

Artículo Original / Original Article

Neuroprotective effect of *Capparis ovata* Desf. var. *palaestina* Zoh. on H₂O₂-induced neurotoxicity in SH-SY5Y cells

[Efecto neuroprotector de *Capparis ovata* Desf. var. *palaestina* Zoh. sobre la neurotoxicidad inducida por H₂O₂ en células SH - SY5Y]

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Abstract: The neuroprotective effect of flower and fruit parts of *Capparis ovata* Desf. var. *palaestina* Zoh. plant was investigated in H₂O₂-induced cytotoxicity in SH-SY5Y cells. The cells were treated with H₂O₂ alone or pretreated with flower (COMFL) and fruit extract (COMFR) of *C. ovata* var. *palaestina*. MTT, xCELLigence, and qualitative and quantitative determination of phytochemical constituents in the extracts by LC-MS/MS methods were employed. COMFL and COMFR had a neuroprotective effect and this effect was stronger when the presence of oxidative stress. The mass spectrums revealed the presence of flavonoids and phenolic acid derivatives in the extracts. According to quantitative analyses, the main compounds were myristoleic acid, apigenin, caffeic acid, caffeic acid-3-glucoside, and 5-cynapoil quinic acid in both COMFL and COMFR and rutin was found in COMFL. The extracts could inhibit H₂O₂ induced neuronal cell death which might be beneficial for the pretreatment of oxidative stress in neurodegenerative diseases.

Keywords: *Capparis ovata*, SH-SY5Y, MTT, xcelligence, LC-MS/MS

Resumen: Se investigó el efecto neuroprotector de flores y frutos de *Capparis ovata* Desf. var. *palaestina* Zoh sobre la citotoxicidad inducida por H₂O₂ en células SH-SY5Y. Las células se trataron con H₂O₂ solo o se pretrataron con extracto de flores (COMFL) y frutos (COMFR) de *C. ovata* var. *palaestina*. Se emplearon MTT, xCELLigence y determinación cualitativa y cuantitativa de constituyentes fitoquímicos en los extractos mediante LC-MS/MS. COMFL y COMFR que tuvieron un efecto neuroprotector y este efecto fue mayor cuando hubo estrés oxidativo. Los espectros de masas revelaron la presencia de flavonoides y derivados del ácido fenólico en los extractos. Según los análisis cuantitativos, los compuestos principales fueron ácido miristoleico, apigenina, ácido cafeico, ácido cafeico-3-glucósido y ácido quínico 5-cinapoil tanto en COMFL como en COMFR y se encontró rutina en COMFL. Los extractos podrían inhibir la muerte celular neuronal inducida por H₂O₂, lo que podría ser beneficioso para el pretratamiento del estrés oxidativo en enfermedades neurodegenerativas.

Palabras clave: *Capparis ovata*, SH-SY5Y, MTT, xcelligence, LC-MS/MS.

Oxidative stress is defined as a change in the balance situation resulting in an increase in the formation of reactive oxygen species (ROS) by exceeding the antioxidant capacity of pro-oxidants. The imbalance between ROS and antioxidants can affect normal cells, causing cell damage, death, and the formation of cancer cells. ROS have been associated with many neurodegenerative diseases such as Alzheimer's Disease (Barnham *et al.*, 2004) and other diseases like cirrhosis (Natarajan *et al.*, 2006), atherosclerosis (Kattoor *et al.*, 2017), and diabetes (Giacco Brownlee, 2010). Excessive ROS production and accumulation damage the structure of cell membranes and the biological functions of lipids, proteins, and DNA, and ultimately cause the initiation of cell apoptosis (Alfadda & Sallam, 2012). Additionally, hydrogen peroxide (H₂O₂)-induced ROS production and release can cause a series of oxidative stress responses, which in turn lead to mitochondrial dysfunction, cell damage, and death (Cheng *et al.*, 2010). Therefore, in recent years, interest in the discovery of natural resources with high antioxidant potency has increased (Elfalleh *et al.*, 2019; Karakaya *et al.*, 2019; Kumbhakar *et al.*, 2019). It has been reported that oxidative stress plays a crucial role in neurodegenerative diseases. H₂O₂ is commonly used to reproduce oxidative stress *in vitro* for the study of various neurodegenerative diseases caused by oxidative stress (Eftekhazadeh *et al.*, 2010; Vuong *et al.*, 2010). Some natural products were reported to prevent oxidative stress in H₂O₂-treated SH-SY5Y cells (Sue-Mian *et al.*, 2010). However, the effects of *C. ovata* var. *palaestina* in H₂O₂-treated SH-SY5Y cells have not been explored.

Capparis L. is one of the widespread genus of the Capparaceae family. The species of this genus are also known as "Caper". There are two species in Turkey (*C. spinosa* and *C. ovata*) and each species is represented by three varieties as *C. spinosa* L. var. *spinosa* L., *C. spinosa* L. var. *inermis* Turra., *C. spinosa* L. var. *aegyptia* (Lam) Boiss, *C. ovata* Desf. var. *palaestina* Zoh., *C. ovata* Desf. var. *herbacea* (Wild) Zoh., and *C. ovata* Desf. var. *canescens* (Coss.) Heywood (Okur *et al.*, 2018a; Okur *et al.*, 2018b; Okur *et al.*, 2018c).

