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The effect of *Cephalaria syriaca* L. seed extract, produced in different planting dates and irrigation conditions, on the quality of kefir drink

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ABSTRACT

Cephalaria syriaca L. is an annual medicinal plant with limited prior research. This study investigated the total phenolic and flavonoid content, antioxidant activity, and antiradical properties of Cephalaria syriaca L. under different ecological conditions in Urmia, Iran, including spring, delayed, and autumn planting dates, as well as full irrigation, supplementary irrigation, and dryland regimes. The extraction process involved mixing 1 g of seed powder with 10 mL of ethanol-water solvent (70:30), followed by shaking at 1000 rpm for 3 hours and sonication at 40°C for 15 minutes. Total phenolics and flavonoids were quantified using high-performance liquid chromatography (HPLC), while antioxidant and antiradical activities were measured via spectrophotometry. Data were analyzed using SAS software and ANOVA. Results revealed that extracts from non-irrigated, autumn-cultivated plants exhibited significantly higher total phenolics (125.31 mg QUE/g), flavonoids (12.20 mg QUE/g), DPPH radical inhibition (37.15%), nitric oxide radical scavenging (35.56%), and superoxide radical inhibition (27.29%). HPLC analysis identified gallic acid, chlorogenic acid, coumaric acid, and quercetin as the primary polyphenols, with concentrations increasing under drought stress. Notably, gallic acid showed the highest content (1222.11 mg QUE/g), while cinnamic acid had the lowest (0.86 mg QUE/g). Organoleptic evaluation of kefir enriched with *C. syriaca* extract demonstrated that the addition of 0.2 mg/mL of extract yielded the best results. These findings underscore the potential of *C. syriaca* as a functional ingredient in pharmaceutical and food products, leveraging its bioactive properties for health benefits.

1.Introduction

Free radicals are highly reactive, unstable molecules that include reactive oxygen species (superoxide and hydroxyl radicals), reactive nitrogen species (nitric oxide), and non-radical species such as nitrous acid and hydrogen peroxide [1,2]. These reactive oxygen species induce oxidative stress in human biological systems, compromising endogenous antioxidant defense mechanisms [3]. Numerous studies have demonstrated that free radicals attack cellular components, leading to physiological dysfunction [3]. Synthetic preservatives commonly used in food products can generate stable free radicals that have been linked to carcinogenesis [4,5]. Consequently, there has been growing interest in plant-derived alternatives due to their lower side effects [6-12]. Essential oils and plant extracts have gained widespread application in pharmaceutical and food industries owing to their well-documented antimicrobial and antioxidant properties [13,14]. *Cephalaria syriaca L*., a member of the *Dipsacaceae* family, represents one of seven identified genera of medicinal plants [15]. Native to eastern and southeastern Turkey, central Anatolia, the eastern Mediterranean, western and central Asia, southern Europe, and northern and southern Africa [16,17], this species has also been reported in Iran [18]. Although frequently classified as a wheat field weed that reduces crop yield [18], *Cephalaria syriaca L*. possesses notable medicinal properties, including sedative and relaxing effects [18]. Phytochemical investigations have revealed that various compounds from *Cephalaria* species including iridoids, flavonoids, triterpenes, alkaloids, and lignans - have been used in traditional medicine for centuries [19,20]. These compounds exhibit diverse biological activities such as antimicrobial, antifungal, antioxidant, and cytotoxic effects [19,20]. Despite these significant therapeutic potentials, comprehensive studies on *Cephalaria syriaca L*. remain limited [20]. This drought-tolerant species shows particular promise for cultivation in arid and semi-arid regions like Iran, where water scarcity is increasingly problematic. The current study represents the first systematic investigation of *Cephalaria syriaca L*. in West Azerbaijan province, Iran, focusing on optimizing cultivation conditions to enhance production of phenolic compounds, flavonoids, and phenolic acids while evaluating its radical scavenging capacity against nitric oxide and superoxide radicals. Given Iran's arid climate and the growing need for drought-resistant crops, this research specifically examines: the effects of different irrigation regimes (full irrigation, supplementary irrigation, and dryland farming), planting dates (spring, delayed, and autumn cultivation), and their combined impact on bioactive compound production in *Cephalaria syriaca L*. under Urmia's ecological conditions. Furthermore, we evaluated the potential application of optimized *Cephalaria syriaca L*. extracts for enriching functional food products, particularly kefir, based on their organoleptic properties and bioactive content.

