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Molecular Biological Effects of *Gundelia tournefortii* (Kenger Herb) Extract on Oxidative Stress and Cellular Responses in *Saccharomyces cerevisiae*

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Keywords
Gundelia tournefortii (Kenger plant), Hydrogen peroxide, Oxidative damage, Protein synthesis, *Saccharomyces cerevisiae*

Abstract: *Gundelia tournefortii* (Kenger plant) is traditionally used for liver diseases, diabetes, chest pain, heart failure, stomach pain, vitiligo, diarrhea, and bronchitis. Additionally, it is known for its purgative, sedative, anti-inflammatory, antiparasitic, antiseptic, and antiemetic properties. This study investigates whether *Gundelia tournefortii* has a protective role against hydrogen peroxide (H_2O_2)-induced oxidative damage in *Saccharomyces cerevisiae*. In this study, four groups were established: Control group; H_2O_2 group (15 millimolar); *G. tournefortii* group (10%); *G. tournefortii* (10%) + H_2O_2 (15 millimolar) group. *S. cerevisiae* cultures were grown at 30°C for 1, 3, 5, and 24-hour intervals. Cell growth periods, lipid peroxidation malondialdehyde (MDA) analysis, glutathione (GSH) levels, and catalase activity (CAT) were measured using a spectrophotometer. The total protein concentration changes of *S. cerevisiae* cultures were determined by SDS-PAGE electrophoresis and calculated using the Bradford method. As a result, in the H_2O_2 group, MDA levels increased, while GSH levels and CAT activity decreased, indicating increased oxidative stress. In the *G. tournefortii* group, MDA levels decreased, while GSH levels and CAT activity increased, suggesting that this plant has a protective effect. The results suggest that *G. tournefortii* extract enhances cellular defense mechanisms by mitigating oxidative stress and promoting protein synthesis in yeast cells.

Gundelia tournefortii (Kenger Otu) Ekstresinin *Saccharomyces cerevisiae'de* Oksidatif Stres ve Hücresel Tepkiler Üzerindeki Moleküler Biyolojik Etkileri

Anahtar Kelimeler
Gundelia tournefortii (Kenger bitkisi), Hidrojen peroksit, Oksidatif hasar, Protein sentezi, *Saccharomyces cerevisiae*

Öz: *Gundelia tournefortii* (Kenger bitkisi), geleneksel olarak karaciğer hastalıkları, diyabet, göğüs ağrısı, kalp yetmezliği, mide ağrısı, vitiligo, ishal ve bronşit için kullanılır. Ayrıca müshil, yatıştırıcı, iltihap giderici, antiparaziter, antiseptik ve kusma önleyici özellikleriyle de bilinir. Bu çalışma, *Gundelia tournefortii'nin Saccharomyces cerevisiae'de* hidrojen peroksit (H_2O_2) kaynaklı oksidatif hasara karşı koruyucu bir rolü olup olmadığını araştırmaktadır. Bu çalışmada dört grup oluşturuldu: Kontrol grubu; H_2O_2 grubu (15 milimolar); *G. tournefortii* grubu (%10); *G. tournefortii* (%10) + H_2O_2 (15 milimolar) grubu. *S. cerevisiae* kültürleri 30°C'de 1, 3, 5 ve 24 saat boyunca büyütüldü. Hücre büyüme periyotları, lipid peroksidasyon malondialdehit (MDA) analizi, glutatyon (GSH) seviyeleri ve katalaz aktivitesi (CAT) bir spektrofotometre kullanılarak ölçüldü. *S. cerevisiae* kültürlerinin toplam protein konsantrasyonundaki değişimler SDS-PAGE elektroforezi ile belirlendi ve Bradford yöntemi kullanılarak hesaplandı. Sonuç olarak, H_2O_2 grubunda MDA seviyeleri artarken, GSH seviyeleri ve CAT aktivitesi azaldı ve bu da oksidatif stresin arttığını gösterdi. *G. tournefortii* grubunda ise MDA seviyeleri azalırken, GSH seviyeleri ve CAT aktivitesi arttı ve bu da bu bitkinin koruyucu etkiye sahip olduğunu gösterdi. Sonuçlar, *G. tournefortii* özütünün oksidatif stresi azaltarak maya hücrelerinde protein sentezini teşvik ederek hücresel savunma mekanizmalarını güçlendirdiğini göstermektedir.

