transactions of Georgian Technical University*, vol. 483, no. 1, pp. 59–63, 2012.
- [3] M. Akhalkatsi, J. Ekhvaia, and Z. Asanidze, "Diversity and genetic erosion of ancient crops and wild relatives of agricultural cultivars for food: implications for nature conservation in

- Georgia (Caucasus),” in *Perspectives on Nature Conservation—Patterns, Pressures and Prospects*, J. Tiefenbacher, Ed., chapter 3, pp. 51–92, InTech, New York, NY, USA, 2012.
- [4] V. Exarchou, N. Nenadis, M. Tsimidou, I. P. Gerothanassis, A. Troganis, and D. Boskou, “Antioxidant activities and phenolic composition of extracts from Greek oregano, Greek sage, and summer savory,” *Journal of Agricultural and Food Chemistry*, vol. 50, no. 19, pp. 5294–5299, 2002.
  - [5] É. P. Kemertelidze, T. G. Sagareishvili, V. N. Syrov, and Z. A. Khushbaktova, “Chemical composition and pharmacological activity of garden savory (*Satureja hortensis* L.) occurring in Georgia,” *Pharmaceutical Chemistry Journal*, vol. 38, no. 6, pp. 319–322, 2004.
  - [6] V. Rodov, Y. Vinokur, N. Gogia, and I. Chkhikvishvili, “Hydrophilic and lipophilic antioxidant capacities of Georgian spices for meat and their possible health implications,” *Georgian Medical News*, no. 179, pp. 61–66, 2010.
  - [7] V. Hajhashemi, A. Ghannadi, and S. K. Pezeshkian, “Antinociceptive and anti-inflammatory effects of *Satureja hortensis* L. extracts and essential oil,” *Journal of Ethnopharmacology*, vol. 82, no. 2–3, pp. 83–87, 2002.
  - [8] C. Uslu, R. M. Karasen, F. Sahin, S. Taysi, and F. Akcay, “Effects of aqueous extracts of *Satureja hortensis* L. on rhinosinusitis treatment in rabbit,” *Journal of Ethnopharmacology*, vol. 88, no. 2–3, pp. 225–228, 2003.
  - [9] C. Sanbongi, H. Takano, N. Osakabe et al., “Rosmarinic acid inhibits lung injury induced by diesel exhaust particles,” *Free Radical Biology and Medicine*, vol. 34, no. 8, pp. 1060–1069, 2003.
  - [10] N. Osakabe, H. Takano, C. Sanbongi et al., “Anti-inflammatory and anti-allergic effect of rosmarinic acid (RA): inhibition of seasonal allergic rhinoconjunctivitis (SAR) and its mechanism,” *BioFactors*, vol. 21, no. 1–4, pp. 127–131, 2004.
  - [11] M. Tavafi and H. Ahmadvand, “Effect of rosmarinic acid on inhibition of gentamicin induced nephrotoxicity in rats,” *Tissue and Cell*, vol. 43, no. 6, pp. 392–397, 2011.
  - [12] R. T. Abraham and A. Weiss, “Jurkat T cells and development of the T-cell receptor signalling paradigm,” *Nature Reviews Immunology*, vol. 4, no. 4, pp. 301–308, 2004.
  - [13] G. Nindl, N. R. Peterson, E. F. Hughes, L. R. Waite, and M. T. Johnson, “Effect of hydrogen peroxide on proliferation, apoptosis and interleukin-2 production of Jurkat T cells,” *Biomedical Sciences Instrumentation*, vol. 40, pp. 123–128, 2004.
  - [14] M. Reth, “Hydrogen peroxide as second messenger in lymphocyte activation,” *Nature Immunology*, vol. 3, no. 12, pp. 1129–1134, 2002.
  - [15] J. W. Baty, M. B. Hampton, and C. C. Winterbourn, “Proteomic detection of hydrogen peroxide-sensitive thiol proteins in Jurkat cells,” *Biochemical Journal*, vol. 389, pp. 785–795, 2005.