2. Materials and Methods

2.1. Materials

The seeds of *Cephalaria syriaca L*. were obtained from Turkey and cultivated in the experimental field of the Department of Plant Production and Genetics, Faculty of Agriculture, Urmia University. The experiment was conducted as a split-plot arrangement within a randomized complete block design (RCBD) with three replications. The first factor consisted of irrigation regimes at three levels (rainfed, supplemental irrigation: applied at two stages (during flowering and seed filling, and full irrigation), while the second factor included different planting dates (autumn planting: October 6, delayed planting: December 6, and spring planting: April 4). Other nutritional materials, including kefir grains, milk, doogh (a traditional Iranian yogurt drink) from Pegah Dairy Company, sugar, and powdered milk, were purchased from the local market in Urmia. Chemical reagents, including acetic acid, hydrochloric acid, chloroform, acetonitrile (HPLC grade), potassium dihydrogen phosphate, and methanol (HPLC grade, Sigma-Aldrich, USA), were supplied by Adoora Teb Co. (Urmia, Iran).

2-2-Methods

1-2-2- Optimization of Extract Extraction from *Cephalaria syriaca L*.

Double-distilled water was prepared using a Fisons apparatus. The extraction of *Cephalaria syriaca L*. seed extract was performed following the method described by Soleimanifard et al. (2019) with minor modifications. In this method, the dried plant

parts were first ground into a fine powder using an electric mill (Sunny, Model SG-80). Subsequently, 1 g of the resulting powder was transferred into a sealed glass vial, and 10 mL of an ethanol-water (70:30) extraction solvent was added. The vial was then placed on an orbital shaker for 3 hours at 1000 × g for thorough mixing, followed by 15 minutes of sonication at 40°C in an ultrasonic bath. The extracted solution was then combined with the previously separated extract and subjected to lyophilization (freeze-drying at −80°C under 0.001 mbar pressure for 24 hours). The dried extract was reconstituted in the same solvent used for extraction, filtered through a 0.45-µm membrane filter, and then used for further analysis [21].

2-2-2-Identification and Quantitative Determination of Phenolic Acids (Polyphenolic Compounds) Using High-Performance Liquid Chromatography (HPLC)

The isolation, identification, and quantitative determination of the phenolic acids investigated in this study were performed using a high-performance liquid chromatography (HPLC) system (Agilent 1100 series, USA) equipped with a 20 µL injection loop, a quaternary solvent gradient pump, a degassing system, a column oven (set at 25°C), and a diode array detector (DAD) adjusted to wavelengths of 250, 272, and 310 nm. A 25 µL microsyringe (SGE, model F-LC25, Australia) was used for sample loading and injection into the HPLC system. For the separation and quantitative measurement of phenolic acids in the extracted samples, the HPLC system was fitted with an octadecylsilane (C18) column (ZORBAX Eclipse XDB, 25 cm in length, 4.6 mm in diameter, and 5 µm particle size) along with a 1 cm guard column. The system was also equipped with dual reciprocating pumps, an oven, an online degasser, a 20 µL sample loop, and a UV/Visible detector (SPD-10 AVP, USA) featuring an 8 µL quartz flow cell. Data acquisition and analysis were conducted using ChemStation software. Prior to use, the HPLC solvents (acetic acid, acetonitrile, and distilled water) as well as the extracted samples were filtered through a 0.45 µm membrane filter (Millipore). To achieve optimal compound separation, a gradient elution program was employed. Initially, the mobile phase consisted of 10% acetonitrile and 90% 1% acetic acid solution at a flow rate of 1 mL/min. Over 5 minutes, the composition was adjusted to 25% acetonitrile and 75% 1% acetic acid solution while maintaining the same flow rate. Subsequently, over the next 10 minutes, the ratio was further modified to 65% acetonitrile and 35% 1% acetic acid solution, still at a flow rate of 1 mL/min. The total separation time was 15 minutes

3-2-2-Total Phenolic Content

The total phenolic content was determined using the Folin-Ciocalteu method with slight modifications [22]. Briefly, 100 mg of the extract powder was dissolved in 1 mL of methanol. Subsequently, 100 µL of the plant seed extract was mixed with 100 µL of 50% Folin-Ciocalteu reagent, 2 mL of 2% sodium carbonate solution, and 2.8 mL of distilled water. The resulting solution was incubated in the dark at room temperature for 1 hour. The absorbance was then measured at 720 nm using a spectrophotometer, with a blank sample as the reference. The total phenolic content of the samples was calculated based on a gallic acid standard curve and expressed as milligrams of gallic acid equivalents per gram of dry plant weight (mg GAE g^{-1}) [23].

