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Research Article

THE MOLECULAR BIOLOGICAL INVESTIGATION OF *ACHILLEA MILLEFOLIUM* (YARROW) EXTRACT ON *SACCHAROMYCES CEREVISIAE*

Saime Delioglu, Ozlem Gok, Seda Beyaz, Abdullah Aslan*

Firat University, Faculty of Science, Department of Biology-Molecular Biology and Genetics Program, Elazıg, Turkiye

**Corresponding Author:*
E-mail: aaslan@firat.edu.tr

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ABSTRACT. Yarrow, whose scientific name is "*Achillea millefolium*", is a flowering plant belonging to the Asteraceae family. Almost all parts of *Achillea millefolium*, a perennial herbaceous plant, are medically useful. It has many bioactive features such as anti-inflammatory, anti-spasmodic, analgesic, blood coagulant, wound healing, anti-hypertension, expectorant and menstrual regulator. Today, Covid-19, caused by severe acute respiratory syndrome, has brought about some studies on using natural therapeutics along with researching drug treatment, as well as developing a vaccine. For this reason, by investigating the natural therapeutic effect of the *Achillea millefolium* extract that we will use in our study, studies on its use as an alternative medicine in Covid-19 will be accelerated. This study aims to examine the protective effects of *Achillea millefolium* plant extract against changes at the cellular level. After the addition of carbon tetrachloride and *Achillea millefolium* extracts, the cultures were allowed to develop at 30°C. It was determined that oxidative stress and lipid peroxidation decreased and catalase and glutathione levels, which are anti-oxidant defense markers, increased after treatment with *Achillea millefolium* extracts. Based on these findings, it can be concluded that *Achillea millefolium* has beneficial properties in protecting the cell against oxidative damage.

Keywords: *Achillea millefolium, oxidative damage, protein synthesis, Saccharomyces cerevisiae.*

INTRODUCTION

Achillea genus is a plant belonging to the Asteraceae family, containing more than 130 perennial plant species native to the Northern Hemisphere from Europe to Asia. It grows in temperate climates and dry or semi-dry habitats. *Achillea millefolium* (yarrow), the most common species growing in temperate climates, has been among the plant species most frequently used in both folk and traditional medicine for over 3000 years. Although it is commonly known as yarrow, it has different local names in various countries. *Achillea* is represented by 46 taxon, 25 of which are special to, in Turkey and 19 species, 7 of which are endemic, in Iran. The plant usually blooms from May to June, with real growing occurring in spring [1].

Fig. 1. *Achillea millefolium (yarrow) [1]*

Achillea millefolium (yarrow) is a small, perennial, tufted plant, 50 cm tall, with a slender rootstock that shoots numerous roots and stolons [2]. The color of its flowers varies from pale yellow, ivory, white and golden yellow. Its flowers are white in color and appear in the form of large anthers, tubular and tongue-shaped. It is important to collect the flowers during the hours when the sun is most intense in order for the therapeutic effect of the essential oils to be at their highest level [1, 3]. *Achillea millefolium* is used for diseases such as diabetes, high blood pressure, kidney stones, muscle pain, acne, bleeding disorders, cough, flu and pneumonia [2, 4]. It is prescribed for the treatment of hepatitis B and C. It is efficient in respiratory tract infections and gastrointestinal disorders. It is a digestive system and carminative. It diminishes fever through sweating [2].

Fig. 2. *Flower, leaf, root and seed image of Achillea millefolium [5]*

Achillea millefolium contains a wide variety of bioactive components such as necessary oil, flavonoids, tannins, alkaloids, glycosides, choline, azulene, kamazulene, salicylic acid, sesquiterpenoids, eucalyptol, terpineol, amino acids [2, 6]. The presence of these components is effective in showing anti-oxidant, anti-diabetic, anti-bacterial, anti-inflammatory, anti-hepatoxic, anti-cancer and anti-tumor properties [1, 7]. It has also been determined by studies that it is a strong anti-oxidant against free radicals and reactive oxygen species [8, 9]. *Achillea millefolium* is a plant frequently used as a fresh or dried

herb in the Middle East. It is used in dissimilar forms such as tea blends, tinctures, tablets and ointments, between other formulations [10].

