Original article

Molecular Biological Investigation of the Effect of Sumac Extract on Protein Synthesis and Development of *Saccharomyces cerevisiae*

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Abstract. *Study objective:* Sumac is a plant that is widely used for various purposes such as industrial, pharmaceutical and nutritional applications. Adding it to food or water as a natural preservative can have a beneficial effect on human health. It has biological activities such as anti-bacterial, anti-fungal and anti-oxidant. In this study, the effect of sumac plant on oxidative damage in *S. cerevisiae* culture caused by copper chloride ($CuCl_2$) was investigated. *Methods:* In this study 4 groups were formed. Groups; Group (1): Control group; Group (2): Sumac group (10%); Group (3): Copper chloride ($CuCl_2$) group (10 mM); Group (4): Copper chloride ($CuCl_2$) (10 mM) + Sumac (10%) group. *S. cerevisiae* cultures were developed at 30°C for 1 hour, 3 hours, 5 hours and 24 hours. Cell growth, lipid peroxidation, MDA (malondialdehyde) analyzes, glutathione (GSH) levels and catalase (CAT) activities were determined by spectrophotometer. Total protein changes were determined by SDS-PAGE electrophoresis and calculated by the Lowry method. *Results:* According to the results obtained; Sumac plant extract which added to *S. cerevisiae* cultures increased cell growth (1, 3, 5 and 24 hours), total protein synthesis (1, 3, 5 and 24 hours), GSH levels (24 hours) and catalase activities (24 hours), decreases the MDA level (24 hours). *Conclusions:* These results show that sumac plant extract reduces oxidative damage in *S. cerevisiae* culture, promotes protein synthesis and has a protective effect to increase cell growth.

Key words: Copper chloride, Protein, *S. cerevisiae*, SDS-PAGE, Sumac extract

Introduction

In recent years, the trend towards natural herbal remedies or natural herbal food sources has increased significantly due to the side effects of chemical drugs. Some fruits and herbs that are grown under natural conditions and do not have any side effects when consumed in certain amounts on a daily basis have been used by people for many years for the treatment of many diseases (1). Sumac, one of these herbs, is a widely used spice to give a lemon flavor to dishes. Although sumac, which comes from the Anacardiaceae family and *Rhus* genus, is used as a powder, it is a fruit in its natural state. It is used in traditional herbal therapy in the Far East and in the Mediterranean region in our country. It is a tree that can grow anatomically up to ten meters high, spreading both by seed and underground with new roots called rhizomes. It contains various phytochemicals such as gallic acid, minerals, terpenoids, linoleic and oleic acids, vitamins, kaempferol and quercetin (2-4). It has economic importance due to its increasing use in the food, cosmetic and pharmaceutical industries, coloring, preserving foods and veterinary applications. It has many effects such as anti-bacterial, anti-carcinogen, anti-diabetic, anti-fungal, anti-oxidant, analgesic, anti-lipidemic and hypoglycemic (2,4). In the food industry as sumac, spices and beverages; it is used in the industry for dyeing fabrics and leathers to yellow color. Sumac leaves are used in the pharmaceutical industry by taking advantage of

their anti-septic, diarrhea and anti-blood properties and their antipyretic properties. In addition, it is also used in the treatment of hemorrhoids, mouth sores, eye disorders, hand and foot cracks. The material obtained after the powdered sumac leaves and roots is a very valuable material used in tanning and dyeing light and thin leathers (3,5). Due to the interactions of organisms with heavy metals, it is important to determine the metal accumulation, biotransformation and excretion in their bodies. Even an increase in the concentration of a single metal in the environment causes toxic effects on organisms. Copper (Cu) is an element that is very common in nature and is needed by organisms in trace amounts. The toxicity of copper is related to its soluble forms, which are Cu^{2+} and $Cu(OH)^2$ (6). Therefore, a model organism called *S. cerevisiae* is used to determine this activity in organisms and to understand the molecular basis of cell functions. This is because this model organism creates a cellular response to oxidative stress and defense functions involved in this response (7,8). In addition, as their genetic structure and cellular properties are well known, their use continues to spread every day and therefore it is one of the most preferred microorganisms in scientific studies (9,10).