1. INTRODUCTION

For centuries, humans have used compounds derived from plants to treat or prevent diseases. The use of medicinal plants and the identification of their roles in treatment processes have continued to gain importance, with their significance steadily increasing over time. Traditionally used plants continue to inspire new ideas and important research in today's pharmaceutical industry. The plants used for treating various ailments and the specific disorders for which they are used have been passed down orally from generation to generation. Recently, there has been a growing interest in herbal supplements and functional food products due to their nutritional and health benefits. The subject of this project, *Gundelia tournefortii*, is an endemic, edible plant belonging to the Asteraceae family, commonly known by various names, including "kenger otu," "haskenger," "akub," and "kanger." It is a plant with well-known health benefits and has a long history of traditional uses. *Gundelia tournefortii* (Kenger plant) is a therapeutic plant known for its antibacterial, anti-inflammatory, hepatoprotective, antiplatelet, and hypolipidemic activities [1,2].

In Turkish folk medicine, it has been used to treat diabetes, act as a painkiller, relieve cramps, treat indigestion, manage hypoglycemia, strengthen nerves, and alleviate migraines. Its stem is particularly known for its hepatoprotective and blood-purifying properties, while its fruit is used to treat vitiligo [2]. *G. tournefortii* is a perennial plant that grows to about 60 cm in height, with branched stems that are almost hairless and spiny, resembling thistles. Its leaves are leathery and tough, with distinct yellow, red, or purple veins arranged alternately. Its flowers can be white, yellow, purple, red, or green. The stems, flowers, leaves, and seeds of *G. tournefortii* can be used as food. In Turkey, the dried plant is stored as winter food. The latex sap of *G. tournefortii* is used for various traditional purposes, while its seeds are considered a coffee alternative and consumed [3].

Figure 1. *Gundelia tournefortii* (Kenger plant) [4]

Saccharomyces cerevisiae (*S. cerevisiae*) is a species of yeast known as the simplest eukaryotic model organism. Yeast cells are exposed to various stresses, and each stress factor has the potential to cause cellular damage. Oxidative stress is one of these stress factors. *S. cerevisiae* cells must cope with the toxic effects of reactive oxygen species (ROS) derived from molecular oxygen. These reactive oxygen species include superoxide anion ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydroxyl radical (OH•), and hydrogen peroxide (H_2O_2). Particularly, hydrogen peroxide (H_2O_2) is a harmful oxidative stress factor for *S. cerevisiae* cells. It accumulates within the cell and targets cellular structures, especially proteins, lipids, and DNA, leading to oxidative damage and disrupting normal cellular functions. Hydrogen peroxide (H_2O_2), as a strong reactive oxygen species (ROS), causes oxidative effects on proteins, lipids, and DNA, leading to oxidative damage in these biomolecules. This damage results in the disruption of cellular functions, harm to genetic material, and imbalance in biological systems. Oxidative damage can prevent cells from performing their normal functions and contribute to the development of pathological conditions such as aging, cancer, cardiovascular diseases, and neurological disorders. H_2O_2 is commonly used in both in vivo and in vitro conditions for modeling oxidative stress, making it a valuable research tool to study the effects of oxidative stress on biological systems [5].

Oxidative stress arises from the imbalance between free radicals and antioxidant defense mechanisms in cells. This imbalance results in the damage caused by reactive oxygen species (ROS) targeting cellular structures. Biomolecules such as DNA, proteins, lipids, and carbohydrates are among the structures most affected by this damage. To counteract the effects of oxidative stress, organisms rely on protective mechanisms such as antioxidant enzymes and reduced glutathione. Lipid oxidation is considered an indicator of cellular stress, and in association with this process, levels of malondialdehyde (MDA) increase [6]. This study aims to investigate the protective effects of *Gundelia tournefortii* (Kenger plant) against hydrogen peroxide (H_2O_2)-induced oxidative damage in the *S. cerevisiae* cell model. Furthermore, the study will evaluate the effects of *G. tournefortii* extracts on antioxidant and oxidative stress parameters, such as cellular development, malondialdehyde (MDA) levels, glutathione (GSH) levels, catalase (CAT) activity, and total protein concentration.

2. MATERIAL AND METHOD

2.1. Material

In this study, MDA, GSH, catalase activity (CAT) analyses were performed using a spectrophotometer device, and protein electrophoresis was performed using an SDS-PAGE mini protein device. Some of the chemical consumables used are as follows; glucose, tryptone, yeast extract, hydrogen peroxide (H_2O_2).

2.2. Experimental Groups

In this study, the therapeutic effects of *Gundelia tournefortii* (Kenger plant) extract against hydrogen peroxide (H_2O_2)-induced damage in *S. cerevisiae* were investigated. Four different groups were established, as follows: 1) Control group, 2) H_2O_2 group (15 millimolar),

3) *G. tournefortii* group (10%), 4) *G. tournefortii* (10%) + H_2O_2 (15 millimolar) group.