Carbon tetrachloride (CCl_4) is a dense, colorless and non-flammable liquid. It has a regular tetrahedron molecular structure. It is insoluble in water and soluble in nonpolar solvents such as alcohol. It can be easily taken into the body by inhalation, swallowing or through the skin and is quickly absorbed from the gastrointestinal tract. The toxicity of CCl_4 shows its effect at the biochemical and cell organelle level. It is a powerful hepatotoxin. It distributes to all tissues and organs and causes changes in the microsomal enzymes of the liver. Thus, it reduces protein synthesis by disrupting amino acid secretion. It can also cause the manufacture of reactive oxygen types that trigger lipid peroxidation in the biological membrane by decrease the activities of antioxidant enzymes [15, 16].

CCl_4 is an organic solvent and has a nonpolar molecular structure. Four chlorine (Cl) atoms are bonded to a carbon molecule in a tetrahedral manner, and with the exchange of H and Cl ions, it becomes a trichloromethane ($CHCl_3$) molecule. CCl_4 is obtained as the product of many chemical reactions. CCl_4, which has a long half-life, decays very slowly. Because it has a stable chemical structure [17].

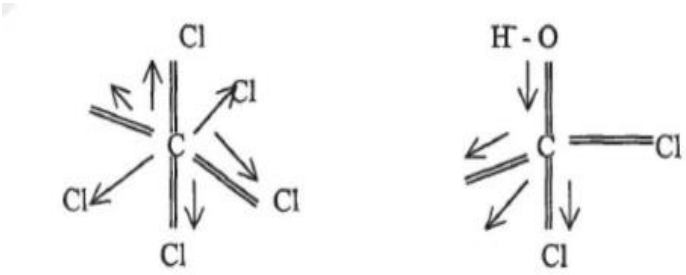

Fig. 3. *Molecular structure of carbon tetrachloride [18]*

MATERIALS AND METHODS

Research Groups of the Study

In this study, the molecular and biological effects of *Achillea millefolium* extract against CCl_4-induced damage in *Saccharomyces cerevisiae* were investigated. 6 groups were created in the study.

Our groups:

Group (1): Control Group
Group (2): Carbon tetrachloride (CCl_4) Group (10 mM)
Group (3): *Achillea millefolium*-10% Group
Group (4): *Achillea millefolium*-30% Group
Group (5): CCl_4 (10 mM) + *Achillea millefolium*-10% Group
Group (6): CCl_4 (10 mM) + *Achillea millefolium*-30% Group

Cell Development Environment of Saccharomyces cerevisiae

For the growth and reproduction of yeasts, 15 g of yeast extract, 15 g of tryptone and 15 g of glucose were weighed and prepared for 500 ml of YEPD. Then, 7 conical flasks were taken (1 conical flask in which *Saccharomyces cerevisiae* was not planted) and 50 ml of the prepared 500 ml medium was added to each of the conical flasks. After being

kept in the autoclave at 121^{o}C for 1 hour, it was removed and cooled. Yeast was planted in each flask next to the burner flame. After waiting in the oven for 20 minutes, yeast proliferation (yeast threshold value) was checked by blank measurement on a spectrophotometer [19].

Preparation of Achillea millefolium (Yarrow) Extract and Carbon tetrachloride (CCl_4)

Achillea millefolium was collected from Keban district of Elazığ province in spring. The collected plants were brought to the laboratory environment and then prepared for the study. Preparation of *Achillea millefolium* (10%) extract; 10 g of *Achillea millefolium* was weighed, brewed in 1000 ml of boiling distilled water, and then filtered through a sterile cheesecloth. Preparation of *Achillea millefolium* (30%) extract; 30 g of *Achillea millefolium* was weighed, brewed in 1000 ml of boiling distilled water, and then filtered through a sterile cheesecloth. In the experimental study, the extract we obtained was added to the flasks labeled with *Achillea millefolium* in the proportions we provided and developed at 30^{0}C. In addition, in the experimental study, 10 mM CCl_4 was added to each of the flasks labeled CCl_4 and developed at 30^{0}C [20].

