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Original article

Evaluating the multifaceted impact of Cephalaria extract on dough quality: Antioxidant, antimicrobial, and cytotoxic properties

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Summary *Cephalaria syriaca*, known as cephalaria, is an annual, pilose plant with pink-purple flowers growing wild in wheat fields. Viscoelastic properties of gluten-free doughs can be improved by adding cephalaria. However, this addition results in some undesirable properties in bread such as bitter taste and dark internal colour. This study aimed to reduce these effects by obtaining cephalaria obtained from distilled water and ethanol extracts and to increase the potential use of extracts in bread production. Different proportions of water and ethanol extracts obtained from oil-free cephalaria were added to bread by creating a model with Mixture Design, and the optimum concentration was determined by farinograph trials. The antioxidative, antimicrobial, and cytotoxic effects of extract and the effect of cephalaria on yeast activity were examined. Cephalaria extracts had no measurable antimicrobial effect and no antifungal effect on *Rhizopus stolonifer*, while an antifungal effect was found against *A. niger* and *P. expansum*. The IC50 value was found to be 4.15 mg mL^{-1} for ethanol extract while the water extract had no effect on cells. The addition of extract had no negative effect on the number of yeasts in sourdough fermentation. The study has improved the usage of cephalaria by using water and ethanol extracts in dough.

Keywords Antioxidant activity, *Cephalaria syriaca*, cytotoxicity, dough properties.

Introduction

The gluten content of the flour provides an increase in the viscoelastic properties of wheat flour dough. With the increase in the gluten content of wheat flour, an increase in the viscoelastic properties of the dough is observed (Elgün *et al*., 2001). The sulfhydryl groups (SH), disulfide bonds (SS), and SS bond formation in gluten structure have a strong effect on the extensographic qualities and dough-kneading properties of wheat flour. SH-SS ratio in flour is an effective and important factor in dough rheology. SS bonds contribute to the formation of the stable structure of the dough (Hayta & Schofield, 2004; Shiau & Yeh, 2004; Karaoğlu, 2006).

Food additives are substances that add some desired properties to foods and become necessary in the industry in the age of new technologies. However, the consumer should not take too many additives into the body as a result of unconscious consumption, and health risks are discussed. For this reason, the tendency towards natural additives has increased day by day. Various cereal seeds and cereal seeds-derived components are used as natural additives. Cephalaria (*Cephalaria syriaca*) as a different species can be shown among the examples (Karahan & Kilincceker, 2019).

Cephalaria (*Cephalaria syriaca*), a species of *Cephalaria*, is a natural plant that grows wild in wheat fields. The fruit of *C. syriaca* resembles a wheat grain in size and shape and it is not toxic, despite being bitter. Cephalaria, which is easy to obtain because it grows naturally, has been used locally for years to strengthen weak flours by adding to bread dough, and it has an important potential in terms of being used as a natural additive in the bakery. Rheological properties of the dough have been stated to improved significantly as a result of the farinograph and extensograph analyses made on the dough with the addition of cephalaria

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products to wheat flour (Karaoğlu, 2006). By confocal microscope imaging, it has been found that there is a homogeneous distribution between cephalaria proteins and gluten, and the interaction between them can be in the form of numerous polar cross-links arising from polar amino acids.

The effects of oily and de-oiled cephalaria flours on the rheological properties of flour mixtures used in whole wheat bread production were previously investigated. As a result of the addition of cephalaria products, a significant change occurred in the rheological properties of the dough and also bread-making quality increased in bread applications made from these flours (Karaoğlu, 2012).

In another study, a significant positive effect on bread volume, crust hardness, and sensory properties has been found even in bread with very low levels of cephalaria flour addition (Karaoglu, 2011).

The effect of cephalaria flour on the quality of wheat damaged by a stink bug was examined in another study. The addition of cephalaria flour has been stated to positively affect the gluten index, falling number, and Zeleny sedimentation values of the stink bug-damaged wheat. The increase in the gluten index value has been attributed to the fact that some oxidizers in the structure of cephalaria flour caused the formation of disulfide bonds (Başar *et al*., 2016).