4-2-2- Total Flavonoid Content

The flavonoid content in different plant parts was determined using an aluminum chloride colorimetric assay. Briefly, 500 µL of the plant seed extract was mixed with 1.5 mL of 80% methanol, 2.8 mL of distilled water, and 100 µL of 1 M potassium acetate solution. After 5 minutes of incubation, 100 µL of 10% aluminum chloride solution was added. The reaction mixture was allowed to stand for 40 minutes at room temperature. The absorbance of the solution was then measured at 415 nm using a spectrophotometer, with a blank sample (containing all reagents except the plant extract, replaced with an equal volume of 80% methanol) as the reference. The total flavonoid content was calculated based on a quercetin standard curve and expressed as milligrams of quercetin equivalents per gram of dry plant weight (mg QE g^{-1}) [22].

5-2-2-Determination of Antioxidant Activity by DPPH Method

To evaluate the antioxidant activity, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was employed [24]. For this

purpose, 50 µL of the plant-methanol extract (containing 0.5 mL of different treatment extracts mixed with 1.5 mL methanol) was combined with 950 µL of DPPH solution. The mixture was then kept in darkness for 40 minutes to allow the reaction to proceed. Subsequently, the optical absorbance was measured at 517 nm using a spectrophotometer.

$$I\ (\%) = \frac{(A_B - A_S)}{A_B} \times 100$$

In Equation 1, AB represents the absorbance of the control sample (containing all reaction components except the test sample), while AS denotes the absorbance of the test sample.

6-2-2- Identification and Quantification of Polyphenolic and Flavonoid Compounds

The polyphenolic and flavonoid compounds in different extracts of *Cephalaria syriaca L.* (seed and leaf) were identified by comparing the retention times of sample peaks with those of reference standards. The analysis followed the method described by Soleimanifard et al. (2019) [21]. For this purpose, a solution of 0.2 mg/mL in methanol was prepared. Subsequently, 20 µL aliquots of the different extracts and relevant standards were separately injected into the HPLC system using a syringe to determine the retention times of various compounds. The quantification of polyphenolic and flavonoid contents in the different extracts was performed by measuring the peak areas and comparing them with those of the corresponding standard curves.

7-2-2-Nitric Oxide Radical Scavenging Activity

The percentage inhibition of nitrite free radicals was calculated according to the method described by Nikokhah et al. (2008) with minor modifications [23]. Briefly, 10 µL of extract was mixed with 0.5 mL of phosphate-buffered saline (10 mM) and 2 mL of sodium nitroprusside (10 mM), followed by incubation at 25°C for 150 minutes. Subsequently, 0.5 mL of the resulting solution was combined with 1 mL of sulfanilic acid (0.33% in 10% glacial acetic acid) and allowed to stand for 5 minutes to complete the reaction. Then, 1 mL of N-(1-naphthyl)ethylenediamine dihydrochloride (0.1%) was added, and the mixture was incubated at 25°C for 30 minutes, resulting in the formation of a diffuse pink color. The absorbance was measured at 540 nm, and the percentage inhibition was calculated using the following formula:

$$\text{NO radical scavenging } \% = \frac{(A_{blank} - A_{sample})}{A_{sample}} \times 100$$

where A_blank represents the absorbance of the mixture without extract, and A_sample denotes the absorbance of the mixture containing the extract.

8-2-2-Kefir Grain Cultivation

In this study, kefir grains (a symbiotic consortium of lactic acid bacteria and yeasts) were cultured for 3 months in pasteurized post-churned milk (1:5 ratio) supplemented with powdered milk (West Azerbaijan province) and either doogh or yogurt (Pegah Dairy) to adjust pH. The cultures were maintained at 25°C in an incubator equipped with an agitator. Aseptic subculturing was performed every 24 hours in sterile containers. Empirical additions of powdered milk, yogurt, or doogh were made to enhance kefir grain growth in the post-churned milk medium. Following 24-hour incubation periods, the kefir grains were separated from the produced kefir. Subsequently, Cephalaria syriaca extract powder was incorporated at concentrations of 0.1, 0.2, and 0.3 mg per 100 mL of kefir, homogenized using a mixer, and refrigerated for subsequent analyses [25].