  - [16] A. Barbouti, P.-T. Doulias, L. Nouis, M. Tenopoulou, and D. Galaris, “DNA damage and apoptosis in hydrogen peroxide-exposed Jurkat cells: Bolus addition versus continuous generation of H<sub>2</sub>O<sub>2</sub>,” *Free Radical Biology and Medicine*, vol. 33, no. 5, pp. 691–702, 2002.
  - [17] R. Chiaramonte, E. Bartolini, P. Riso et al., “Oxidative stress signalling in the apoptosis of Jurkat T-lymphocytes,” *Journal of Cellular Biochemistry*, vol. 82, no. 3, pp. 437–444, 2001.
  - [18] Y. Saito, K. Nishio, Y. Ogawa et al., “Turning point in apoptosis/necrosis induced by hydrogen peroxide,” *Free Radical Research*, vol. 40, no. 6, pp. 619–630, 2006.
  - [19] E. Kolettas, C. Thomas, E. Leneti et al., “Rosmarinic acid failed to suppress hydrogen peroxide-mediated apoptosis but induced apoptosis of Jurkat cells which was suppressed by Bcl-2,” *Molecular and Cellular Biochemistry*, vol. 285, no. 1–2, pp. 111–120, 2006.
  - [20] Y.-G. Hur, Y. Yun, and J. Won, “Rosmarinic acid induces p53-dependent apoptosis in Jurkat and peripheral T cells via mitochondrial pathway independent from fas/fas ligand interaction,” *Journal of Immunology*, vol. 172, no. 1, pp. 79–87, 2004.
  - [21] R. J. Ruch, S.-J. Cheng, and J. E. Klaunig, “Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea,” *Carcinogenesis*, vol. 10, no. 6, pp. 1003–1008, 1989.
  - [22] N. Zamzami, S. A. Susin, P. Marchetti et al., “Mitochondrial control of nuclear apoptosis,” *Journal of Experimental Medicine*, vol. 183, no. 4, pp. 1533–1544, 1996.
  - [23] H. Aebi, “Catalase,” in *Methods of Enzymatic Analysis*, H. U. Bergmeyer, Ed., vol. 2, pp. 673–684, Academic Press, New York, NY, USA, 2nd edition, 1974.
  - [24] P. Kakkar, B. Das, and P. N. Viswanathan, “A modified spectrophotometric assay of superoxide dismutase,” *Indian Journal of Biochemistry and Biophysics*, vol. 21, no. 2, pp. 130–132, 1984.
  - [25] U. Justesen, “Negative atmospheric pressure chemical ionisation low-energy collision activation mass spectrometry for the characterisation of flavonoids in extracts of fresh herbs,” *Journal of Chromatography A*, vol. 902, no. 2, pp. 369–379, 2000.
  - [26] B. Blazics, *Analysis of Medicinal Plant Phenolics by Coupled Tandem Mass Spectrometry [Ph.D. dissertation]*, Semmelweis University, Budapest, Hungary, 2010.
  - [27] T. Askun, G. Tumen, F. Satil, S. Modanlıoğlu, and O. Yalcin, “Antimycobacterial activity of some different *Lamiaceae* plant extracts containing flavonoids and other phenolic compounds,” in *Understanding Tuberculosis—New Approaches to Fighting Against Drug Resistance*, P. J. Cardona, Ed., chapter 14, pp. 309–336, InTech, New York, NY, USA, 2012.
  - [28] F. Mosaffa, J. Behravan, G. Karimi, and M. Iranshahi, “Antigenotoxic effects of *Satureja hortensis* L. on rat lymphocytes exposed to oxidative stress,” *Archives of Pharmacological Research*, vol. 29, no. 2, pp. 159–164, 2006.
  - [29] H. J. Lee, H.-S. Cho, E. Park et al., “Rosmarinic acid protects human dopaminergic neuronal cells against hydrogen peroxide-induced apoptosis,” *Toxicology*, vol. 250, no. 2–3, pp. 109–115, 2008.