Studies have been carried out on the use of cephalaria as a natural additive in bread making, however, the use of cephalaria in this way gives the bread bitterness after a certain level and also causes a darkening in the colour of the bread, which affects its consumption negatively. Therefore, these problems are seen as the most important obstacles to the commercial use of cephalaria. This study aimed to use water and ethanol extracts obtained from cephalaria instead of whole cephalaria flour and strengthening effects on the dough were determined to minimise these problems. Thus, the most efficient combination and quantity of extracts were determined. The determined extract combination was added to the bread flours and the farinograph and extensograph properties were examined. Additionally, the antioxidative, antimicrobial, and cytotoxic activities of the extract were examined. At the same time, the interaction between cephalaria and dough was examined by a confocal microscope, and finally, the effect of cephalaria on yeast activity during dough fermentation was investigated.

Materials and methods

The cephalaria used in the study was grown and harvested under organic production in the lands of Ziya Organik Tarım Corp. in Büyükçekmece, Istanbul. After the cephalaria seeds arrived in the laboratory, they were manually cleaned of foreign materials and stored in plastic boxes at 4 °C in cold storage. Flour samples used for the dough were provided by Istanbul Metropolitan Municipality Halk Ekmek Corp. Flour was stored at 4 °C in plastic containers. All experiments were performed repeatedly.

Physicochemical properties of Cephalaria

Moisture determination and ash analysis were carried out according to a previous study (Büyüktuncel, 2012). In the ash analysis, oil-reduced ground cephalaria samples were burned in porcelain crucibles at 900 °C in an ash furnace (DAIHAN Scientific Co., Ltd. Seoul, Korea). After the process, the %-ash content was calculated on the dry matter (Elgün *et al*., 2001). The amount of protein in the cephalaria samples was determined by the Kjeldahl method (Elgün *et al*., 2001). The fat concentration of cephalaria samples was determined using the Soxhlet method. Hexane was used as a solvent (Büyüktuncel, 2012).

Three grams of oil-free cephalaria samples were weighed and put into beakers to determine crude fibre. Then, 50 mL of 5% H_2SO_4 and 150 mL of water were added. The mouth of the samples was well sealed and stirred for 30 min with the help of a magnetic stirrer to prevent evaporation (IKA-Werke GmbH & Co.KG, Staufen, Germany). At the end of the period, the samples were filtered through filter paper and washed with hot water until they lost their acid effect. After adding 50 mL of 5% NaOH to the sample remaining on the paper, it was stirred again for 30 min, and this time it was thoroughly washed with hot water to reduce the effect of the base. The part remaining on the filter was left on a pre-tared filter paper and washed first with distilled water, then with 95% ethanol. Then, they were taken to a drying oven at 105 °C (Memmert, Schwabach, Germany) to dry. The dried samples were weighed (Elgün, 1999). The amount of starch found in cephalaria samples was determined according to the Ewers method proposed by a previous study (Elgün *et al*., 2002). The determination of grain weight in cephalaria grains was made according to a method specified previously (Elgün *et al*., 2002).

Physicochemical properties of flours

Sedimentation test, and wet and dry self-determination were carried (AACC, 1990; Dokuzlu & Özkaya, 2013). The amount of protein in the flour samples was determined by the Kjeldahl method (Elgün *et al*., 2001).

Preparation of the Cephalaria extract

A ground oil-free cephalaria was used to obtain water and ethanol extracts from the cephalaria. Extraction processes were carried out according to a previous

method (Abbas *et al.*, 2012). For the extraction, temperature and time factors were taken into account, and optimum temperature and time composition were determined. Trials were carried out at 50 °C and 80 °C for 4, 8, and 12 h. After extraction, the solution was centrifuged at 1000 rpm for 10 min, and then the supernatant was poured into glass petri dishes.

Properties of Cephalaria extract

Antimicrobial activity

Antibacterial activity of cephalaria extracts was determined against 2-g positive (*Listeria monocytogenes and Staphylococcus aureus*) and 2-g negative (*Escherichia coli* O157: H7 and *Salmonella enterica* subsp. *enterica* serovar typhimurium) bacteria by the agar disk diffusion method (Mueller Hinton Agar, Merck, Germany) (Murray *et al.*, 1995). Antifungal activity of cephalaria extracts against baker's yeast *Saccharomyces cerevisiae* was also tested by the same agar diffusion method (Malt Extract Agar, Merck, Germany). For this purpose, 0.5 g of the cephalaria extract was weighed and dissolved in 5 mL of sterile distilled water. The mixtures were kept in an ultrasonic water bath at room temperature for 5 min to ensure thorough dissolution. An antimicrobial substance (cephalaria extract) was impregnated on the 10 mm diameter discs (4 pieces) and the discs were placed equidistant from each other. The petri dishes prepared in this way were incubated at 37 ± 0.1 °C for bacteria and 25 °C for yeast for 24 h. At the end of the period, inhibition zones formed on the medium were evaluated in millimetres. Standard discs immersed in sterile distilled water without extracts were used as controls for comparison.