2.3. Application of *Gundelia tournefortii* (Kenger plant) Extract and Hydrogen Peroxide (H_2O_2) to the Culture

Preparation of *S. cerevisiae* Culture Medium: The medium required for yeast growth was prepared using YEPD medium. For this medium, 7.5 g of yeast extract, 7.5 g of tryptone, and 7.5 g of glucose were mixed per 250 mL solution. The prepared medium was transferred into five Erlenmeyer flasks, 50 mL each, and sterilized in an autoclave at 121°C for 1 hour. After sterilization, the flasks were allowed to cool at room temperature. Once cooled, 800 µl of yeast was added to each flask, and the cultures were incubated in an incubator for 20 min. After the incubation period, yeast development was assessed by optical density measurements. Preparation of 10% *Gundelia tournefortii* (Kenger plant) Extract: 10 g of *G. tournefortii* (Kenger plant) was added to 100 mL of boiling distilled water and left to steep for 15-20 min. After the steeping process, the mixture was filtered through a sterile cloth and was ready to be added to the culture medium.

Figure 2. *Gundelia tournefortii* (Kenger plant) and Extract

2.4. Malondialdehyde (MDA) Analysis

Malondialdehyde (MDA) reacts with TBA to form a pink-colored compound. The concentration of MDA is determined by measuring the absorbance of this compound at 535 nm using a spectrophotometer. 0.4 mL of supernatant was mixed with 1.6 mL of Tris-KCl buffer, and 0.5 mL of 30% TCA was added. After 0.5 mL of 0.75% TBA was added and boiled, then centrifuged at 4000 rpm. The clear supernatant was collected, and the absorbance was measured at 532 nm [7,8].

2.5. Glutathione (GSH) Analysis

The GSH analysis was performed according to the Ellman [9] method. Reduced glutathione reacts with 5,5'-dithiobis 2-nitrobenzoic acid to form a yellow-green colored compound. The light intensity of this compound was spectrophotometrically measured at 412 nm [9,10].

2.6. Catalase (CAT) Activity Assay

The method developed by Aebi was used to measure CAT (catalase) activity. The measurement principle is based on the light absorption properties of hydrogen peroxide (H_2O_2) at a wavelength of 240 nm [11,12].

2.7. Total Protein Concentration Measurements (Bradford Method)

Total protein concentration was determined spectrophotometrically at 595 nm using the Bradford method. The protein amount in S. cerevisiae groups was calculated comparatively using BSA protein standards [13,14].

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

After taking 1 mL of the culture sample, it was centrifuged at 13,000 rpm for 5 min. The resulting pellet was resuspended with 500 µl of TEA (Tris-EDTA Acetic Acid) buffer (pH: 7.5). The cells were disrupted using a Bandelin Sonopuls sonicator (Germany) at power level 2, with two rounds of sonication, each lasting 10 seconds. After sonication, the sample was incubated on ice for 5 min. and then centrifuged again at 13,000 rpm for 10 min. The resulting pellet was mixed with an equal amount of sample buffer (Sample Amplification Buffer) to prepare the sample for SDS-PAGE [15,16].

2.9. SDS-PAGE (Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis) Analysis

Before loading the protein samples from *Saccharomyces cerevisiae* cultures into the wells, they were boiled for 5 min. after the addition of an equal amount of SDS-PAGE Sample Amplification Buffer (SAB) dye. A 1x tank buffer was used for electrophoresis. Afterward, a 30 mA current was applied until the blue band of bromophenol blue (tracking dye) reached the end of the gel, allowing the movement of proteins in the gel to be tracked. After electrophoresis, the gel was stained with Coomassie Brilliant Blue for 30 min. to 1 hour at room temperature. The gel was then washed with a stain remover solution until the protein bands became visible. Gel images were taken, and the protein bands between the groups were analyzed [17, 18].

2.10. Statistical Analysis

The statistical data of the study were presented as mean ± standard error (Mean ± Sd). The analyses were repeated 3 times. Comparisons between the groups were made using One-Way Analysis of Variance (ANOVA), and the analyses were performed using the Statistical Package for Social Sciences (SPSS, SPSS Inc., Chicago, IL, USA) software version 25.0. Statistical significance was determined at $p<0.05$.

3. RESULTS

In this study, the protective effects of *Gundelia tournefortii* (Kenger otu) extract against H_2O_2-induced oxidative stress were investigated through biochemical parameters such as malondialdehyde (MDA), catalase (CAT), glutathione (GSH) levels, cell growth, and protein concentration. The obtained results show that *G. tournefortii* significantly reduced the oxidative damage

caused by H_2O_2 and played an effective role in protecting the cells. The findings are as follows:

As presented in Table 1 and Figure 3, the H_2O_2-treated group exhibited significantly lower cell growth at all time points compared to the control group ($p<0.05$). This result supports the hypothesis that H_2O_2 inhibits cell growth. However, the *G. tournefortii* + H_2O_2 group showed significantly higher cell growth compared to the H_2O_2-only group ($p<0.05$). The data in Table 1 demonstrate that while H_2O_2 inhibits cell growth significantly, *G. tournefortii* extract mitigates this inhibition and preserves cell viability.