The aim of this study is to investigate the protective effects of sumac plant against copper chloride ($CuCl_2$) induced oxidative damage in *S. cerevisiae*.

Material and Methods

Research Groups

In this study, the protective effect of sumac plant against the damage caused by copper chloride ($CuCl_2$) in *S. cerevisiae* was investigated. 4 groups were formed in the study.

Our groups;
Group (1): Control group
Group (2): Sumac group (10%)
Group (3): Copper chloride ($CuCl_2$) group (10 mM)
Group (4): Copper chloride ($CuCl_2$) (10 mM) + Sumac (10%) group.

Application of Sumac and $CuCl_2$ to the Culture

Growth medium of *S. cerevisiae*: YEPD (7.5 g yeast extract, 7.5 g tryptone, 7.5 g glucose per 250 ml) was prepared for the growth and propagation of the yeast. Then, 5 flasks were taken and 50 ml of 250 ml of prepared medium was added to each flask. After waiting at 121° C in the autoclave for 1 hour, it was removed and cooled. In addition to the burner flame, 800 µl of yeast was planted in each flask. After 20 min. in the oven, blind measurement was made. For the preparation of 10% Sumac plant; 10 g of sumac was weighed. It was brewed in 100 ml of boiling distilled water for 10-15 min. It was then used by filtration through a sterile cheesecloth. Then, $CuCl_2$ and sumac extract were added to the other flasks removed from the oven besides the burner flame. It was developed at 30°C by adding 10 ml of sumac filtrate and 0.5 grams of $CuCl_2$ according to the content of the groups (11).

Cell Growth Measurements

Culture samples were grown at 30°C for 1 hour, 3 hours, 5 hours and 24 hours (overnight) and were measured using a spectrophotometer at 600 nm (OD_{600}) wavelength (11). These samples, which were measured in spectrophotometer, were then prepared separately for protein electrophoresis, MDA, GSH, total protein analysis and catalase activity parameters, necessary analyzes were made, and the results were interpreted and expressed in the article.

Protein Isolation for SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis)

After taking 1 ml of the culture sample and centrifuging at 13000 rpm for 5 min. the pellet was dissolved in 500 µl TEA (Tris-EDTA Acetic Acid) (pH: 7.5). The cells were kept in ice for 5 min. after being disintegrated twice at power 2 for 10 seconds with a sonicator (Bandelin Sonopuls, Germany). Then it was centrifuged at 13000 rpm for 10 min. and the pellet part was removed. For SDS-PAGE studies, an equal amount of sample was mixed with the staining solution and made ready to use for electrophoresis (12).

SDS-PAGE Analysis

Protein samples of *S. cerevisiae* cultures were boiled for 5 min. After, added of an equal amount of SDS-PAGE SAB (Sample Amplification Buffer) dye before loading into the wells. 1 x tank buffer was used for electrophoresis and a current of 30 mA was applied until the blue band of the dye (bromophenol blue), which allows the movement of proteins in the gel to be monitored, reached the end of the gel. After electrophoresis, the gel was stained with Coommasie blue for 30 min. to 1 hour at room temperature. Then, the protein bands in the gel were washed with a dye remover solution until the protein bands became visible and the protein bands between the groups were examined by taking the gel images (13,14).

Total Protein Density Measurements with Lowry Protein

Lowry protein assay kit using, total protein density was performed using spectrophotometer at 650 nm (OD_{650}) according to the kit method.

Malondialdehyde Analysis

Malondialdehyde in analysis, after adding 0.5 µl of sample to the test tube and 0.5 ml of distilled water into the blind tube, 2.5 ml of 20% TCA (Trichloroacetic acid) and 1 ml of TBA (Thiobarbituric Acid) were added to all test tubes. Then it was cooled after waiting for 30 min. in a boiling water bath at 90° C. After adding 4 ml of n-butanol-pyridine mixture and vortexing, it was centrifuged at 3000 rpm for 10 min. At the end of this process, the upper phase part was removed and 532 nm wave measurement was carried out in the spectrophotometer. Results were recorded in nmol/ml (15).

Catalase Activity Assay

For catalase measuring, two tubes are taken and 1.4 ml of 30 mM H_2O_2 is added to the blank tube and 0.1 ml of phosphate buffer is added on it. 1.4 ml of 30 mM H_2O_2 and 0.1 ml of enzyme are added to the sample tube and mixed with vortex. Absorbances at 240 nm are read twice at 30 second intervals and thus the activity is determined (16).