Determination of Saccharomyces cerevisiae Cell Development Measurements

To determine cell growth, culture example were grown at 30°C for 1 hour, 3 hours, 5 hours and 24 hours and their absorbance was measured at 600 nanometer wavelength (OD_{600}) [19].

Lipid Peroxidation Malondialdehyde (MDA) Analysis

0.8 ml of phosphate buffer, 0.025 ml of BHT (Butylated hydroxytoluene) and 0.5 ml of 30% TCA (Trichloroacetic acid) were added to 0.2 ml of sample. The tubes were mixed by vortex and kept on ice for two hours. It was then centrifuged at 2000 rpm for 15 minutes. 1 ml of the supernatant was taken and transferred to another tube. 0.075 ml of 0.1 M EDTA and 0.25 ml of 1% TBA were added and mixed. After waiting in the water bath for 15 minutes, the absorbance was read at 532 nanometer on the spectrophotometer [21, 22].

Saccharomyces cerevisiae Glutathione (GSH) Activity Measurement Analysis

1 ml of phosphate buffer and 0.125 µl of DTNB were added to 100 µl of sample and vortexed. 1 ml phosphate buffer, 0.125 µl DTNB and 100 µl distilled water were added to the blank tube and mixed. The absorbance of the mixtures was read at 412 nanometer within 3-4 minutes [22, 23]. After the necessary measurements were made on the spectrophotometer, the values obtained were calculated in micrograms by substituting them according to the formula obtained according to the standard curve data.

Saccharomyces cerevisiae Catalase (CAT) Activity Determination

For catalase measurement analysis, first 0.2 ml of culture homogenate was taken and mixed with 1.2 ml of phosphate buffered saline. The reaction was started by taking 1 ml of 30 mM hydrogen peroxide solution. The absorbance was measured in a spectrophotometer at 30-second intervals at a wavelength of 240 nm and the results were recorded as U/ml [24, 26].

Pellet and Supernatant Total Protein Intensity (Bradford) Measurements

The standards obtained by creating Bowin serum albumin (BSA) protein standards and the total protein quantity in *Saccharomyces cerevisiae* groups corresponding to this standard value were determined according to the Bradford method [14]. After the necessary measurements were made on the spectrophotometer according to the Bradford method, the values obtained were calculated in nmol/ml by substituting them according to the formula obtained according to the Bowin serum albumin (BSA) protein standard curve data.

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

In our study, the gels prepared for analysis of our protein samples using the SDS-PAGE method were imaged using the gel imaging system (Mini-Protean Tetra Cell–Bio-Rad). After growing at 30 mA, the gels were stained with Coommasie blue. Then, protein bands between groups were analyzed according to the gel image [24, 28]. After loading 19 microliters of protein samples into the wells on the previously prepared loading gel, the electric current was cut off after ensuring that the blue band visible during the separation of proteins in the gel reached the bottom of the gel, the gel between the glass plates was taken and stained with commassie dye, and the necessary comments were made after the bands became clear.

Statistical Analysis

Our results are shown as mean ± standard deviation. Statistical analysis was performed by One-Way ANOVA Post *Hoc* Tukey test followed by multiple comparison using GraphPad Prism 5. $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Natural products, particularly obtained from plants, keep going to issue new and substantial clues in the drug exploration process. The primary step in drug invention has been to document ingredients traditionally used to remedy a condition. Information about important medicinal plants and practices is passed on orally from one generation to the next. Documenting such information facilitates future studies on medicinal plant safety and validation effectiveness [1]. For this reason, research was conducted on *Saccharomyces cerevisiae* to determine the anti-oxidant effect of *Achillea millefolium* (yarrow), which we used in our study. *Saccharomyces cerevisiae* is a budding yeast used in the manufacture of fermentative foods, beverages and biofuels. It is used as a cell factory for the making of pharmaceuticals and other essential biochemical compounds [29]. *Achillea millefolium*, which has been medically investigated as one of the alternative drugs in recent years, has wide biological activity due to its ability to regulate various enzymes and cell receptors and its anti-oxidant properties. It has also been determined that it has very valuable biological activities such as anti-oxidant, anti-diabetic, anti-cancer, anti-inflammatory and anti-bacterial [30].