Table 1. Cell Growth (1h, 3h, 5h, 24h)

Groups	1h	3h	5h	24h (Overnight)
Control	0.624 ± 0.04[a]	0.743 ± 0.06[b]	0.935 ± 0.07[b]	1.266 ± 0.04[b]
G. tournefortii	0.697 ± 0.03[c]	0.808 ± 0.05[d]	1.263 ± 0.05[d]	1.615 ± 0.05[d]
H_2O_2	0.515 ± 0.02[a]	0.558 ± 0.01[a]	0.596 ± 0.03[a]	1.013 ± 0.02[a]
G. tournefortii + H_2O_2	0.585 ± 0.03[b]	0.579 ± 0.02[c]	0.644 ± 0.05[c]	1.135 ± 0.03[c]

[a,b,c,d] The differences between the groups carrying different letters are statistically significant ($p<0.05$). One-way ANOVA Post Hoc Tukey Test.

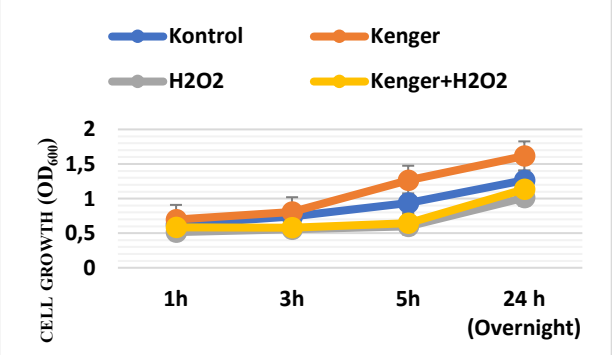

Figure 3. Time-Based Changes in *S. cerevisiae* Cell Development (1, 3, 5, and 24 Hours)

MDA is a marker of oxidative stress as an indicator of lipid peroxidation. As shown in Table 2 and Figure 4 the H_2O_2 group exhibited significantly higher MDA levels compared to the Control group (0.44 ± 0.06 nmol/mL vs. 0.33 ± 0.02 nmol/mL, $p<0.05$). This confirms that H_2O_2 increases oxidative stress. The *G. tournefortii* + H_2O_2 group, however, showed significantly lower MDA levels compared to the H_2O_2 group (0.37 ± 0.04 nmol/mL vs. 0.44 ± 0.06 nmol/mL, $p<0.05$). This indicates that *G. tournefortii* significantly reduced the H_2O_2-induced MDA increase, demonstrating a protective effect against lipid peroxidation. This suggests that *G. tournefortii* reduces H_2O_2-induced oxidative stress and prevents lipid peroxidation.

Table 2. Malondialdehyde (MDA) Level

Groups	MDA Level (nmol/mL)
Control	0.33 ± 0.02[c]
G. tournefortii	0.31 ± 0.03[c]
H_2O_2	0.44 ± 0.06[a]
G. tournefortii + H_2O_2	0.37 ± 0.04[b]

a–c: The differences between the groups carrying different letters in the same column are statistically significant ($p<0.05$). One-way ANOVA Post Hoc Tukey Test

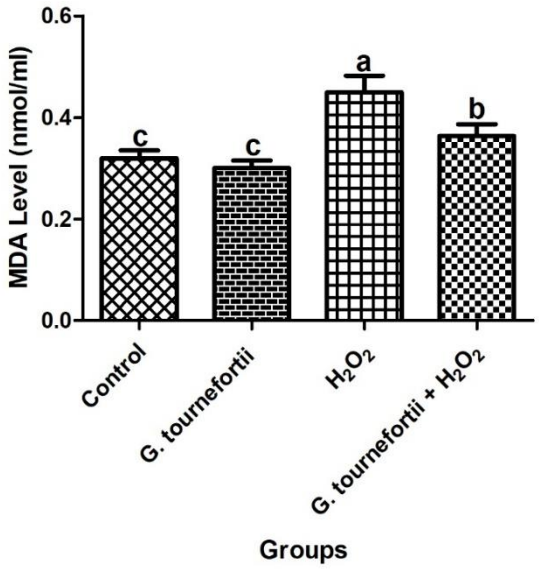

Figure 4. MDA results

Glutathione (GSH) is an important molecule that neutralizes free radicals in cells, preventing oxidative damage, and contributes to the body's defense system.

According to the data presented in Table 3 and Figure 5 the H_2O_2 group exhibited significantly lower glutathione levels compared to the Control group (1.61 ± 0.05 µg/mL vs. 2.08 ± 0.12 µg/mL, $p<0.05$). This confirms that H_2O_2 increases oxidative stress by decreasing glutathione levels. On the other hand, the *G. tournefortii* + H_2O_2 group showed significantly higher glutathione levels compared to the H_2O_2 group (2.09 ± 0.10 µg/mL vs. 1.61 ± 0.05 µg/mL, $p<0.05$). These findings suggest that *G. tournefortii* supports antioxidant defense mechanisms by preventing the reduction of glutathione induced by H_2O_2.