Glutathion Activity Assay

0.1 ml of culture sample was mixed with 0.4 ml of TCA (10%) solution, after vortexing for 20 seconds and centrifuging at 3000 rpm for 5 min. 0.1 ml of supernatant was taken into a clean tube and 0.9 ml of distilled water, 2 ml of Tris buffer (0.4M pH: 8.9) and 0.1 ml of DTNB (5,5'-Dithiobis-(2-Nitrobenzoic Acid)) solution were added. The resulting yellow color was read against distilled water in a spectrophotometer at a wavelength of 412 nm (17,18).

Statistical Analysis

Statistical analysis of our studies was evaluated with SPSS 22 package program. In addition, One Way Anova *Post Hoc* Tukey LSD tests were applied to determine the intragroup differences. Data are expressed as mean ± standard deviation (SD). In terms of the reliability of the statistical analysis of our studies, all measurements were made in 3 repetitions.

Results

Malondialdehyde (MDA) Analysis Results

When we look at the data in Table 1 and Figure 1A, 1B, it was seen that the MDA level decreased in the $CuCl_2$ + Sumac groups, while the amount of MDA increased in the $CuCl_2$ groups. ($p < 0.05$). These results show us that the sumac plant has a protective effect against lipid peroxidation.

Table 1. MDA analysis results

Groups	MDA Levels (nmol/ml)
Control	4.18 ± 0.01^{c}
Sumac	4.01 ± 0.02^{c}
$CuCl_2$	5.24 ± 0.02^{a}
$CuCl_2$ + Sumac	4.24 ± 0.02^{b}

$^{a-c}$: Among the groups which bearing of different letter are significant ($p<0.05$). One way Anova Post Hoc LSD test

Table 2. CAT activity

Groups	CAT Activity (U/ml)
Control	88.88 ± 1.00[a]
Sumac	91.82 ± 1.00[a]
$CuCl_2$	30.35 ± 1.00[c]
$CuCl_2$ + Sumac	58.80 ± 1.00[b]

[a-c]: Among the groups which bearing of different letter are significant (p<0.05). One way Anova Post Hoc LSD test

Catalase (CAT) Activity Results

As a result of this study, it was observed that the CAT activity of the $CuCl_2$ + Sumac group was higher than the $CuCl_2$ group. However, the difference between CAT levels was statistically significant in all other groups. ($p < 0.05$) (Table 2 and Figure 1C, 1D).

Glutathione (GSH) Analysis Results

According to the results of our study, the GSH level was higher in the $CuCl_2$ + Sumac group compared to the $CuCl_2$ group. (Table 3 and Figure 1C, 1E). GSH and CAT levels increasing show us that the sumac plant has anti-oxidant activity.

Cell Development Measurement Results

In Table 4 and Figure 1F, there is a statistically significant difference between the groups depending on the development times at different times ($p < 0.05$). It was determined that the cell growth increased in the groups given sumac compared to the $CuCl_2$ group.

Total Protein Density (Lowry) Measurements

When the total protein results are given in Table 5,6 and Figure 1C, Figure 1G and Figure 1H is examined, we can say that the sumac plant increases protein synthesis in *S. cerevisiae*. Especially when compared with the $CuCl_2$ group, it is observed that protein synthesis increased at a high rate in the $CuCl_2$ + Sumac group.

SDS-PAGE Analysis Results

SDS-PAGE gel images show that the protein concentration increased significantly in the $CuCl_2$ + Sumac group compared to the $CuCl_2$ group, depending on the time (1, 3, 5 and 24 hours). As a result of this study, it was concluded that the sumac plant increased the protein density and development of *S. cerevisiae* despite the negative effects of $CuCl_2$ (Figure 2A, 2B, 2C and 2D).