The antifungal activities of cephalaria extracts on *Rhizopus stolonifer*, *Aspergillus niger*, and *Penicillium expansum* were measured. For that purpose, the extract diluted 10 times with sterile distilled water was added to wells on Malt Extract Agar, and a 100-fold diluted mould spore was inoculated right in the middle of the petri dish. After 3–5 days of incubation at 25 °C, the antifungal effect was determined by measuring the mould-free zone around the wells.

Antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) test method of Brand-Williams *et al.* (1995) was modified and applied for the determination of antioxidant activity (Brand-Williams *et al.*, 1995). 0.5 g of the sample was mixed with 10 mL of distilled water until the sample was thoroughly dissolved. 0.1 mL of these samples were taken into each tube and distilled water was used instead of the sample as a control. Alcohol was used as solvent and control for ethanol extract by the same method. At 20 s intervals, 4.9 mL DPPH solution (0.1 mM) was added to each tube and vortexed. The samples were kept at room temperature for 30 min in a dark environment and then the absorbance was measured at 517 nm in the spectrophotometer. DPPH capture activity was expressed as %-inhibition.

Cytotoxic and anticancer activity

Cytotoxic activity was detected in human umbilical and endothelial cells (HUVEC) by modifying a previous method (Aisha *et al.*, 2012). Cells were maintained in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM-F12, Sigma-Aldrich, Merck, Germany) medium supplemented with 10% heat-inactivated fetal bovine serum in a 25 cm^2 flask. Flasks were incubated at 37 °C in a humidified incubator with 5% CO_2 atmosphere (Panasonic Healthcare Co., Tokyo, Japan). Cells were harvested with 0.05% trypsin/EDTA (Fisher Scientific (Austria) GmbH - Dresdner Straße 89 - A-1200 Wien) and subcultured about twice a week. Cell counts were assessed by standard cell counting procedures using a haemocytometer.

The viability test of 2,3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxyanilide (XTT) was used to determine the cytotoxic and anticancer activity. Serial dilutions were prepared using a cell medium by dissolving cephalaria water extract in dimethyl sulfoxide (DMSO) and ethanol extract in phosphate-buffered saline (PBS). Mediums containing no cephalaria extract were used as controls.

Cells were transferred to 24-well plates at a concentration of 5×10^4 cells per well and incubated for 48 h in the presence of different concentrations of cephalaria extract (25.4 mg mL^{-1} µg mL^{-1}). After the process, the absorbances were measured with a spectrophotometer at 450 nm (Elisa reader Elx reader Elx800, Biotek, Vermont, USA). % cell viability was calculated using the formula: Cell viability (%) = (Optical density sample/Optical density Control) × 100.

Determination of the effect of cephalaria extracts on yeast fermentation

For this purpose, 3% commercial baker's yeast was added to the dough samples and kneaded in the dough kneader for 5 min. Then the yeast dough samples were subjected to fermentation for 3 h in the conditioning cabinet set at 70% relative humidity and 30 °C temperature. The number of *S. cerevisiae* in the dough was determined at the beginning and at the end of fermentation by the spread plate method (Halkman, 2005).

Determination of the effect of cephalaria extracts on sourdough fermentation

Firstly, for sourdough production, the basic dough formulation that does not contain cephalaria was prepared and incubated at 25 °C for 15 h. This sourdough was added to the dough with cephalaria at a rate of 10% and kneaded in a mixer for 5 min. It was

then fermented for 3 h at 70% relative humidity and 30 °C. The total number of yeasts in the dough at the beginning and end of fermentation was determined by the spread plate method (Halkman, 2005).

Determining the most effective extract ratio by modelling

A 2-factor (Ethanol:Water extract rate and total extract amount) trial design was used to determine the water and ethanol extract ratios to be used to strengthen the viscoelastic structure of the dough. Seventeen trial points were selected consisting of various combinations of these two factors. It was envisioned that the amount of extract should be given between 0.1 and 0.6%. Water and ethanol extract ratios were given to complement each other to 1 (100%). According to these values, the optimum ethanol, water ratio, and extract amount were determined. Optimization was carried out using the Design-Expert program. The cubic model was used in the modelling of the results, and the terms considered to be insignificant were removed from the model with the backward elimination method. 3D graphics of the obtained models were obtained using the Design-Expert software. Wheat flour with a sedimentation value of 34 ± 0.5 was used in the optimization study.