Table 3. Glutathione (GSH) Level

Groups	**GSH Seviyesi (µg/mL)**
Control	2.08 ± 0.12[a]
G. tournefortii	2.09 ± 0.10[a]
H_2O_2	1.61 ± 0.05[c]
***G. tournefortii* + H_2O_2**	1.92 ± 0.06[b]

a–c: The differences between the groups carrying different letters in the same column are statistically significant ($p<0.05$). One-way ANOVA Post Hoc Tukey Test

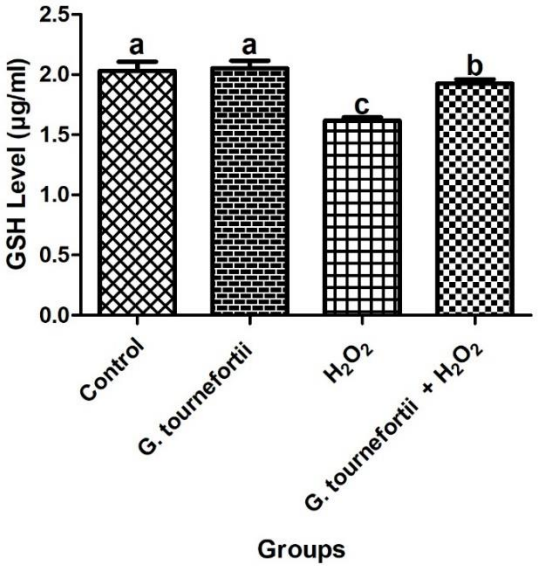

Figure 5. GSH results

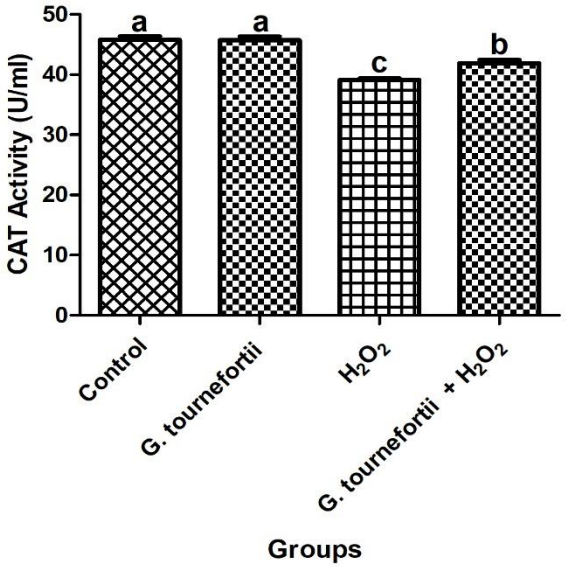

Figure 6. CAT results

Catalase is an important enzyme that converts hydrogen peroxide, which can accumulate in cells and cause oxidative damage, into water and oxygen. According to the data presented in Table 4 and Figure 6, the H_2O_2 group exhibited significantly lower catalase activity compared to the Control group (39.32 ± 0.56 U/mL vs. 45.76 ± 1.05 U/mL, $p<0.05$).

This result indicates that H_2O_2 suppresses antioxidant defense. In the *G. tournefortii* + H_2O_2 group, however, significantly higher catalase activity was observed compared to the H_2O_2 group (45.68 ± 1.03 U/mL vs. 39.32 ± 0.56 U/mL, $p<0.05$). These findings suggest that *G. tournefortii* supports antioxidant defense by preventing the reduction of catalase activity caused by H_2O_2. *G. tournefortii* significantly reversed the decrease in catalase (CAT) and glutathione (GSH) levels induced by H_2O_2, thereby strengthening cellular defense.

Table 4. Catalase (CAT) Activity

Groups	CAT Seviyesi (U/mL)
Control	45.76 ± 1.05[a]
G. tournefortii	45.68 ± 1.03[a]
H_2O_2	39.32 ± 0.56[c]
***G. tournefortii* + H_2O_2**	41.89 ± 0.95[b]

a–c: The differences between the groups carrying different letters in the same column are statistically significant ($p<0.05$). One-way ANOVA Post Hoc Tukey Test

As shown in Table 5, 6 and Figures 7, 8, and 9 the H_2O_2 group exhibited significantly lower protein density in both the supernatant and pellet images compared to the control group ($p<0.05$). This confirms that H_2O_2 inhibits protein synthesis. The *G. tournefortii* + H_2O_2 group, however, showed significantly higher protein density in both the supernatant and pellet images compared to the H_2O_2 group ($p<0.05$). This suggests that *G. tournefortii* reduces the inhibition of protein synthesis caused by H_2O_2 and supports protein synthesis.