9-2-2-Organoleptic Evaluation

The sensory characteristics of kefir beverage treatments were assessed by a 12-member trained panel. Panelists received standardized training to evaluate key product attributes including: color homogeneity, extract powder dispersibility, beverage consistency, fresh taste, appropriate aroma, and overall acceptability. The evaluation employed a 5-point hedonic scale (5=excellent, 4=good, 3=average, 2=poor, 1=very poor). Between sample assessments, panelists cleansed their palates with plain bread and water to prevent sensory fatigue [21].

10-2-2-Statistical Analysis

Data were recorded and compiled in Excel prior to statistical processing. A split-plot arrangement within a randomized complete block design (RCBD) with three replications was analyzed using SAS version 9.4. Variance analysis (ANOVA) was performed for all traits, followed by mean separation using Duncan's

multiple range test at α=0.05 significance level. All graphical representations were generated using Excel software.

3-Discussion and Conclusion

1-3-Optimization of Extraction Methods for *Cephalaria syriaca L*. Extracts

Modern multi-solvent systems are widely employed to optimize the extraction of phenolic compounds from medicinal plants. Ethanol has been recognized as both a safe and effective solvent for phenolic compound extraction, making it particularly suitable for recovering these phytochemical groups, especially when intended for nutraceutical (food-drug) applications [26]. Comparative analysis of ethanol-water and acetone-water solvent mixtures revealed that extracts obtained from drought-stressed plants treated with ethanol-water and acetone-water contained 22.6 and 18.4 mg/g gallic acid equivalents, respectively, while fully irrigated plants yielded 14.8 and 11.3 mg/g. The data demonstrated that a 70:30 ethanol-water mixture achieved the highest absorption at the characteristic wavelength for gallic acid (720 nm) in non-irrigated plants. These findings align with prior studies investigating the effects of irrigation regimes and biofertilizers on morphological traits, essential oil content, and phenolic/flavonoid levels in *Foeniculum vulgare*, where the highest essential oil yield and total phenolic content were observed under well-irrigated conditions [27]. Similarly, research on the influence of sowing date and chemical fertilizers on moisture content, flavonoids, and germination indices in *Lallemantia iberica* seeds reported peak flavonoid concentrations (0.264 mg/g) in crops sown on March 5th, reflecting a 63.97% increase compared to October 15th planting [28]. Collectively, these results underscore the critical role of solvent selection and agronomic practices in maximizing bioactive compound extraction efficiency.

2-3-Phenolic and Flavonoid Content

Table 1 presents the analysis of variance (ANOVA) results for the chemical and antioxidant properties of *Cephalaria syriaca*. The highest phenolic content was observed in non-irrigated (21.96 mg/g gallic acid equivalent), supplemental irrigation (17.62 mg/g), and full irrigation (14.25 mg/g) treatments, respectively. A similar trend was noted for flavonoid content, with the highest levels recorded in non-irrigated (1.89 mg/g gallic acid equivalent), supplemental irrigation (1.51 mg/g), and full irrigation (1.2 mg/g) treatments (Figure 1). Previous studies have confirmed the antioxidant activity of *Cephalaria syriaca* extracts [29, 30].

This plant contains numerous phenolic compounds that exhibit a direct correlation with its antioxidant activity. These compounds function by scavenging free radicals through their hydroxyl groups [31]. Plant phenolics are secondary metabolites synthesized under favorable environmental conditions via the shikimic acid pathway and phenylpropanoid metabolism [32]. These compounds play significant physiological and ecological roles in plant-environment interactions, including pollinator attraction, protection against biotic and abiotic stressors, reproduction, anti-herbivory properties, antimicrobial activity, and as previously mentioned, antioxidant functions [33]. Consequently, drought stress has been shown to increase phenolic content in Cephalaria syriaca, consistent with findings from other studies. Given that the antioxidant activity is associated with flavonoid content in various parts of Cephalaria species [33], and considering that the growth of these plant parts is influenced by irrigation regimes, the significant effect of water availability on this trait was expected.