Various studies showed that *Capparis* L. (Capparaceae) plant has a strong antioxidant effect (Mansour *et al.*, 2016; Yu *et al.*, 2017). *C. ovata* is one of the endemic plants in Turkey which popularly used as food. The antidiabetic (Okur *et al.*, 2018a;

Okur *et al.*, 2018c), antioxidant (Kalantari *et al.*, 2018), hepatoprotective, (Kalantari *et al.*, 2018), anticancer (Rathee *et al.*, 2012), wound healing (Okur *et al.*, 2018b), anti-neuroinflammatory (Ozgun-Acar *et al.*, 2016), and antinociceptive (Arslan & Bektas, 2010; Arslan *et al.*, 2010) effects have been demonstrated in several studies. Traditional uses have been summarized, and reported (Olagan *et al.*, 2020). The roots, leaves, buds, fruit, bark and seeds of various *Capparis* species were used in folk medicine for different medicinal purposes (Tlili *et al.*, 2011). The whole plant was used for rheumatism. Roots were used as diuretic, astringent, and tonic (Rahnavard & Razavi, 2017).

Here, we investigated whether *C. ovata* var. *palaestina* prevented oxidative stress-induced cytotoxicity of SH-SY5Y cells. For this purpose, the possible protective effect of both COMFL and COMFR were evaluated in the oxidative damaged SH-SY5Y induced by H₂O₂ by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and xCELLigence real-time cell analysis *in vitro*. Additionally, the phytochemical profile of the extracts was established by LC-MS/MS.

MATERIAL AND METHODS

Plant material

C. ovata var. *palaestina* flower buds were collected from the last week of August from Akpınar Village in Adıyaman Province, which is located within the borders of Türkiye. After drying under suitable conditions, it was stored in a cool and dry environment before extraction.

Preparation of the extracts

Dried flowers and fruits after pulverization were extracted separately using 70% methanol in Soxhlet (continuous extraction) apparatus for 8 h. The extract was evaporated in a rotavapor under reduced pressure at 38°C to obtain crude methanol extract of the flower and fruits.

SH-SY5Y cell line

The SH-SY5Y cell line (ATCC®, CRL-2266™) is a widely used model system for investigating neuronal cell death caused by oxidative stress, evaluating the neuroprotective effects of natural products and the pharmacological and toxicological potential of acetylcholinesterase inhibitors (Garcimartín *et al.*, 2017; Sun *et al.*, 2017; An *et al.*, 2019; Liu *et al.*,

2019). The SH-SY5Y cell line was cultured in DMEM medium (Sigma, D5796) containing 10% fetal bovine serum (FBS) (Biocrom, S 0115) and 50 µg/mL penicillin and 100 µg/mL streptomycin (Biological Industries, 03-031-1C). These cells were grown in a 37°C incubator in humidified 5% CO₂. The medium was replaced every 2–3 days. The cells were trypsinized with 0.25% trypsin-ethylenediamine tetra acetic acid (EDTA) (Biological Industries, 03-052-1B) when the cells reached 80-90% confluency.

After culture in DMEM for 24 h, the cells were pretreated with the extracts (4, 8, 16, 32, 65, 125, and 250 µg/mL) for 4 h and then co-treated with H₂O₂ (200 µM) for 72 h.

Investigation of cell viability by MTT method

SH-SY5Y cells (1 × 10⁴ cells/well) were seeded and cultured into a 96-well plate for 24 h. The cells were pretreated with the extracts with serum-free DMEM for 4 h, and then co-treated with H₂O₂ for 72 h.

The cell viability was determined by MTT assay according to the developed protocol (Mosmann, 1983). MTT (0.5 mg/mL) was transferred to the cell cultures, and the cells were incubated for an additional 4 h. The supernatant was removed, DMSO (150 µL/well) was added, and the plates were shaken for 15 min. The absorbance was measured using an absorbance reader (Synergy HT, BioTek) with a wavelength of 570 nm.

Cytotoxicity assay using xCELLigence real-time cell analyses (RTCA)

xCELLigence is a device that measures cell viability in real-time via the Cell Index (CI). In cell culture medium, the effects of the substances applied to the E-plates, which are suitable for the device and coated with gold on the bottom surface of the wells, on time-dependent cell viability can be examined. Cells attached to the surface of the e-plate affect the impedance of the array of electrodes covering the base. This effect is converted to CI by xCELLigence software. The software of the device transfers the cell viability simultaneously to the computer program by calculating CI in the determined period and thus cell profiles are obtained (Moodley *et al.*, 2011; Dwane *et al.*, 2013).

Effects of COMFL and COMFR on cell growth curves were assessed with the RTCA system

xCELLigence. According to MTT assay results, novel concentrations were determined to evaluate its effects on cell growth and proliferation of COMFL (4, 8, 16, 32, and 65 µg/mL) and COMFR (125 and 250 µg/mL), separately.