Table 5. Protein Density Level (Supernatant)

Groups (Supernatant)	Total Protein Level (nmol/mL)
Control	4.50 ± 0.17[b]
G. tournefortii	4.57 ± 0.18[a]
H_2O_2	3.19 ± 0.08[d]
***G. tournefortii* + H_2O_2**	3.93 ± 0.12[c]

a–d: The differences between the groups carrying different letters in the same column are statistically significant ($p<0.05$). One-way ANOVA Post Hoc Tukey Test

Table 6. Protein Density Level (Pellet)

Groups (Pellet)	Total Protein Level (nmol/mL)
Control	5.37 ± 0.07[a]
G. tournefortii	5.42 ± 0.06[a]
H_2O_2	4.32 ± 0.03[c]
***G. tournefortii* + H_2O_2**	4.95 ± 0.05[b]

a–c: The differences between the groups carrying different letters in the same column are statistically significant ($p<0.05$). One-way ANOVA Post Hoc Tukey Test

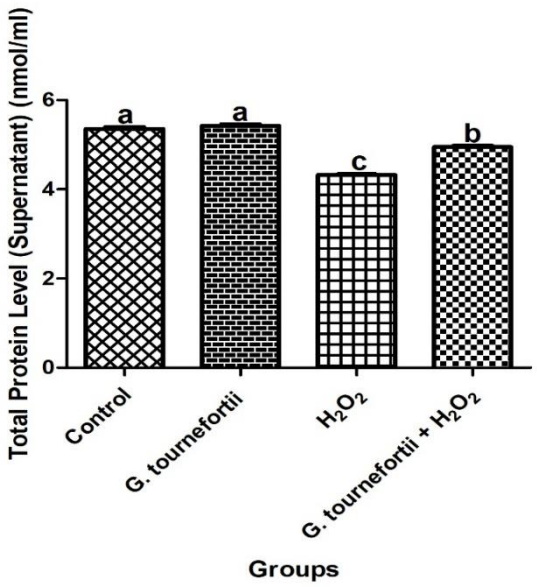

Figure 7. Supernatant Protein Density

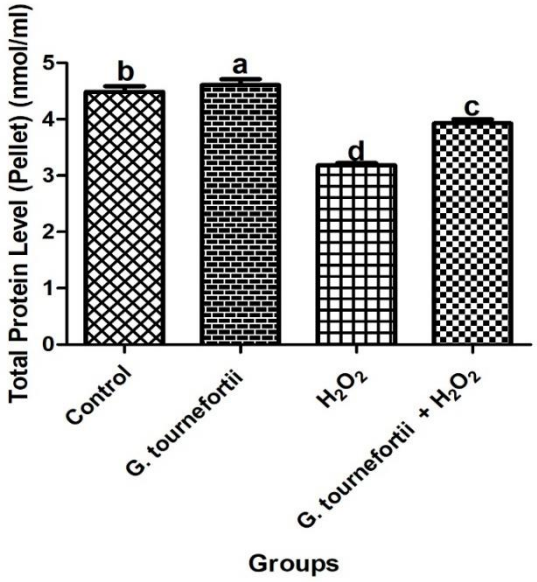

Figure 8. Pellet Protein Density

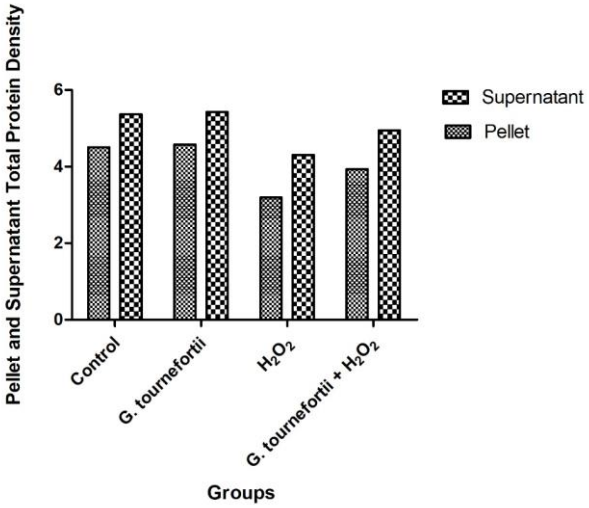

Figure 9. Pellet anad Supernatant Protein Density

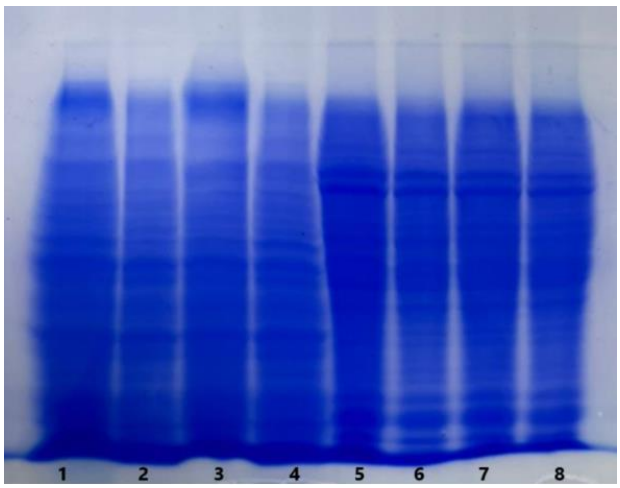

Figure 10. Groups (Supernatant); 1: Control; 2: *G. tournefortii*; 3: H2O2; 4: *G. tournefortii* + H2O2; Groups (Pellet); 5: Control; 6: *G. tournefortii*; 7: H2O2; 8: *G tournefortii* + H2O2

The SDS-PAGE gel image of the supernatant and pellet proteins obtained after subjecting *S. cerevisiae* cells to different Groups was examined (Figure 10):

Band-1; Control-Supernatant: In the Control group, distinct bands corresponding to proteins normally produced by the cells under normal conditions were observed at different molecular weights. This indicates normal protein expression.

Band-2; *G. tournefortii*-Supernatant: In the cells treated with *G. tournefortii*, a band similar to the Control group was observed. This shows that *G. tournefortii* has no effect on extracellular protein secretion.

Band-3; H_2O_2-Supernatant: A significant decrease in band intensity was observed in the cells treated with H_2O_2. This indicates that H_2O_2 inhibits extracellular protein secretion and the cells secrete fewer proteins in response to stress.

Band-4; *G. tournefortii* + H_2O_2-Supernatant: An increase in band intensity was observed in cells treated with both *G. tournefortii* and H_2O_2. This indicates that *G. tournefortii* protects cells from the harmful effects of H_2O_2.

Band-5; Control-Pellet: In the pellet image of the Control group, distinct bands corresponding to intracellular proteins were observed. This represents the intracellular proteins produced by the cells under normal conditions.

Band-6; *G. tournefortii*-Pellet: In the cells treated with *G. tournefortii*, a band similar to the Control group was observed. This indicates that *G. tournefortii* has no effect on intracellular protein expression.

Band-7; H_2O_2-Pellet: A significant decrease in band intensity was observed in the cells treated with H_2O_2. This shows that H_2O_2 inhibits intracellular protein expression and that the cells produce fewer proteins in response to stress. The decrease in low molecular weight proteins is particularly notable.

Band-8; *G. tournefortii* + H_2O_2-Pellet: An increase in band intensity was observed in the cells treated with both *G. tournefortii* and H_2O_2. This indicates that *G. tournefortii* partially reverses the decrease in protein expression caused by H_2O_2 and protects the cells.