When the cell development results of our study at different times were examined, it was observed that there was a meaningful difference among the groups depending on the dissimilar development times ($p<0.05$). It was observed that the groups given *Achillea millefolium*-10% and *Achillea millefolium*-30% extracts increased cell development

against the negative effect of CCl_4 depending on time. It was determined that cell development was least in the group given CCl_4 (Fig. 4).

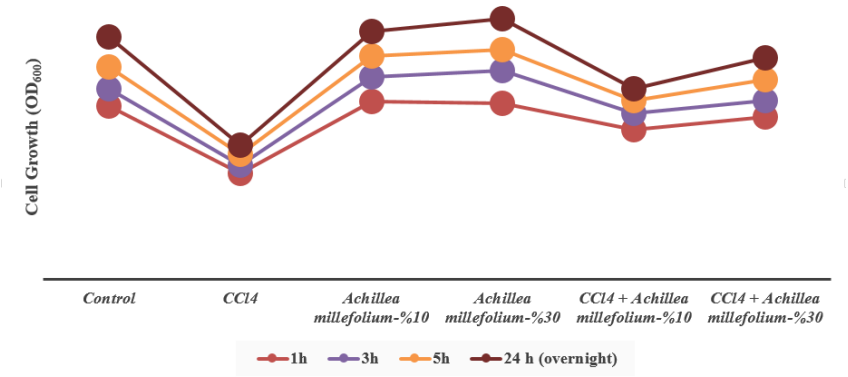

Fig. 4. *Cell development of Saccharomyces cerevisiae at different times in Achillea millefolium extract*

When we examined the MDA results of *Achillea millefolium* in Fig. 5, it was defined that the MDA level was at the highest level in the group given CCl_4, and the MDA level decreased significantly in the CCl_4 + *Achillea millefolium* -10% and CCl_4 + *Achillea millefolium* -30% treatment groups. It was defined that MDA levels were lowest in the control, *Achillea millefolium*-10% and *Achillea millefolium*-30% groups. These results show us that *Achillea millefolium* has a preventive effect to lipid peroxidation.

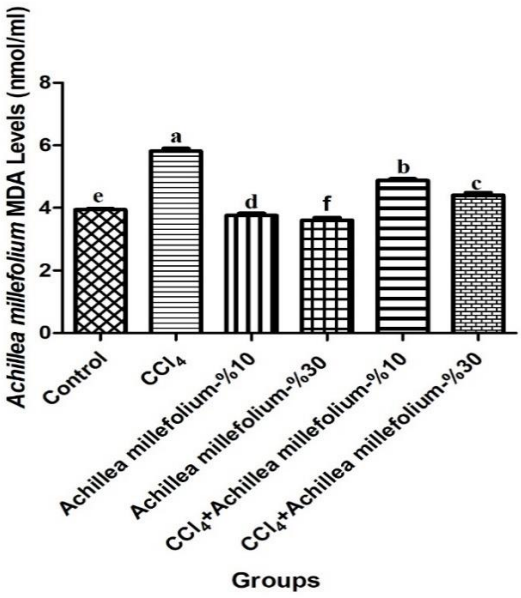

Fig. 5. *MDA level between groups*

When we examined the GSH levels as a result of our study, it was observed that the GSH level was at the lowest level in the CCl_4 group, and the GSH level was at the highest level in the Control, *Achillea millefolium*-10% and *Achillea millefolium*-30% groups.