Discussion

Sumac is a fruit belonging to the *Rhus* genus and the Anacardiaceae family. Produced by grinding dried fruits, it is used as a popular food spice. The most important components of sumac are tannins, flavonoids, phenolic acids, essential oils and fatty acids. It has many properties such as anti-oxidant, anti-inflammatory, anti-microbial, anti-viral and anti-fungal properties. However, in traditional medicine, this herb has been used to treat diseases such as hemorrhoids, ulcers, diarrhea, liver diseases, urinary system disorders, diabetes and cancer (19, 20). In this study, it was investigated whether the sumac plant has a protective role against $CuCl_2$ damage in *S. cerevisiae*.

Hariri et al. (21) evaluated the effects of sumac on oxidative stress, inflammation, and depression in overweight or obese women with depression. They noted that sumac may have beneficial effects on obesity management in combination with restricted diet through possible regulatory effects on oxidative stress in overweight or obese depressed women. Akkoyun and Karadeniz (22) investigated the protective effects of ellagic acid (EA) on rat kidneys exposed to nicotine (N) during fetal period. They found that MDA level decreased significantly and GSH level increased in groups given EA. Momeni et al. (23) reported that sumac fruit extract significantly reduced the expression of IL-1β and IL-18 cytokines on joints and had an anti-inflammatory effect. Aslan et al. (17) reported that EA had anti-oxidative and anti-inflammatory effects against carbon tetrachloride (CCl_4) -induced kidney damage in rats. Zhou et al. (24) evaluated the preventive effect of sumac extract in mice on liver