In brief, SH-SY5Y cells were seeded at a density of 1 × 10⁴ cells/well of a 96-well E-plate (Catalog No. 05232368001; ACEA Biosciences, Inc., San Diego, CA, USA). After 24 h of incubation of the cells, both of the extracts were applied 4 replicates at the specified concentrations. The real-time cellular analysis system monitored the proliferation of cells every 15 min. for about 24 h. During the log growth phase, the cells were treated with different concentrations as mentioned above and monitored continuously for 72 h.

EC₅₀ value was defined by the extracts at 72 h. The RTCA software performs a curve-fitting of selected “sigmoidal dose-response equation” to the experimental data points and calculates the logarithmic half-maximum effect of concentration (log [EC₅₀]) values at a given time point based on log of concentration producing a 50% increase of CI value relative to solvent control CI value (100%), expresses as log EC₅₀ (Moodley *et al.*, 2011; Dwane *et al.*, 2013).

Statistical analysis

Statistical analysis were performed using GraphPad Prism 8.2.1 statistical software (GraphPad Software, Inc., San Diego, CA, USA). All experiments were performed in triplicates. The data were presented as the means ± standard deviation (S.D.). Statistical analysis was carried out using one-way ANOVA followed by the Dunnett test, with *p*<0.05 considered statistically significant.

Phytochemical analyses by LC-MS/MS

The LC-MS/MS conditions: Shimadzu LCMS-8040 triple quadrupole mass spectrometer (ESI) was used to analyze chemical compounds in extracts of flowers and fruits. For this purpose the extracts were solved in methanol and the final concentration of solutions was 10 µg/mL. The samples were injected in Column Restek C-18 (150 mm × 4.6 mm, 3 µm) at the flow rate of 0.5 mL/min and the injection volume of 1 µL. The mobile phase was the mixture of methanol: acetonitrile (70:30, v/v) (A) and water (B) as 80% solvent A and 20% solvent B.

RESULTS

It investigated whether *C. ovata* var. *palaestina* prevented oxidative stress-induced apoptosis of SH-SY5Y cells. For this purpose, the possible protective effect of COMFL and COMFR was evaluated in the oxidative damaged SH-SY5Y induced by H₂O₂ *in vitro*. The protective effects of plant extracts were investigated by xCELLigence real-time cell analysis and MTT cell viability method.

Effect of the extracts on H₂O₂-induced neurotoxicity in SH-SY5Y cells

As a result of the literature review, in *in vitro* studies modeled with H₂O₂, it is seen that oxidative stress is

induced by H₂O₂ at concentrations of 3 mM, 250, and 500 μM (Vygodina & Konstantinov, 1988; Chetsawang *et al.*, 2006). Considering the literature, the appropriate H₂O₂ concentration is required to create an optimum damage model in the SH-SY5Y cell line is determined by general dose scanning using the MTT method. As a result of general dose screening with the MTT method, no cytotoxic effects were observed at 100 and 150 μM concentrations (Figure No. 1). The IC₅₀ of H₂O₂ was calculated as 200 μM ($p < 0.01$) and used this concentration which is also in line with the literature, as used to create oxidative stress in another experiment (Figure No. 1).

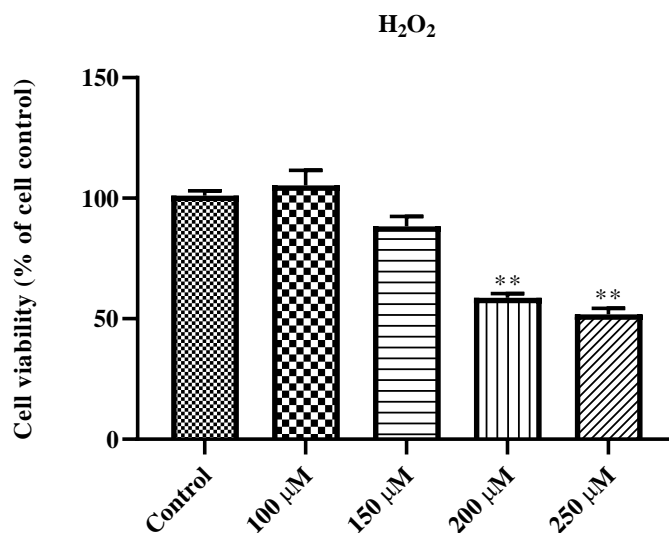


Figure No. 1

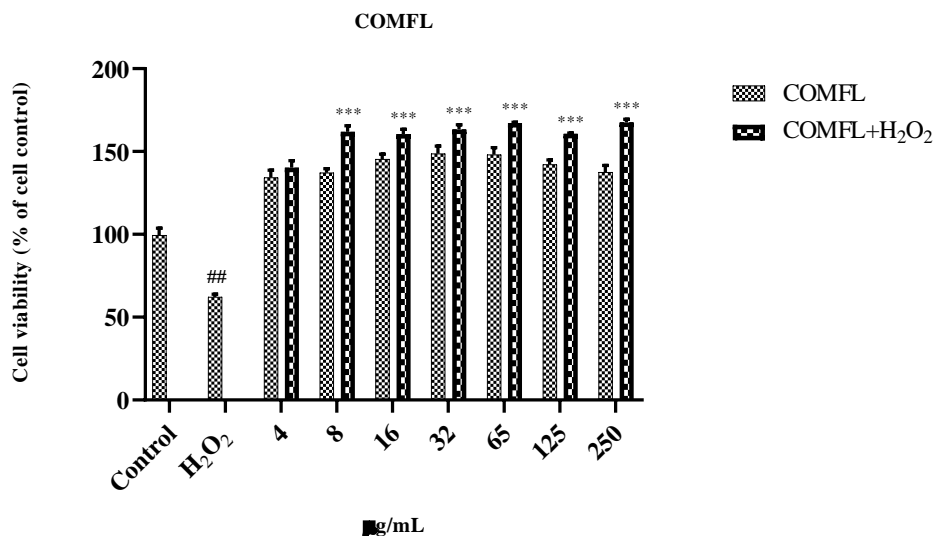
The cell viability of H₂O₂ treated SH-SY5Y cells