However, GSH level was found to be statistically meaningful among all groups ($p<0.05$) (Fig. 6). When we examined the CAT levels, it was found that CAT levels rised in the CCl_4 + *Achillea millefolium*-10% and CCl_4 + *Achillea millefolium*-30% treatment groups compared to the CCl_4 group. It was established that the highest CAT activity was in the group given *Achillea millefolium*-30% (Fig. 7). The rise in GSH level and CAT activity shows us that *Achillea millefolium* protects *Saccharomyces cerevisiae* against oxidative damage.

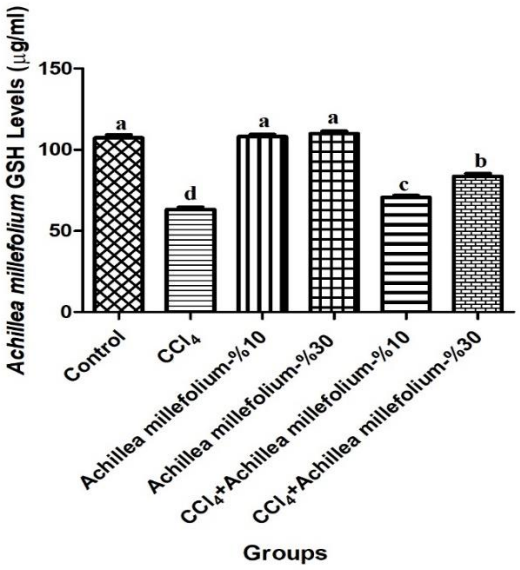

Fig. 6. *GSH level among groups*

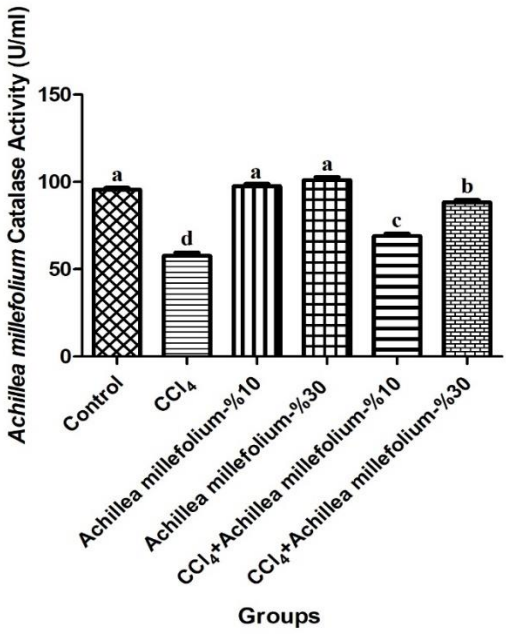

Fig. 7. *CAT activity among groups*

When the pellet total protein intensity results given in Fig. 8 and the supernatant total protein intensity results given in Fig. 9 are studied we can speak that *Achillea millefolium* rise protein synthesis in *Saccharomyces cerevisiae*. It is observed that the total protein intensity rises significantly in the *Achillea millefolium*-10% and *Achillea millefolium*-30% groups, mainly when confront to the CCl_4 group. It was determined that the lowest pellet and supernatant total protein density was in the group given CCl_4.