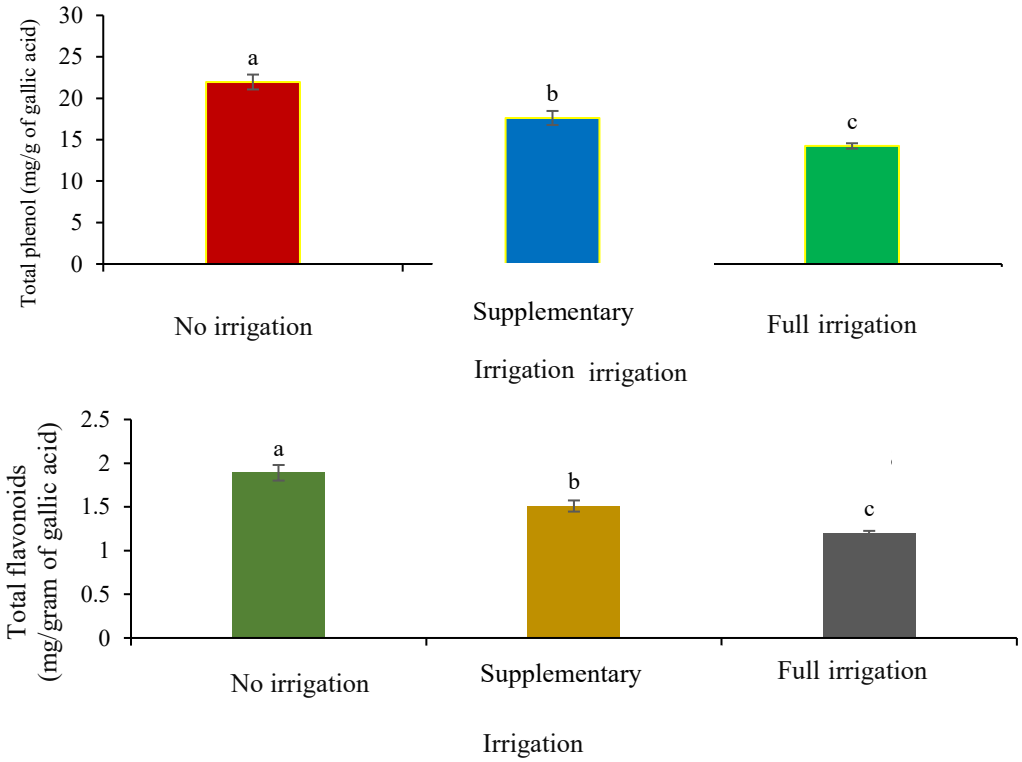

Fig 1. The results of the comparison of the simple effect of irrigation regimes on the content of phenol (a) and total flavonoid (b) of ***Cephalaria syriaca*** **L**.

Table 1. Variance analysis of biochemical traits of *Cephalaria syriaca* L. under different irrigation regimes and planting dates

Sources of change	degrees of freedom	Mean square				
		Total phenol	Total flavonoids	DPPH radical	Nitric Oxide	superoxide radical
Block	2	11.27	0.061	44.59	48.23	7.62
Irrigation regime	2	134.4	1.092	638.5	716.3	288.8
Error a	4	3.26	0.029	14.18	13.67	4.51
date of cultivation	2	11.25	0.083	62.39		33.2
Irrigation × planting date	4	3.66	0.047	27.60	81.4	9.8
error b	12	3.71	0.027	15.34	38.77	7.74
coefficient of variation (%)		10.73	10.68	20.39	32.21	13.22

ns, * and ** are non-significance and significance respectively at the probability level of five and one percent.

3-3-DPPH Radical Scavenging Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is one of the most widely used methods for evaluating the antioxidant potential of plant extracts. In this study, the highest DPPH radical scavenging activity was observed in drought-stressed (non-irrigated) plants (28.16%), followed by supplemental irrigation (18.05%) and full irrigation (11.43%) treatments. Additionally, autumn cultivation (21.67%) and delayed planting (19.53%) exhibited the strongest radical inhibition capacity (Figure 2). The enhanced DPPH scavenging activity under drought stress was expected, as antioxidants mitigate oxidative damage by neutralizing free radicals, thereby reducing physiological deterioration and enhancing tissue resistance to environmental stresses and microbial infections [35]. Previous studies have confirmed the DPPH inhibitory properties of *Cephalaria syriaca* L. [18]. In line with this, Rahimi et al. (2019) reported a scavenging activity range of 47.10% to 60.16% [30], which is higher than the values observed in this study. However, the

minimum inhibition rate (18.8%) reported by Kavak and Baştürk (2020) [36] aligns closely with our findings. Given its strong antioxidant capacity, *Cephalaria syriaca* L.shows promise for preventing foodborne diseases and could be utilized in functional food or nutraceutical applications [18].