Groups were given as percentage change compared to the control group. The values were analyzed by One-way ANOVA and post-hoc Dunnet test in GraphPad Prism 8.2.1 program. Data are presented as the mean ± SD. ** $p < 0.01$ vs. the control group

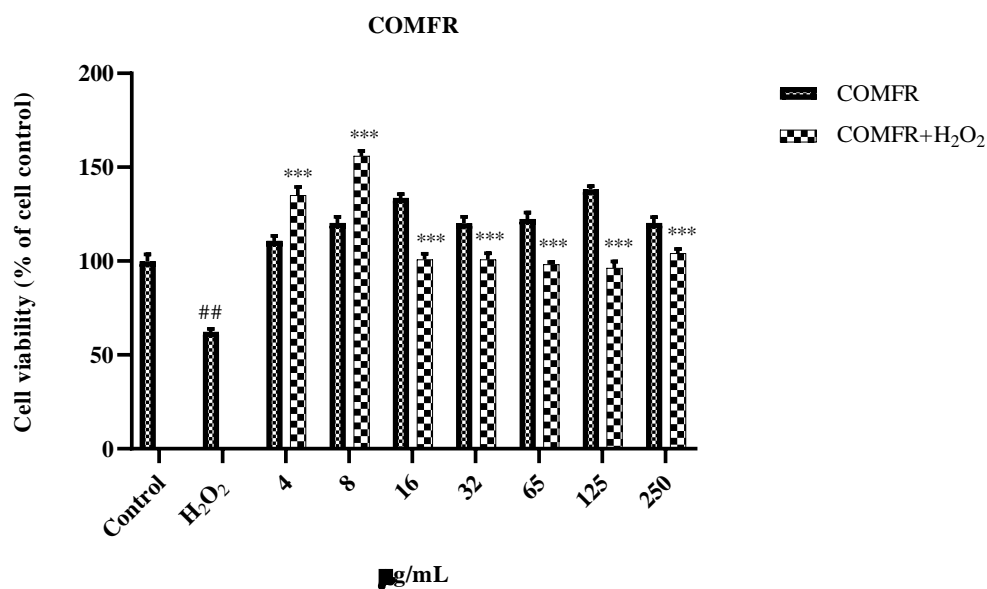
The SH-SY5Y cells were pretreated with the extracts (4, 8, 16, 32, 65, 125, and 250 μg/mL) for 4 h and then incubated with H₂O₂ (200 μM) for 72 h. It was found that the extracts had no toxic effect on SH-SY5Y cells at all concentrations (Figure No. 2a & No. 2b). However, the cell viability was significantly ($##p < 0.01$) reduced in H₂O₂-treated cells compared to controls (Figure No. 1, Figures No. 2a and No. 2b).

The pre-treatment with COMFL and COMFR increased the viability of SH-SY5Y cells vs. the control group at whole applied concentrations of the

extracts (4, 8, 16, 32, 65, 125, and 250 μg/mL) (Figures No. 2a and 2b). The result showed that pre-treatment with COMFL and COMFR significantly ($***p < 0.001$) reduced H₂O₂-induced cell death at whole applied concentrations (4, 8, 16, 32, 65, 125, and 250 μg/mL) vs. the H₂O₂-treated group (Figures No. 2a and No. 2b). Taken together, these results revealed that both COMFL and COMFR exerted a neuroprotective effect against H₂O₂-induced neurotoxicity in SH-SY5Y cells.



(a)



(b)

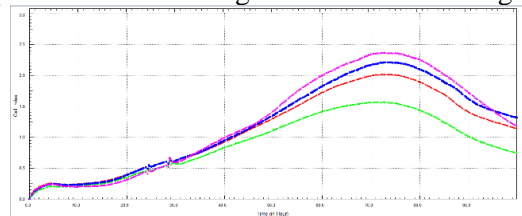
Figure No. 2

a) Effect of COMFL on H₂O₂-induced neurotoxicity in SH-SY5Y cells. After being pretreated with COMFL (4, 8, 16, 32, 65, 125, and 250 µg/mL) for 4 h, SH-SY5Y cells were stimulated with H₂O₂ (200 µM) for 72 h. Cell viability was measured by MTT assay. b) Effect of COMFR on H₂O₂-induced neurotoxicity in SH-SY5Y cells. After being pretreated with COMFR (4, 8, 16, 32, 65, 125, and 250 µg/mL) for 4 h, SH-SY5Y cells were stimulated with H₂O₂ (200 µM) for 72 h. Cell viability was measured by MTT assay. Groups were given as percentage change compared to the control group. The values were analyzed by Two-way ANOVA and Sidak's multiple comparisons test in GraphPad Prism 8.2.1 program. Data are presented as the mean ± SD. ##*p*<0.01 vs. the control group, ****p*<0.001 vs. the extract-treated group