4. DISCUSSION

Recent studies have shown that *G. tournefortii* possesses anticancer, anti-inflammatory, antioxidant, anti-cholesterolemic, antibacterial, hepatoprotective, and hypoglycemic effects [19].

Abu Lafi et al. [20] examined the anticancer effects of the *Gundelia tournefortii* plant on human colon cancer HCT-116 cell lines. The study found that the methanol and hexane extracts exhibited significant antitumor effects on cancer cells, while the aqueous extract was ineffective. Additionally, the phytochemical profiles of these extracts were analyzed using gas chromatography-mass spectrometry, and it was determined that 6 out of 27 natural compounds were active against cancer cells. Among these compounds were sitosterol, stigmasterol, lupeol, gitoxigenin, α-amyrin, and artemisinin. These findings suggest that *Gundelia tournefortii* may be a potential source for cancer treatment. Bati et al.[6] investigated the effects of *G. tournefortii* extract on DNA damage, biochemical parameters, and antioxidant activities in rats fed a high-calorie diet. The study was conducted by dividing Wistar albino male rats into four groups and monitoring them for 12 weeks. The results indicated that *G. tournefortii* extract positively reduced DNA damage in brain tissue and improved MDA levels by affecting lipid peroxidation in liver tissue. Furthermore, the plant extract was found to significantly improve antioxidant parameters and demonstrated potential anti-obesity effects by targeting obesity. Vazquez et al. [21] investigated the effects of melatonin on cellular defense against oxidative stress in *S. cerevisiae* cells. The aim of the study was to understand how melatonin affects endogenous cellular defense systems by examining cell viability, glutathione levels (reduced GSH and oxidized GSSG), reactive oxygen species (ROS) production, and the expression of genes related to antioxidant defense systems. The study observed changes in glutathione levels after melatonin was applied to the cells; GSH levels decreased, while GSSG levels increased. Furthermore, melatonin-activated genes are related to the antioxidant defense system, leading to a stronger cellular defense against oxidative stress. In cells subjected to oxidative stress with H_2O_2, melatonin treatment reduced ROS accumulation and increased GSSG levels, thereby reducing cell damage. As a result, melatonin was found to exhibit antioxidant properties in *S. cerevisiae* cells and strengthen cellular defense systems. Aslan [15] studied the protective effects of goji berry against oxidative damage induced by chromium ($K_2Cr_2O_7$) in *S. cerevisiae*. The study showed that goji berry extract reduced $K_2Cr_2O_7$-induced oxidative stress, preserved cell viability, and increased antioxidant enzyme activity. Amer et al. [3] investigated the effects of *G. tournefortii* on hepatocellular carcinoma (HCC). They found that *G. tournefortii* treatment increased tumor size in HCC mice, inhibited cell proliferation, reduced inflammation, and increased antioxidant levels. Furthermore, the treatment inhibited the p53 and phosphorylated PI3K/AKT/mTOR signaling pathways, leading to significant changes in HCC cells. The study suggested that *G. tournefortii* exhibited anticancer and antiproliferative effects and could be a potential therapeutic option for liver cancer.