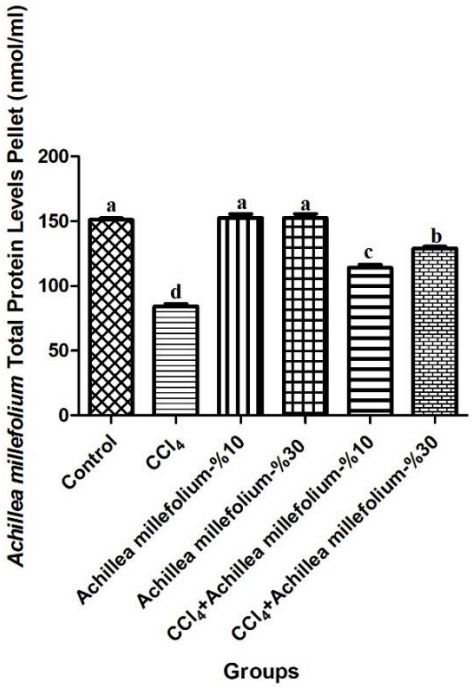

Fig. 8. *Pellet total protein densities between groups*

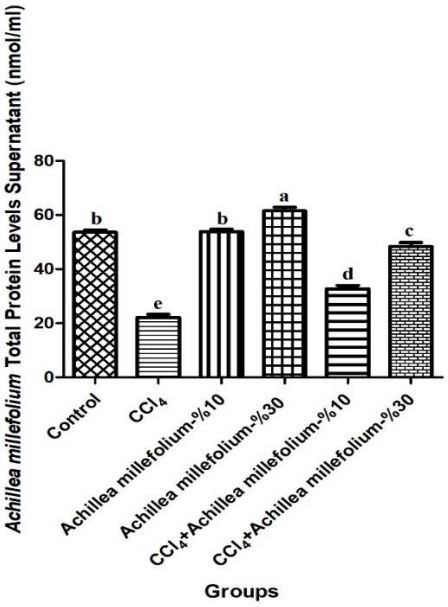

Fig. 9. *Supernatant total protein densities between groups*

When the SDS-PAGE gel image (pellet) was examined, it was concluded that the protein concentration raised meaningful in the *Achillea millefolium*-30% group compared to the CCl_4 group. It was defined that the protein concentration decreased in the CCl_4 administered group due to the damage caused by CCl_4. As a result of this study, according to our data, it was determined that *Achillea millefolium* raised the protein density of *Saccharomyces cerevisiae*, spite of the negatory effects of CCl_4 (Fig.10).

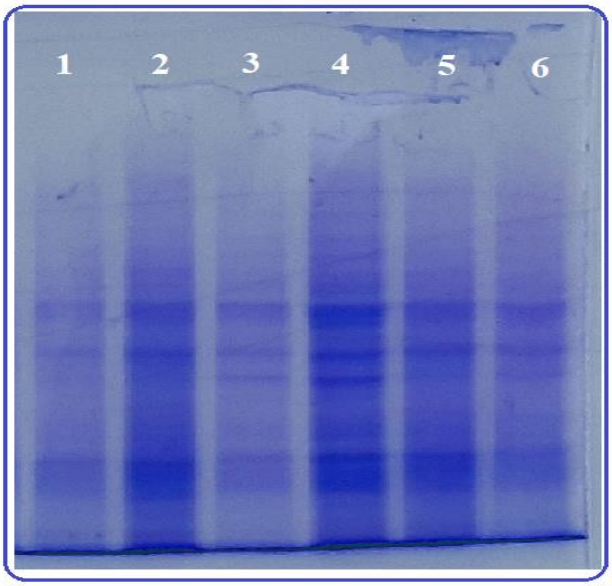

Fig. 10. *SDS-PAGE pellet protein bands: (1: Control, 2: Achillea millefolium 10%, 3: CCl_4, 4: Achillea millefolium 30%, 5: CCl_4 +Achillea millefolium 30%, 6: CCl_4 + Achillea millefolium 10%)*

As a result of this research, it was conclusions that *Achillea millefolium* extract raised the growth of *Saccharomyces cerevisiae* despite the negative effects of CCl_4. *Achillea millefolium* has been shown to have various biological and pharmacological effects in the literature. Milutinovic et al. [31], determined in their study the effect of polyphenol-rich plant extracts, especially yarrow, on the growth of probiotic and pathogenic microorganisms. They stated that these plant extracts suppressed the growth of pathogenic Candida yeast. Ayoobi et al. [6], researched the effect of aqueous extract of *Achillea millefolium* on patients with relapsing-remitting MS (RR-MS). They stated that 1-year oral practice of the aqueous extract of *Achillea millefolium* provided a important improvement in clinical outcomes in RR-MS patients. Vahid et al. [32], observed that plasma nitric oxide metabolites reduced significantly after Achillea millefolium application in chronic kidney in patients. They stated that higher doses and long-term *Achillea millefolium* plant application may make these changes more meaningful. Hajhashemi et al. [33], found that *Achillea millefolium* and *Hypericum perforatum* ointments relieved the level of perineal pain, wound redness and edema, and their consumption may be beneficial in episiotomy treatment. Jonsdottir et al. [34], stated that extracts obtained from *Achillea millefolium* and *Menyanthes trifoliate* plants have anti-inflammatory effects on human dendritic cells and contain compounds that can reduce autoimmune responses.