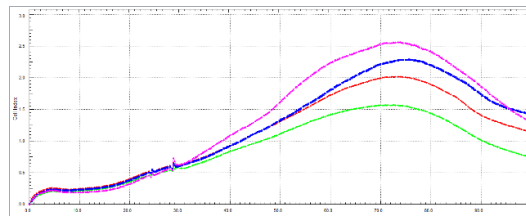
xCELLigence test results

RTCA graphs showed that CI increased in the same manner in all wells within 24 h after cell cultivation (Figures No. 3a-g and No. 4a-g). The CI value was remarkably increased in all groups at all concentrations (4, 8, 16, 32, 65, 125, and 250 $\mu\text{g/mL}$) both COMFL and COMFR. COMFL and COMFR extracts protected the cells against oxidative damage

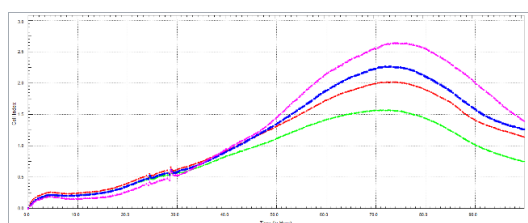
caused by 200 μM H_2O_2 at all concentrations and showed a proliferative effect while increasing CI (Figures No. 3a-g and No. 4a-g). When the MTT results (as seen in Figure No. 2a & No. 2b) and CI values (Figures No. 3a-g and No. 4a-g) were evaluated together, the results seemed to be compatible with each other.



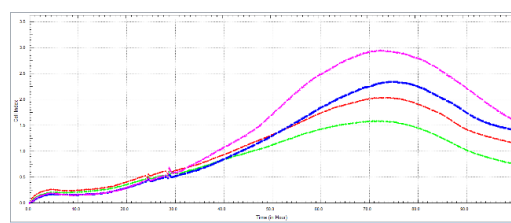
(a)



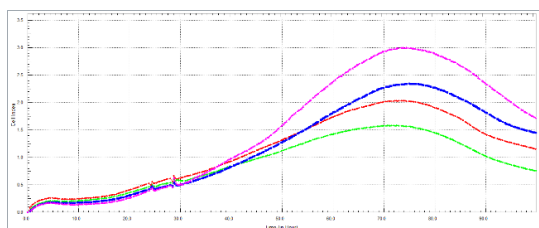
(b)



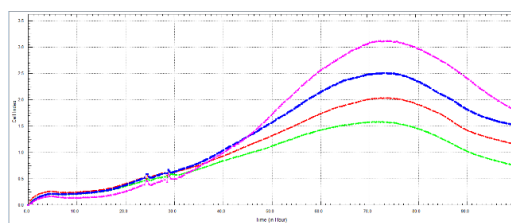
(c)



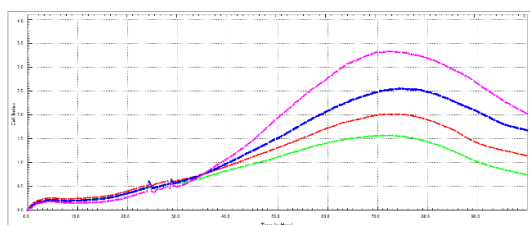
(d)



(e)



(f)



(g)

Figures No. 3

- a) Concentration and time-dependent CI alteration of COMFL extract at a concentration of; a) 4 $\mu\text{g/mL}$ b) 8 $\mu\text{g/mL}$ c) 16 $\mu\text{g/mL}$ d) 32 $\mu\text{g/mL}$ e) 65 $\mu\text{g/mL}$ f) 125 $\mu\text{g/mL}$ g) 250 $\mu\text{g/mL}$
 b) Red: Control, Green: H_2O_2 , Indigo: COMFL, Pink: COMFL + H_2O_2