  - [30] S. Qiao, W. Li, R. Tsubouchi et al., “Rosmarinic acid inhibits the formation of reactive oxygen and nitrogen species in RAW264.7 macrophages,” *Free Radical Research*, vol. 39, no. 9, pp. 995–1003, 2005.
  - [31] T. Lapidot, M. D. Walker, and J. Kanner, “Can apple antioxidants inhibit tumor cell proliferation? Generation of H<sub>2</sub>O<sub>2</sub> during interaction of phenolic compounds with cell culture media,” *Journal of Agricultural and Food Chemistry*, vol. 50, no. 11, pp. 3156–3160, 2002.
  - [32] B. Halliwell, “Oxidative stress in cell culture: an underappreciated problem?” *FEBS Letters*, vol. 540, no. 1–3, pp. 3–6, 2003.
  - [33] L. R. Fukumoto and G. Mazza, “Assessing antioxidant and prooxidant activities of phenolic compounds,” *Journal of Agricultural and Food Chemistry*, vol. 48, no. 8, pp. 3597–3604, 2000.
  - [34] N. Kitsati, D. Fokas, M. D. Ouzouni, M. D. Mantzaris, A. Barbouti, and D. Galaris, “Lipophilic caffeic acid derivatives protect cells against H<sub>2</sub>O<sub>2</sub>-induced DNA damage by chelating intracellular labile iron,” *Journal of Agricultural and Food Chemistry*, vol. 60, no. 32, pp. 7873–7879, 2012.

- [35] V. E. Kagan, B. Gleiss, Y. Y. Tyurina et al., "A role for oxidative stress in apoptosis: oxidation and externalization of phosphatidylserine is required for macrophage clearance of cells undergoing Fas-mediated apoptosis," *Journal of Immunology*, vol. 169, no. 1, pp. 487–499, 2002.
- [36] E. Saita, Y. Kishimoto, M. Tani et al., "Antioxidant activities of *Perilla frutescens* against low-density lipoprotein oxidation in vitro and in human subjects," *Journal of Oleo Science*, vol. 61, no. 3, pp. 113–120, 2012.
- [37] K. Itoh, T. Inoue, K. Ito, and S. Hirohata, "The interplay of interleukin-10 (IL-10) and interleukin-2 (IL-2) in humoral immune responses: IL-10 synergizes with IL-2 to enhance responses of human B lymphocytes in a mechanism which is different from upregulation of CD25 expression," *Cellular Immunology*, vol. 157, no. 2, pp. 478–488, 1994.
- [38] K. Ostrowski, T. Rohde, S. Asp, P. Schjerling, and B. K. Pedersen, "Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans," *Journal of Physiology*, vol. 515, no. 1, pp. 287–291, 1999.
- [39] M. J. Kim, J. Ohn, J. H. Kim, and H.-K. Kwak, "Effects of freeze-dried cranberry powder on serum lipids and inflammatory markers in lipopolysaccharide treated rats fed an atherogenic diet," *Nutrition Research and Practice*, vol. 5, no. 5, pp. 404–411, 2011.
- [40] R. L. Zhang, W. D. Luo, T. N. Bi, and S. K. Zhou, "Evaluation of antioxidant and immunity-enhancing activities of *Sargassum pallidum* aqueous extract in gastric cancer rats," *Molecules*, vol. 17, no. 7, pp. 8419–8429, 2012.
- [41] M. Mueller, S. Hobiger, and A. Jungbauer, "Anti-inflammatory activity of extracts from fruits, herbs and spices," *Food Chemistry*, vol. 122, no. 4, pp. 987–996, 2010.
- [42] J. L. Martindale and N. J. Holbrook, "Cellular response to oxidative stress: signaling for suicide and survival," *Journal of Cellular Physiology*, vol. 192, no. 1, pp. 1–15, 2002.



**Hindawi**  
Submit your manuscripts at  
<http://www.hindawi.com>