Preparation of bread dough containing cephalaria extract

The farinograph and extensograph properties of the control dough sample without cephalaria and of the cephalaria dough samples with optimum properties and amounts of extract (0.56%, 80:20% ethanol: water extract) were examined.

Farinograph and extensograph trials

Farinograph and extensograph trials were made based on the AACC 54–21 coded method. The following parameters were determined with the farinograph: Percentage of water required to achieve a dough consistency of 500 Brabender units (BU), stability (residence time of dough at 500BU consistency value), stirring tolerance index (difference in consistency between maximum peak height and 5 min next peak height) and elasticity (bandwidth of curve at maximum consistency); extensibility with the extensograph, maximum resistance, energy and the number of proportions (Elgün *et al.*, 2002).

Statistical analysis

Statistical analysis was carried out by using the JMP 6 program, and whether there was a difference between groups was tested with one-way analysis of variance (ANOVA), Tukey HSD ($P < 0.05$) using the test parameter.

Table 1 Cephalaria physicochemical analysis results

Parameters	Mean ± SD
1000 grains (g)	17.75 ± 1.43
Moisture (%)	6.08 ± 0.68
Ash (%)	5.34 ± 0.45
Protein (%)	15.54 ± 0.78
Oil (%)	25.14 ± 3.30
Starch (%)	6.98 ± 0.34
Crude fibre (%)	25.54 ± 0.41

Results and discussion

Physicochemical analysis results of Cephalaria

The physicochemical properties of the cephalaria seed are given in Table 1. According to the schedule, it was determined that the average 1000 grain weight of seed was 17.75 g, moisture content was 6.08%, ash content was 5.34%, protein content was 15.54%, the oil content was 25.14%, starch content was 6.98%, and the crude fibre content was 25.54%.

The average crude protein content of the cephalaria samples obtained from various regions was found to be 20.03% in a study (Altınığne & Saygın, 1985). While Kayseri has the lowest protein content value at 16.4%, the protein content of the cephalarias obtained from Konya (22.5%) was the highest. In another study, the average 1000 seed weight of cephalaria was determined as 15.29 g (Katar *et al.*, 2012). Oil content was found to be 22.41%, 25.3%, and 24.9–25.8% in previous studies, these results were compatible with the present study (Çiller, 1977; Yazıcıoğlu & Karaali, 1983; Katar *et al.*, 2012).

Sedimentation results and protein values of the flour used

The sedimentation value was 17 ± 0.3 mL in the flour used for modelling. According to previous studies, If the sedimentation value of flour is 36 mL and above, it is considered very good; if it is between 25 and 36 mL, it is considered good; if it is between 15 and 24 mL, it is considered medium; if it is 15 mL and below, it is considered poor (Dokuzlu & Özkaya, 2013). The protein content was (%) 7.00 ± 0.02 in the flour used for modelling.

Extract yield

Due to the bitterness problem of cephalaria (the transition of glycosides to the solvent to change the taste) and the demand for light colour, 50 °C was found to be sufficient and suitable for extraction. The extraction

Table 2 Trial points used for cephalaria extract optimization

Model	A (%)	B (%)	C (g in 100%)	Stability (mm: ss)	Water absorption (%)
1	50	50	0.35	1.42	60.0
2	100	0	0.35	2.39	59.5
3	100	0	0.22	2.24	59.6
4	25	75	0.22	1.57	59.9
5	100	0	0.60	8.56	59.2
6	100	0	0.10	1.57	60.0
7	25	75	0.47	2.03	59.8
8	50	50	0.60	3.2	59.5
9	0	100	0.35	1.55	59.8
10	0	100	0.10	2.18	59.6
11	0	100	0.60	2.24	59.8
12	0	100	0.10	1.55	59.5
13	75	25	0.47	2.39	59.8
14	75	25	0.22	2.13	59.8
15	50	50	0.10	2.05	59.8
16	0	100	0.60	2.34	59.7
17	100	0	0.60	10.58	59.0

A = Ethanol extract, B = Water extract, C = Total cephalaria amount (g in 100%).

time was determined as 12 h since the extraction efficiency increases as the time increases.

After the extraction processes, the extract yield was found 21.5 ± 0.26% and 17.1 ± 0.46% for water and ethanol extracts, respectively.