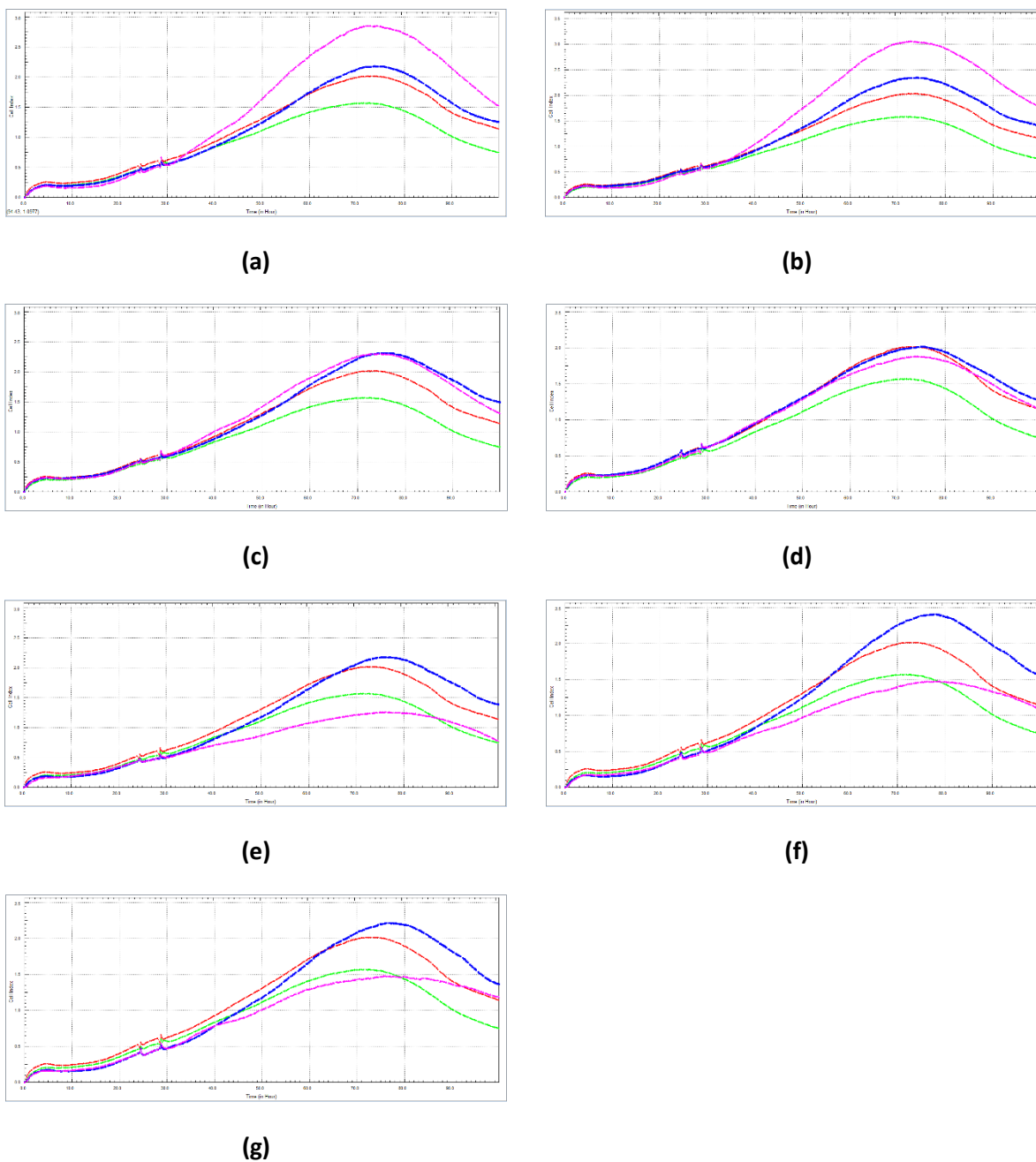


Figure No. 4
Concentration and time-dependent CI alteration of COMFR extract at a concentration of; a) 4 µg/mL b) 8 µg/mL. c) 16 µg/mL d) 32 µg/mL e) 65 µg/mL f) 125 µg/mL g) 250 µg/mL
Red: Control, Green: H₂O₂, Indigo: COMFR, Pink: COMFR+ H₂O₂

Phytochemical analyses by LC-MS/MS

The identification of compounds in COMFL and COMFR of species was carried out by registered mass spectra fragmentation patterns, NIST (National Institute of Standards and Technology) mass spectral database (version 2.3, USA), and literature data.

According to the results, myristoleic acid, apigenin (Paşayeva *et al.*, 2020a), caffeic acid, caffeic acid-3-glucoside, and 5-cynapoil quinic acid (Paşayeva *et al.*, 2020b) were detected in both flower and fruit extract and rutin (Paşayeva *et al.*, 2020b) was found in flower extract (Table No. 1 and Figure No. 5).