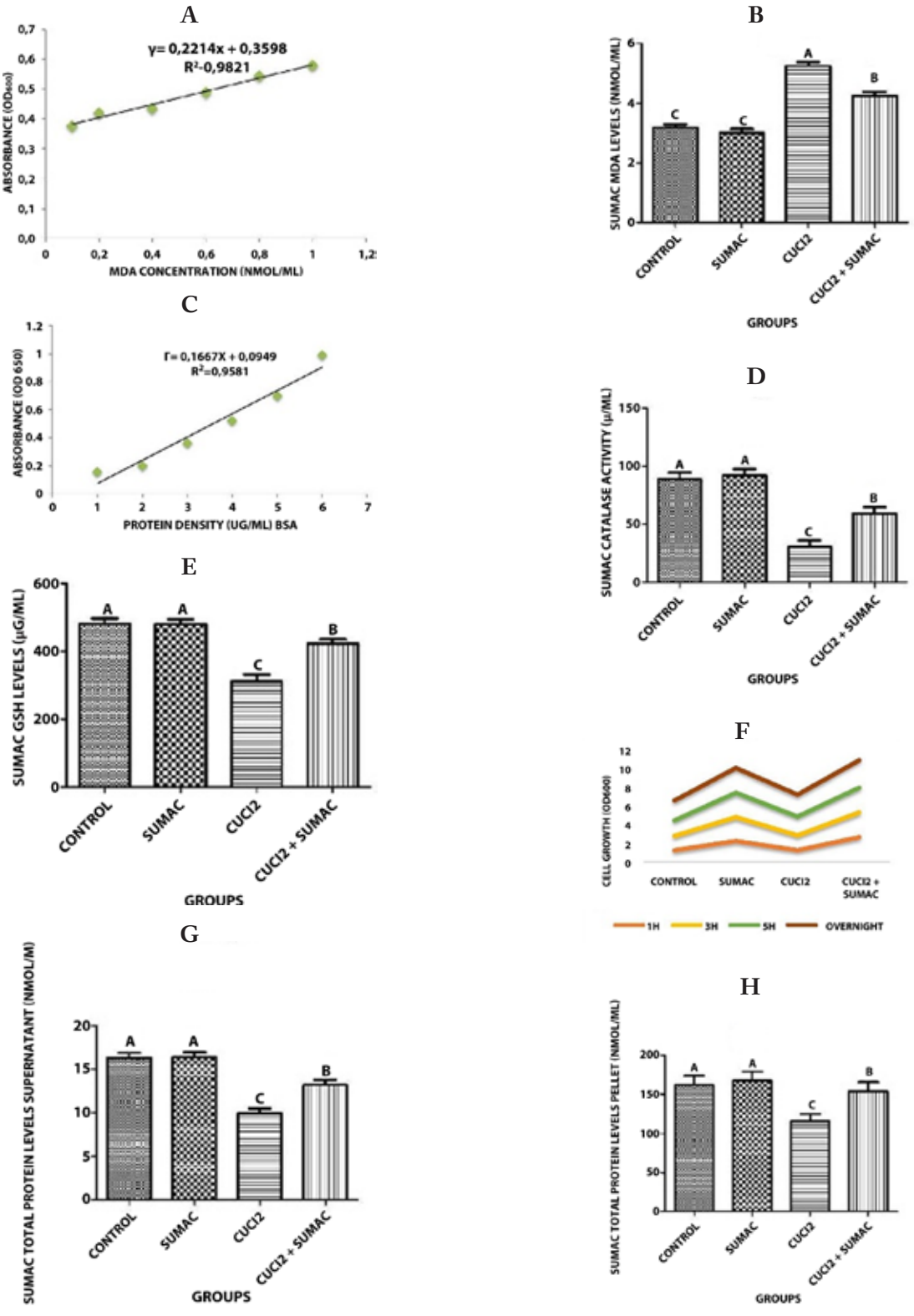

Figure 1. A: MDA standard, B: MDA level between groups, C: Standard protein of bradford bovine serum albumin (BSA), D: Catalase activity between groups, E: GSH level between groups, F: *S. cerevisiae* cell development at different times, G: Supernatant protein pellet density between groups, H: Total protein pellet density between groups.

fibrosis caused by CCI_4. They noted that sumac extract can be used as a functional food to prevent liver fibrosis or alleviate liver fibrosis.

Beyaz et al. (15) compared the protective effect of black mulberry (*Morus nigra* L.) and cranberry (*Cornus mas* L.) fruits in terms of molecular biological and biochemical parameters. They stated that MDA level decreased while cell development and total protein synthesis increased in the Black Mulberry + Cranberry + H_2O_2 group (1, 3, 5 and 24 hours) compared to the H_2O_2 group. Aslan [25] stated that mulberry extract increased cell growth by providing significant protection against H_2O_2 damage in *S. cerevisiae*. Chong et al. (26) investigated the effects of EGCG (Epigallocatechin-3 gallate) one of green tea polyphenols, on DNA repair pathways in *S. cerevisiae* and concluded that EGCG is highly effective in maintaining genome stability by activating DNA repair pathways. Aslan et al. (27) investigated the potential effect of EA in the treatment of pancreatic injury. They found that Nrf-2 and caspase-3 protein expressions, catalase activities and GSH levels increased, TNF-α, NF-κB, Bcl-2, VEGF and Akt protein expressions and MDA levels decreased in EA + CCl_4 group. Plavcova et al. (28) stated that turmeric has an anti-inflammatory effect in *S. cerevisiae*. Guan et al. (29) concluded that selastrol, which has antioxidant and anti-inflammatory effects, reduces the oxidative stress in skeletal muscle that occurs in diabetic rats. Beyaz et al. (30) investigated whether *curcumin* has a protective role against the damage caused by H_2O_2 in *S. cerevisiae* They determined that turmeric has a stimulating effect on cell growth and total protein synthesis in *S. cerevisiae* culture and stated that it has a protective role. Aslan et al. (31) indicated that according to the control group, pomegranate juice (PJ) increased fatty acid synthesis, vitamin content and cell density in PJ group. They stated that more banding was observed in PJ groups compared to the control. Beyaz et al. (32) investigated the protective role of EGCG against the damage caused by H_2O_2 in *S. cerevisiae*. EGCG decreased MDA levels in the EGCG groups compared to the H_2O_2 group. Abdallah et al. (33) stated that sumac inhibits the migration capacity of cervical cancer cells and has an inhibitory

Table 5. Supernatant protein density

Groups (Supernatant)	Protein Density (nmol/ml)
Control	16.32 ± 1.00^{a}
Sumac	16.39 ± 1.00^{a}
$CuCl_2$	9.95 ± 1.00^{c}
$CuCl_2$ + Sumac	12.20 ± 1.00^{b}

[a–c]: Among the groups which bearing of different letter are significant (p<0.05). One way Anova Post Hoc LSD test

Table 3. GSH Levels

Groups	GSH Levels (µg/ml)
Control	481.52 ± 2.00^{a}
Sumac	480.88 ± 2.00^{a}
$CuCl_2$	311.95 ± 2.00^{c}
$CuCl_2$ + Sumac	422.23 ± 2.00^{b}