Gok et al. [21], investigated the effect of *Aloe vera* gel on cisplatin-induced oxidative damage in Saccharomyces cerevisiae culture. They stated that *Aloe vera* gel raised cell growth, total protein synthesis, CAT and GSH levels, and decreased MDA levels. Gok et al. [35], examined the effect of sumac plant on oxidative injury in *Saccharomyces cerevisiae* culture. They showed that sumac plant reduces oxidative damage in yeast culture and has an increasing effect on yeast cell growth. Beyaz et al. [36], investigated the therapeutic effects of bee pollen in *Saccharomyces cerevisiae* culture. As a result of the study, they found that cell development, total protein synthesis and GSH level raised in the bee pollen groups compared to the $CuCl_2$ group, while the MDA level decline. Aslan [20], investigated the protective effect of *Goji berry* in *Saccharomyces cerevisiae* culture against oxidative injury caused by chromium. He stated that GSH levels and catalase activities increased and MDA levels decreased in the treatment group compared to the chromium group.

Eker et al. [37], (2022) stated that *Achillea millefolium* extract meaningful reduced the frequency of spontaneous and oxytocin-induced uterine contractions depending on the dose. Aslan and Can [38], stated in their study that orange juice increased cell growth and protein synthesis by inhibiting oxidative damage in *Saccharomyces cerevisiae* against H_2O_2-induced oxidative stress. Tadic et al. [8] showed in their research that yarrow extracts have a significant anti-inflammatory property. Gok et al. [24], stated that palm leaf decrease oxidative damage in *Saccharomyces cerevisiae* culture and has a protective role to support protein synthesis. Zöngür [39] detected a high amount of 1,8-cineole in the essential oil composition of plants growing in the Sivas region. 1,8-cineole (74%), which is abundant in the essential oil component of eucalyptus species, was also found to a significant extent in *Achillea millefolium* plants (19.33%), although to a lesser extent than in eucalyptus species. Additionally, as a result of the research, it was seen that the essential oil of the plant showed anti-microbial and anti-fungal properties, inhibiting the growth of all tested species. He stated that the data obtained in his study could be a source for future biotechnological, biodiversity, pharmaceutical and medical studies. Ozsahin et al. [40] stated that there were differences in some vitamins and fatty acids of different molasses species on *Saccharomyces cerevisiae*.

CONCLUSION

In line with the data we obtained as a result of this study, it was determined that *Achillea millefolium* (Yarrow) has a protective role against oxidative damage in *Saccharomyces cerevisiae* culture, thanks to its strong anti-oxidant properties. We can say that *Achillea millefolium* is very effective against oxidative damage, increases total protein synthesis and cell development, and protects the cell opposite oxidative injury. According to the data we obtained from the study, the fact that *Achillea millefolium* has a high anti-oxidant capacity makes us think that the positive effects it has on *Saccharomyces cerevisiae* may also have on humans. These results can be a guiding reference for new studies in the future with the contributions we will make to the literature in terms of eliminating the deficiencies in the literature.

Conflict of Interest. The authors declared that there is no conflict of interest.

Authorship Contributions. Concept: A.A., O.G., S.B., Design: A.A., O.G., S.B., Data Collection or Processing: O.G., S.B., S.D., Analysis or Interpretation: O.G., S.B., S.D., Literature Search: O.G., S.B., S.D., Writing: A.A., O.G., S.B., S.D.

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