The optimum extract ratio results from modelling

Ethanol, water, and their amounts were found to be effective in stability and water absorption. Table 2 shows the trial points used for optimization. In the model, the highest stability and water absorption level was 83% ethanol, 17% water phase (completed to 100%) and the amount was 0.56%. This optimum ratio was used in further analyses.

In the results obtained from the variance analysis, the model was found to be significant compared to Duncan ($P < 0.05$), and the coefficient (R^2) was determined as 0.9669. Therefore, the cubic model has been seen to be very compatible with the data obtained.

Model equation; A = Ethanol extract, B = Water extract, C = Total Cephalaria amount (g in 100%).

Equations in the model;

$$\begin{aligned} Stability = &+2.36 \times A + 1.66 \times B - 1.88 \times A \times B \\ &+ 1.29 \times A \times C + 0.26 \times B \times C - 5.65 \\ &\times A \times B \times C + 3.22 \times A \times C^2 + 0.43 \times B \\ &\times C^2 - 2.87 \times A \times B \times C^2 + 2.67 \times A \times C^3 \end{aligned} \quad (3.1)$$

$$\begin{aligned} Water\ absorption = &+59.54 \times A + 59.78 \times B \\ &+ 1.29 \times A \times B + 0.082 \times A \\ &\times C - 0.20 \times B \times C + 0.13 \times A \\ &\times B \times C + 0.017 \times A \times C^2 \\ &- 0.13 \times B \times C^2 - 1.11 \times A \\ &\times B \times C^2 - 0.54 \times A \times C^3 \\ &+ 0.30 \times B \times C^3 \end{aligned} \quad (3.2)$$

Table 2 shows the water absorption and stability values of the trial extracts. Considering the stability values, the highest values were observed to be found in the dough containing 100% ethanol extract at 0.6% with 8.56 (mm: ss) and 10.58 (mm: ss); on the other hand, the lowest rates were found in 100% water extract at 0.35% and 0.10% with 1.55 (mm: ss).

Considering the water absorption values, it was determined that the highest value was the 1st model with a 60 (%) water absorption value and the 6th model with a 100% ethanol ratio.

With farinograph trials carried out with the aim of ethanol: water concentration and the optimization of the total extract ratio to be added to the dough, farinograph trials were made at the trial points specified in Table 2. The effect of ethanol and water extracts on the water absorption and stability of the dough is seen in Fig. 1.

As seen in Fig. 1a, the stability increased due to the increase in the ethanol extract ratio. With the increase in the percentage of total ethanol extract in the extract added to the dough, the stability increased by +2.36 times and the water extract increased by +1.66 times according to the data obtained from the Design-Expert program. In Fig. 1b, the effect of the extracts on the water absorption of the dough is seen. It was clear from the parabolic graph that the ethanol above water absorption and the increase in water extract affected the result almost equally.

As a result of the modelling work, the optimum ethanol: water extract concentration was found as 83:17 (%) and the total extract ratio was 0.56% in terms of the stability and water absorption capacity of the dough. This extract rate was used in the further experiments.

In previous studies, it was found that flours with good bread quality were those with high elasticity and resistance in the farinograph and extensograph (Boz, 2008). It was found that as the rate of grain with a stink bug increased, there were significant differences in the farinograph values of flours. Compared to the control flour, it was seen that as the ratio of stink bugs

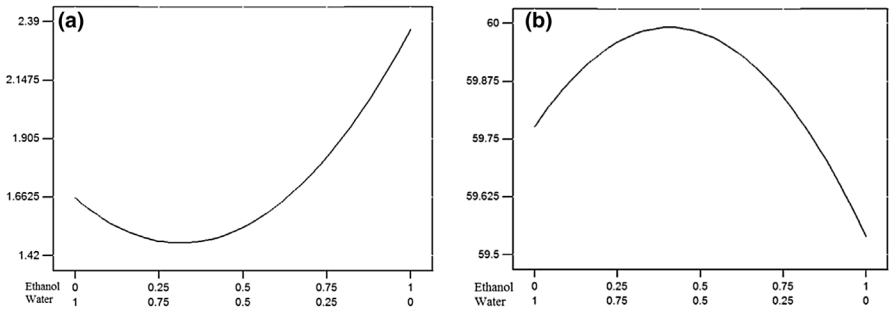

Figure 1 Effects of ethanol (a) and water extracts (b) on the stability and water absorption properties of the dough.

increased in formulations, water absorption increased while dough stability decreased. Cephalaria flour reduced the water absorption value of the dough. This situation may indicate that the cephalaria has low hydration capacity. According to the studies, the softening degree of flour decreases as the cephalaria content increases (Karaoğlu, 2006; Boz, 2008).