[a–c]: Among the groups which bearing of different letter are significant (p<0.05). One way Anova Post Hoc LSD test

Table 6. Pellet protein density

Groups (Pellet)	Total Protein Density (nmol/ml)
Control	162.32 ± 2.00^{a}
Sumac	167.48 ± 2.00^{a}
$CuCl_2$	138.67 ± 2.00^{c}
$CuCl_2$ + Sumac	207.65 ± 2.00^{b}

[a–c]: Among the groups which bearing of different letter are significant (p<0.05). One way Anova Post Hoc LSD test

Table 4. Cell development measurement results

Groups	1h	3h	5h	24h (Overnight)
Control	1.27 ± 0.02^{d}	1.55 ± 0.02^{c}	1.66 ± 0.02^{b}	2.14 ± 0.03^{a}
Sumac	2.28 ± 0.02^{d}	2.57 ± 0.02^{c}	2.63 ± 0.02^{b}	2.65 ± 0.02^{a}
$CuCl_2$	2.61 ± 0.02^{d}	2.62 ± 0.02^{c}	2.64 ± 0.02^{b}	2.72 ± 0.02^{a}
$CuCl_2$ + Sumac	1.73 ± 0.02^{d}	2.16 ± 0.02^{c}	2.45 ± 0.02^{b}	2.58 ± 0.02^{a}

[a,b,c,d]: Among the groups which bearing of different letter are significant (p<0.05). One way Anova Post Hoc LSD test