Hachem et al. [22] examined the effects of *G. tournefortii* extract on liver and kidney damage induced by PCOS. PCOS is an endocrine disorder that increases the risk of kidney and liver damage, and the long-term use of current treatment methods can lead to undesirable side effects. The study explored the potential of *G. tournefortii* to improve liver and kidney damage by regulating oxidative stress pathways. The researchers found that *G. tournefortii* modulated oxidative stress and inflammation, alleviating damage in these organs. In an experiment conducted on mice, the extract treatment reduced ALT and AST levels by 50% and decreased the oxidant marker MDA by 65%. Additionally, superoxide dismutase (SOD) and catalase (CAT) activities returned to normal. Pro-inflammatory cytokines IL-1β and TNF-α were reduced by 80% and 68%, respectively. The study concluded that *G. tournefortii* reduced inflammation in the kidneys by regulating the NLRP3 inflammasome and improved liver and kidney health by reducing oxidative stress. These findings suggest that *G. tournefortii* has complementary potential in the treatment of PCOS.

Karatas [23] evaluated the antioxidant compounds and oxidative stress-related parameters in *G. tournefortii* (thistle). The study determined the levels of vitamin A, vitamin E, vitamin C, MDA, reduced GSH, and oxidized glutathione (GSSG) in the plant. These analyses revealed that the thistle plant exhibited healthy development and was particularly rich in vitamin C and reduced GSH. Additionally, the low MDA level suggested that the plant had not encountered oxidative stress, further indicating healthy growth. The results showed that *G. tournefortii* possesses antioxidant properties, and these properties could provide potential health benefits.

Beyaz et al. [24] evaluated the effects of black mulberry (*Morus nigra* L.) and cornelian cherry (*Cornus mas* L.) fruits on oxidative damage, focusing on molecular biological and biochemical parameters. The study examined parameters such as cell development, MDA levels, reduced GSH, and total protein synthesis. They found that both black mulberry and cornelian cherry fruits played a significant role in reducing oxidative stress. Specifically, in the *Black Mulberry + Cornelian Cherry* + H_2O_2 group, cell development was enhanced, and MDA levels were reduced compared to the H_2O_2-only group. Since MDA is a marker of oxidative stress, this finding suggests that black mulberry and cornelian cherry fruits reduce cellular oxidative damage. Additionally, GSH levels were significantly higher in the black mulberry and cornelian cherry groups compared to the H_2O_2 group. In addition, Aslan [25], Aslan et al. [26] and Beyaz et al. [27] indicated that Mulberry Juice, kiwi fruit juice and epigallocatechin-3 gallate have protective effect against

oxidative stress. In conclusion, black mulberry and cornelian cherry fruits were found to have protective properties against oxidative stress and support cellular growth and protein synthesis, providing beneficial effects on health.

5. CONCLUSION

In conclusion, it has been determined that *G. tournefortii* extract exhibits a strong protective effect against H_2O_2-induced oxidative stress. This extract significantly reduced MDA levels, which are the end product of lipid peroxidation caused by H_2O_2, while showing a significant increase in antioxidant enzyme markers, such as GSH and CAT levels. Furthermore, *G. tournefortii* extract alleviated the detrimental effects of H_2O_2 on protein synthesis and had a positive effect on cell development by increasing cell density. These findings suggest that *G. tournefortii* extract could be an effective natural compound in preventing biological damage caused by oxidative stress and may be considered a potential therapeutic option.

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Conflict of Interest
No potential conflict of interest was reported by the author(s).

Data Availability Statement
All data from the present study were inserted in this manuscript.

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