In also our study, it was observed that the stability values of flours with added cephalaria extract generally increased as the extract ratio increased (Table 2).

In a study, cephalaria addition was shown to be effective on farinograms, but this effect caused a more important change in extensograph values. It was observed that as the level of grain with stink bug increased, the dough stability value generally decreased at all cephalaria levels. According to the results of the study, flours with 1% cephalaria additive have the highest dough stability at the level of 5% and 10% grain with a stink bug, while the best result was shown by flours with 1.5% cephalaria additive at the level of 15% grain with stink bug (Başar *et al*., 2016). The use of cephalaria products (WCSF and DCSF) as an additive to whole meal bread flours was shown to be very effective on dough rheological properties and bread-making quality properties. With the addition of cephalaria products, a more positive effect was observed on the extensograph properties of the dough compared to the farinograph properties. It has been suggested that the reason for this is that the extensograph and rheofermentometer take longer to operate than the farinograph, and that the effect properties of cephalaria products on dough develop better over time (Karaoglu, 2011). However, in our study, since the extract contained the active ingredient of cephalaria at a higher rate and its effect was easier to manifest in the dough, changes in farinograph properties were also observed and based on that, the optimum extract amount and ratio was found (Table 2).

In another study, no change was observed in the taste and colour of the bread when the seeds and pulp of cephalaria were added at a rate of 0.5% (Altınığne & Saygın, 1985). Above this rate, on the other hand, bitterness was felt in the taste of the bread, and colour differences were observed in the interior of the bread that was not suitable for consumer preference. However, the taste of the bread made with flours containing 1.0% and 1.5% of cephalaria crude oil was not bitter and their volume was increased (Altınığne & Saygın, 1985). In also our study, since it was added as an extract, there was no significant change in colour and more positive visual results were obtained compared to cephalaria flour.

Evaluation of Cephalaria extract properties

Antimicrobial activity

In vitro antimicrobial activity tests were performed on 4 bacteria, 1 yeast, and 4 moulds. Test results are shown in Table 3. Cephalaria extracts had no measurable antimicrobial effect on any of the bacteria and yeast (*S. cerevisiae*). The extracts showed no antifungal effect on *Rhizopus stolonifer* mould, while an antifungal effect was found against *A. niger* and *P. expansum* moulds. The antifungal effect of ethanol extract was higher for both moulds compared to water extract and the highest antifungal effect was on *P. expansum*. Any zone was formed in any of the control samples.

The zone formation of the cephalaria ethanol extract was present in both moulds in comparison with the control, whereas it was seen that the effect on *P. expansum* was higher.

Antioxidant activity

Antioxidant activity of cephalaria extracts was tested against DPPH radical. Test results are shown in Table 3. Antioxidant activity of extracts were statistically same ($P < 0.05$) Ethanol extract provided the highest inhibition with 31.76%, and the inhibition of water extract was 31.39%. When the inhibition rates were examined, both extracts possessed antioxidant effects and these effects were very close to each other. In a study, the antioxidant activity of *C. syriaca* seeds obtained from several locations in Turkey was investigated. They showed that the DPPH inhibitory activity

Table 3 Antimicrobial and antioxidant activity of the cephalaria extracts

	Antimicrobial activity (mm)		
Bacteria	**Cephalaria ethanol extract**	**Cephalaria water extract**	**Control**
Listeria monocytogenes	–	–	–
Staphylococcus aureus	–	–	–
E. coli O157:H7	–	–	–
Salmonella thyphimurium	–	–	–
Moulds			
Rhizopus stolonifer	–	–	–
Aspergillus niger	5.00 ± 0.00	4.00 ± 0.00	–
Penicillium expansum	8.00 ± 0.00	6.00 ± 0.00	–
Yeast			
Saccharomyces cerevisiae	–	–	–

Sample	**Antioxidant activity (%)**
Cephalaria ethanol extract	31.76 ± 0.46[a]
Cephalaria water extract	31.39 ± 0.45[a]

Differences between samples for the indicated analysis are represented by different lowercase letters within the same column, with a significance level of $P < 0.05$.