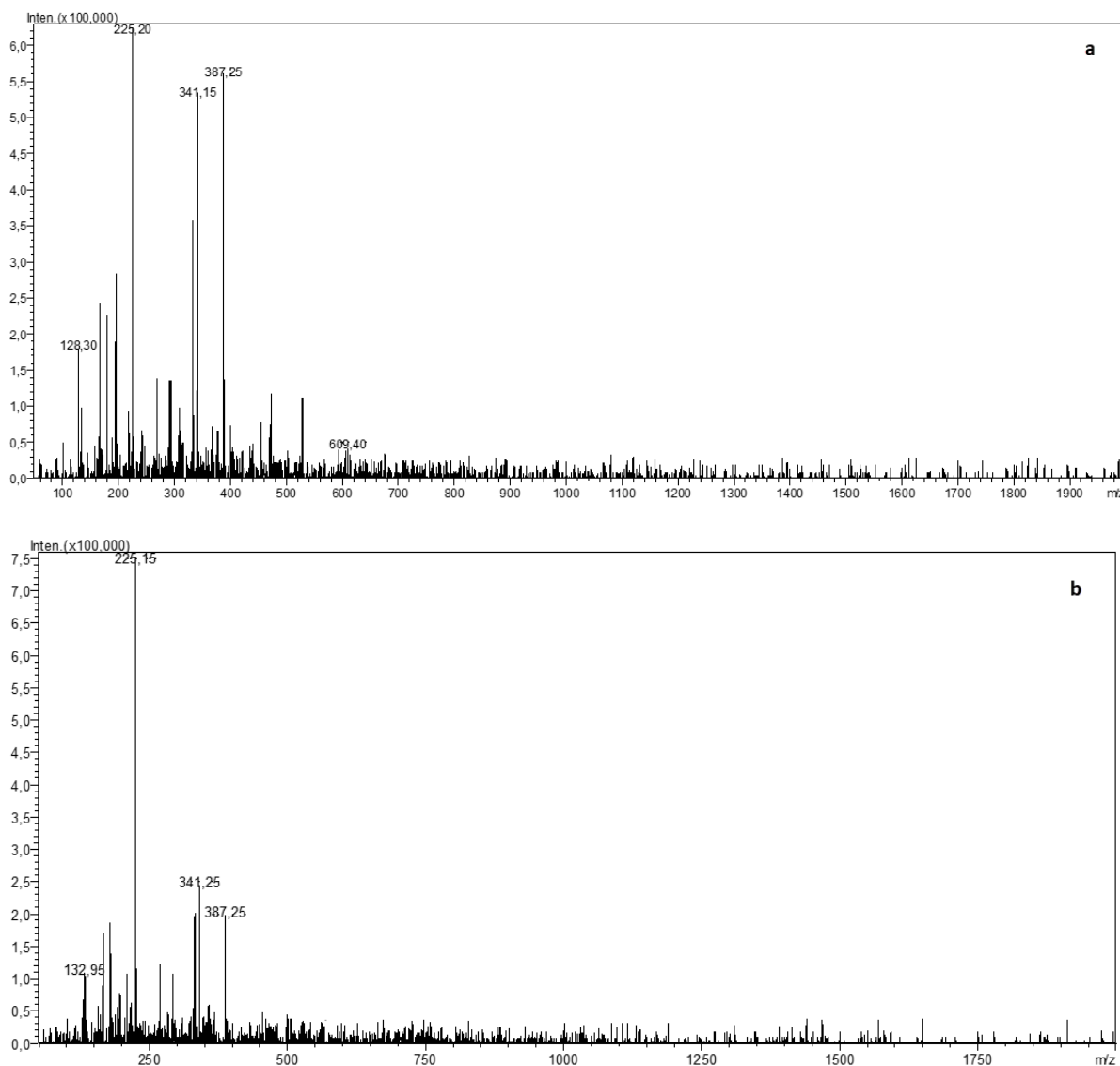


Figure No. 5

**a) Mass spectra of the flower of *C. ovata* var. *palaestina* b) Mass spectra of the fruit extracts
 b) of *C. ovata* var. *palaestina***

Table No. 1
Mass spectral characteristics and identity of compounds in TFE sub-extract

[M-H] (m/z)	MS/MS (m/z)	Compounds	COMFL	COMFR	References
225	181,165	Myristoleic acid	+	+	Paşayeva et al., 2020a
269	-	Apigenin	+	+	Paşayeva et al., 2020a
179	135, 87	Caffeic acid	+	+	Paşayeva et al., 2020b
341	179,135	Caffeic acid-3-glucoside	+	+	Paşayeva et al., 2020b
387	223,191,179	5-cynapoil quinic acid	+	+	Paşayeva et al., 2020b
609	301	Rutin	+	-	Paşayeva et al., 2020b

DISCUSSION

In the present study, we reported the neuroprotective effect of COMFL and COMFR in an *in vitro* model of oxidative stress based on the exposure of SH-SY5Y human neuroblastoma cells to H₂O₂. Additionally, the phytochemical profile of the extracts was established by LC-MS/MS, for the first time.

SH-SY5Y cells have many characteristics of human neuron cells and, therefore, are used to *in vitro* mimic where oxidative stress is increased. Many studies have demonstrated that oxidative stress, associated with mitochondrial dysfunction, contributes to the development of neurodegenerative diseases (Albers & Beal, 2000; Sayre et al., 2001; Jomova et al., 2010).

Free radicals that cause oxidative damage to play a critical role in the development of many diseases like arteriosclerosis (Kattoor et al., 2017), rheumatic diseases (Hassan et al., 2011), cancer (Sosa et al., 2013), and neurodegenerative diseases such as Parkinson's and Alzheimer's (Jomova et al., 2010). The incidence of these diseases increases with aging, especially as a result of the deterioration of redox homeostasis due to aging. The most critical radicals that cause oxidative damage and play a role in the development of all these diseases are ROS and reactive nitrogen products (RNS) (Ögüt & Atay, 2012). Although H₂O₂ is not included in the free

radical class, it is associated with ROS derivatives because it occurs in pathological and physiological conditions in living organisms and is easily converted to free radicals in the organism (Karabulut & Gülay, 2016). There are studies in the literature with the role of H₂O₂ in neurodegenerative diseases such as Parkinson's and Alzheimer's (Tabner et al., 2002; Tabner et al., 2005).