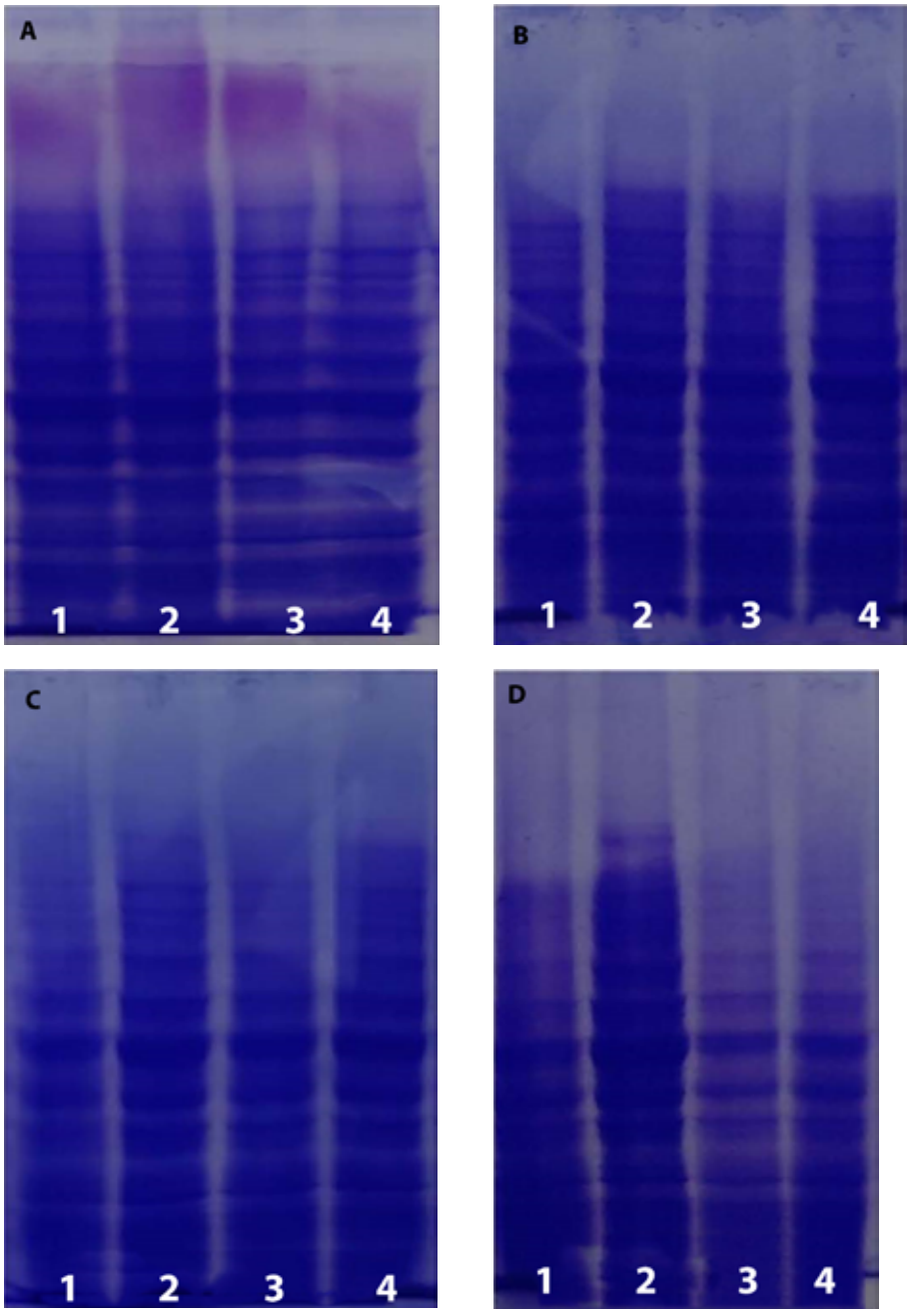

Figure 2. A: SDS-PAGE pellet protein bands (1h)**:** Bands 1: Control; 2: Sumac; 3: $CuCl_2$; 4: Sumac + $CuCl_2$, B: SDS-PAGE pellet protein bands (3h)**:** Bands 1: Control; 2: Sumac; 3: $CuCl_2$; 4: Sumac + $CuCl_2$, C: SDS-PAGE pellet protein bands (5h)**:** Bands 1: Control; 2: Sumac; 3: $CuCl_2$; 4: Sumac + $CuCl_2$, D: SDS-PAGE pellet protein bands (24h)**:** Bands 1: Control; 2: Sumac; 3: $CuCl_2$; 4: Sumac + $CuCl_2$.

effect on cell growth. They also found that sumac is an anti-proliferative agent in the treatment of cervical cancer. Aslan et al. (34) they stated that Royal jelly provides protection against heart damage and has the potential to be developed for the treatment of heart diseases. Gok et al. (35) reported that persimmon leaf reduced oxidative damage and increased cell growth in *S. cerevisiae* culture. Gok et al. (36) found that EA

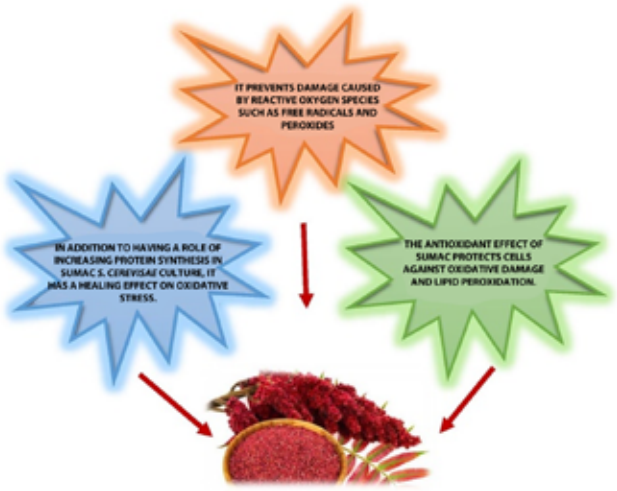

Figure 3. The effect of sumac plant on *S. cerevisiae* cell development

significantly reduced oxidative damage and had a positive effect on yeast growth. Aslan et al. (37) point out that milk thistle induces apoptosis in liver damaged rats and Aslan et al. (38) emphasized that black cumin has protective effect against carbontetrachloride-induced lung damage in rats.

Conclusion

Sumac plant has many properties against lipid peroxidation including anti-oxidant, anti-inflammatory and anti-microbial. As a result of our experimental study, it was determined that the application of sumac plant extract provides protection against damage by eliminating the oxidative damage caused by $CuCl_2$ in *S. cerevisiae*. As a result of biochemical and molecular biological analyzes, we can say that it increases cell development and total protein synthesis and protects the *S. cerevisiae* cell against oxidative damage (Figure 3).

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Conflicts of Interest: There is no conflict of interest between the authors.

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