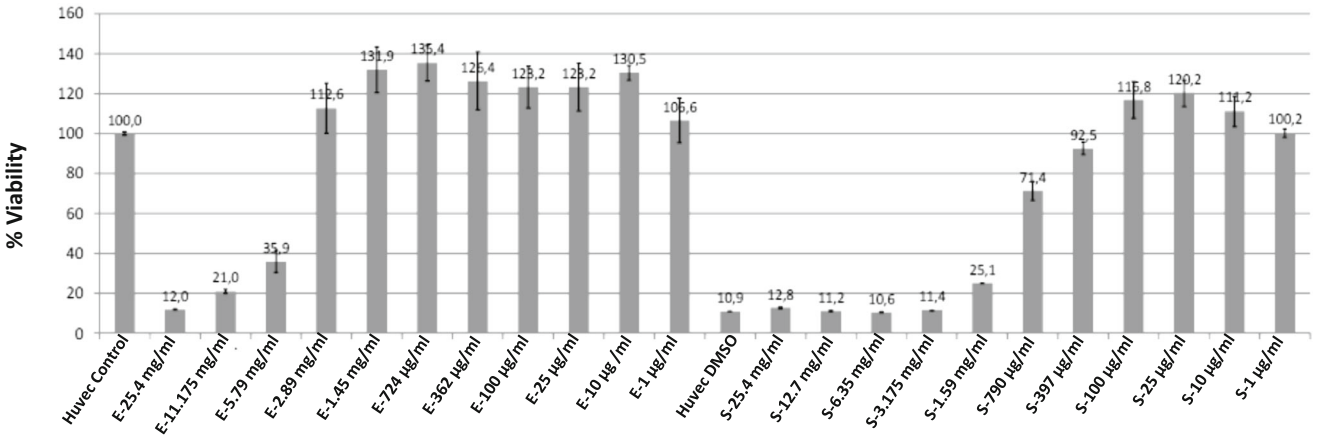

Figure 2 The cytotoxic effect of water and ethanol extracts of cephalaria on the viability of the HUVEC cell line. E, ethanol extract; S, water extract.

of the extracts was between 18.8% and 67.3%. (Kavak & Baştürk, 2020).

Cytotoxic activity

The cytotoxic activity of the extracts was determined using the XTT test on human-derived HUVEC, respectively. Cell viability test results performed with the HUVEC cell line are shown in Fig. 2.

At the end of 48 h of incubation, HUVEC cell viability was observed to be dependent on the cephalaria extract concentration. In water extract, since DMSO was used to dissolve substances, DMSO was used as negative control. DMSO seemed to affect the viability of cells by about 10%. Therefore, it was concluded that water extract did not exhibit a cytotoxic activity on cells, on the contrary, seemed to accelerate cell growth below 1.59 mg mL^{-1}. However, ethanol extract, which is dissolved in water, showed a cytotoxic effect, and the IC_{50} was found to be 4.15 mg mL^{-1}.

The effect of Cephalaria on yeast activity during dough fermentation

The change in the population of *S. cerevisiae*, which is baker's yeast, was investigated during yeast fermentation with the addition of extract and flour to the dough. The *S. cerevisiae* numbers at the beginning and

Table 4 The change of *Saccharomyces cerevisiae* and total yeast during dough fermentation with the addition of baker's yeast in cephalaria-added flour

Sample	The number of *S. cerevisiae* before fermentation (log cfu g^{-1})	The number of *S. cerevisiae* after fermentation (log cfu g^{-1})
Control	4.24 ± 0.11^{a}	7.82 ± 0.05^{b}
%0.56 extract	4.28 ± 0.05^{a}	7.69 ± 0.18^{b}
Sample	**The number of total yeast before fermentation (log cfu g^{-1})**	**The number of total yeast after fermentation (log cfu g^{-1})**
Control	4.48 ± 0.09^{a}	6.48 ± 0.18^{b}
%0.56 extract	4.81 ± 0.25^{a}	6.50 ± 0.10^{b}

Differences between samples for the indicated analysis are represented by different lowercase letters within the same column, with a significance level of $P < 0.05$.

end of fermentation of the dough produced with the addition of baker's yeast are shown in Table 4. The number of yeast before fermentation was between 4.00 log CFU g^{-1} and 4.50 log CFU g^{-1}. During fermentation, there was an increase in the number of yeasts both in the control and dough with extract. Moreover, the addition of the cephalaria extract did not cause a significant inhibition of yeast fermentation in the dough.