The flower buds, leaves, and fruits of the caper species have been used by different cultures throughout human history for their medicinal effects and also as food like pickles, salad, condiments, or snacks (Rivera et al., 2003). *Capparis* species are generally known to be rich in glucosinolates, tannins, alkaloids, phenolic acids, and flavonoids (Matthäus & Özcan, 2005; El-Ghorab et al., 2007; Tlili et al., 2011). In addition to phenolic components, *Capparis spinosa* was found to contain α , γ -tocopherol, high levels of minerals, and significant vitamin C (Tlili et al., 2010; Aliyazicioglu et al., 2013). Caper mainly contains the following chemical compounds; capparine A, capparine B, apigenin, kaempferol, rutin, quercetin, β -carotene, and glucocapparin (Gull et al., 2015). Accordingly, it has been proven by studies that various biological effects indicated in different species of the genus are caused by these compounds (Rathee et al., 2012; Aichi-Yousfi et al., 2016;)

In a study conducted to investigate the

antioxidant effect of caper, 70% ethanol extract prepared from *C. spinosa* leaves and fruits were applied on human sperm in different doses (15, 30, and 45 ppm) without any therapeutic chemicals. As a result of the study, it was determined that ethanol extracts have a high antioxidant effect on human sperm even at low doses (Rad *et al.*, 2021). In a study, 70% ethanol extract of aerial parts of *C. spinosa* was prepared and its cardioprotective effect was examined in H9c2 (rat cardiomyocyte cell) cell damage induced by doxorubicin. Doxorubicin was administered at 4 h following the administration of the extracts. Extracts were administered at different concentrations (6, 12, 25, 50, 100, and 200 µg/mL) before the drug and significantly reduced the induction of apoptosis in cells and significantly increased cell viability. As a result, it was concluded that the extract reduces oxidative stress in H9c2 cells and showed a protective effect on cells (Mousavi *et al.*, 2016). In another study, the anti-inflammatory effect of methanol extract of *C. spinosa* flower buds was investigated in human articular chondrocyte inflammation induced by interleukin-1 β (IL-1 β). As part of the study, the plant extract was applied simultaneously with IL-1 β (10 ng/mL) at concentrations of 10, 100, and 200 µg/mL. Pro-metalloproteases and prostaglandin E2 (PGE2) levels in cells for 120 h were determined to decrease. It has been concluded that *C. spinosa* extract has an anti-inflammatory effect at the specified concentrations; as a result that, methanol extract of *C. spinosa* can therefore be used for protective purposes in osteoarthritis (Panico *et al.*, 2005). According to another study, methanol extracts of both the aerial and root parts of *C. spinosa* were found to have higher total bioactive contents and comparatively higher antioxidant potential. The study revealed that the presence of phenolic, alkaloid, glucosinolate, and flavonoid derivatives (Saleem *et al.*, 2021)

There are some studies on the chemical composition of *C. ovata* var. *palaestina*. According to these studies rutin (Okur *et al.*, 2018a; Okur *et al.*, 2018b), stachydrine, and isoquercitrin (Okur *et al.*, 2018c) were detected in different extracts of species. Based on these reports we can say that our results supported the previous studies. Moreover, myristoleic acid, caffeic acid derivatives, and apigenin were identified first in this study. Although the antioxidant potential of *C. ovata* depends on the type and amount of flavonoids, the scientific literature on this subject

is insufficient (Wojdyło *et al.*, 2019).

There is a study investigating the chemical content of *C. spinosa* Linn. plant and its protective effect against oxidative stress induced by H₂O₂ (Moufid & Farid, 2015). However, there are no studies in the literature on the chemical content of the *C. ovata* var. *palaestina* and its protective effect against oxidative damage. For this reason, the results that we can directly compare to our study are not available in the literature.

Our results revealed that COMFL and COMFR reduced cellular toxicity in H₂O₂-induced SH-SY5Y cells. Pre-treatment with the extracts showed the neuroprotective effects suggesting that extracts-mediated protection can be due to the chemical composition of the extracts. The difference in neuroprotective effects of extracts may be explained by the difference in phytochemical compositions. So COMFL contains a rutin compound unlike COMFR and the protective effect of this compound was reported previously (Wang *et al.*, 2012; Enogieru *et al.*, 2019).

The extracts neuroprotection may be associated with signaling pathways and need to be further explored neurotoxicity models *in vitro* will be a suitable way detailing COMFL and COMFR neuroprotection and its therapeutic potential in the context of neurodegenerative diseases.

CONCLUSION

To the best of our knowledge, this is the first report of the phytochemical analysis of *C. ovata* var. *palaestina* and *in vitro* neuroprotective effect of COMFL and COMFR. In conclusion, one of the endemic species *Capparis ovata* var. *palaestina* Zoh. was found neuroprotective against H₂O₂-induced neurotoxicity in SH-SY5Y cells. This effect was stronger than the absence of oxidative stress. Furthermore, the identified compounds myristoleic acid, caffeic acid derivatives, and apigenin are the possible contributors to the neuroprotective effects of COMFL and COMFR suggesting an interesting potential for further neuroprotective mechanism studies of this valuable plant. In the light of the obtained data, these results suggest that COMFL and COMFR are potential candidates for the prophylactic of oxidative stress-related neurodegeneration and consequent pathological conditions.

The results show that this endemic plant can be a potential source in studies related to

neurodegeneration and that this effect is uncommon due to its neuroproliferative effect.

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