Table 4 shows the increase in the number of yeasts in the dough during sourdough fermentation. The number of yeast ranging between 4.00 and 5.02 log CFU g^{-1} before fermentation exhibited an average 2–3 log increase during fermentation. Likewise, the addition of extract did not affect the number of yeasts negatively in sourdough fermentation.

The effect of Cephalaria extract on farinograph and extensograph properties

The results of the farinograph trials on dough produced using cephalaria extract are shown in Table 5. Any positive effect of extract addition on water absorption capacity compared to the control was observed, however, considering the stability, there was a significant increase with the addition of 0.56% extract compared to the control. Increasing the stability is important in terms of providing better quality dough production and gaining a stable structure to the dough.

The extensograph analysis results of the dough samples containing cephalaria extract are given in Table 5. The extensograph analysis gives information about the structure of the dough and the improvement of the bread-making properties. With the addition of cephalaria extract, not only the sprawling and loosening properties of doughs made from especially weak flours was positively improved, but also it was ensured that the dough reached the energy required for making bread. For bread-making, dough must have a certain energy value. Cephalaria extract increased this energy value effectively. While the energy value was 82 cm^2 in control, it increased to 105 cm^2 with the addition of 0.56% cephalaria extract. The number of rates gives information about the bread quality of the dough. As can be seen in Table 5, the number of rates has also increased with the addition of the cephalaria extract.

Especially in summer, it is not desired to sprawl the dough and it is desired to have a more tough structure. The resistance to elongation is an indicator of this feature of the dough. The higher the resistance, the more stable the dough is. When Table 5 was examined, it was seen that the effect of the extract of the cephalaria on the resistance to elongation was much higher than the control.

Table 5 The farinograph and extensograph properties of dough containing cephalaria samples

Samples	Farinograph	Parameters		
	DDT (mm:ss)	WAC %	S (mm:ss)	DS (FE)
Control	01:25	54.1	02:16	77
%0.56 extract	01:42	53.8	02:41	63

Samples	Extensograph			Parameters		
	Duration (min)	Energy (cm^2)	Elongation resistance (BU)	Elongation (mm)	Maximum Resistance (BU)	The number of rates (BU mm^{-1})
Control	45	82	319	153	412	2.1
	90	86	389	141	470	2.8
	135	85	415	140	486	3
%0.56 extract	45	105	672	106	755	6.3
	90	116	1163	81	1191	14.4
	135	96	1061	72	1078	14.8

BU, Brabender unit; DDT, development time; DS, degree of softening; S, stability; WAC, water absorption capacity.

Conclusion

Since the addition of cephalaria extract significantly increases the stability of the dough, it has been observed that even small amounts of cephalaria extract can be effective in strengthening weak flours.

In studies conducted with the addition of cephalaria flour, it was found that there was a slight increase in stability compared to the addition of extract while water absorption decreased. However, in this study, it was observed that cephalaria extract yield was much higher in flours with similar standards, compared to its flour, since its extract was used instead of cephalaria flour.

While the extracts did not show any antibacterial activity against the bacteria tested, a relative effect was observed on the moulds *Penicillium expansum* and *Aspergillus niger*. This result suggests that the extract addition to the dough can be effective in delaying mould growth in bread. Another important finding is that the extracts do not have an inhibitory effect on *Saccharomyces cerevisiae* used as baker's yeast. Thus, it has been understood that the extract of the cephalaria added to flour will not negatively affect the dough fermentation. The antioxidant activity of the extracts is a positive finding in terms of the nutritiousness and bioactivity of bread. In cytotoxicity tests, it is a positive finding that cephalaria extracts have an accelerating effect on HUVEC cells at low concentrations. Herewith, it was understood that cephalaria can accelerate regeneration in cells. Moreover, it is observed that the addition of cephalaria extract (a mixture of water and ethanol extract) at the optimum level improves the dough properties quite significantly. With the results obtained, it was determined that the extract of cephalaria gave extra positive properties to the dough in many aspects.

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Author contributions

Elif Şeyma Bağdat: Conceptualization; investigation; funding acquisition; writing – original draft; methodology; validation; formal analysis; data curation; visualization; writing – review and editing. **Fatih Bozkurt:** Supervision; methodology. **Özge Kahraman Ilıkkan:** Supervision; review and editing. **Osman Sagdic:** Supervision, Review and editing. **Fatih Törnük:** Project administration; methodology; funding acquisition; supervision; resources.

Data availability statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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