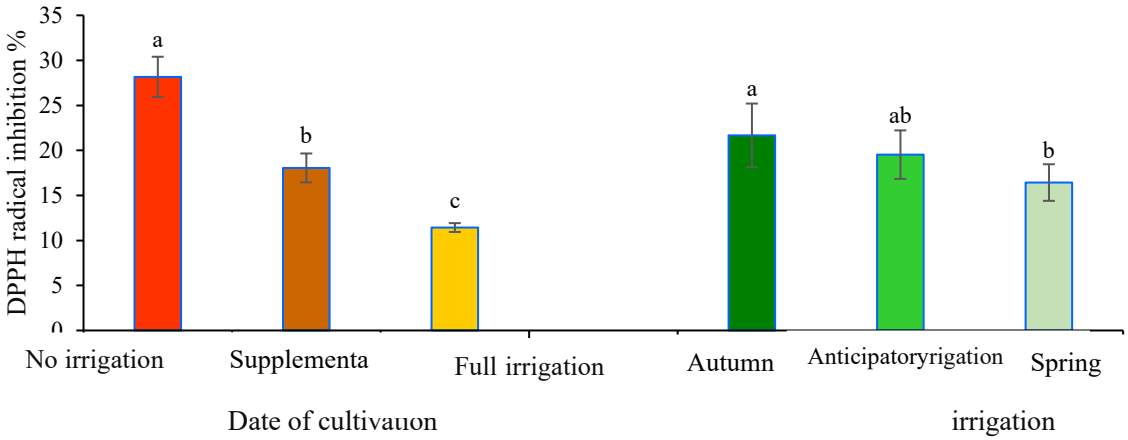

Fig 2. Simple effects of irrigation regime and cultivation date on the changes in DPPH radical scavenging properties of *Cephalaria syriaca* L.

4-3-Identification and Quantification of Polyphenolic Compounds

Table 2 presents the different polyphenolic compounds identified in *Cephalaria syriaca* L.under various experimental treatments. Based on the total average, the major compounds included gallic acid (1221.8 mg QUE/g), chlorogenic acid (169.1 mg QUE/g), coumaric acid (154.6 mg QUE/g), and crocin (61.66 mg QUE/g). Minor compounds consisted of caffeic acid (14.13 mg QUE/g), rutin (11.31 mg QUE/g), rosmarinic acid (9.12 mg QUE/g), apigenin (6.83 mg QUE/g), and cinnamic acid (0.47 mg QUE/g) (Table 2). While gallic acid content showed minimal variation between treatments, chlorogenic acid reached its highest level in autumn cultivation under drought stress (255.3 mg QUE/g) and its lowest in spring cultivation with full irrigation (101.4 mg QUE/g). The results clearly demonstrate that both drought stress and delayed cultivation increase coumaric acid content in *Cephalaria syriaca* L.. Crocin levels varied significantly (0-141.1 mg QUE/g), with the highest values observed in autumn cultivation under drought stress and the lowest in spring cultivation with full irrigation. A similar trend was noted for other compounds. The unique properties of polyphenols—such as blood sugar regulation, cardiovascular protection, and anticancer effects—combined with their high concentrations in *Cephalaria syriaca* L., highlight this plant's potential as a valuable nutraceutical resource. The observed variations in polyphenolic content across different planting dates likely stem from climatic differences induced by experimental treatments. Moreover, increased polyphenolic content under drought stress directly correlates with enhanced antioxidant capacity. Previous studies report that drought stress elevates flavonoid (e.g., luteolin, apigenin) and phenolic acid (e.g., chlorogenic acid) levels in medicinal plants such as St. John's wort [37], sage [38], and cumin [39]. These changes appear to result from abiotic stress-induced modulation of key genes and molecular mechanisms in the phenylpropanoid pathway.

Table 2. Polyphenol compounds of *Cephalaria syriaca* L. in the studied treatments (mg $QUEg^{-1}$)

Irrigation	cultivation	CA	RA	Ru	GA	ChA	Co	Qu	CiA	A
No irrigation	Autumn	25.33	13.25	19.17	1222.1	255.3	248.4	141.1	0.86	12.75
No irrigation	Anticipatory	19.41	11.17	16.61	1222.0	231.9	203.4	132.0	0.6	10.08
No irrigation	Spring	17.51	9.67	16.46	1221.9	197.4	173.9	118.3	0.5	8.05
Supplementary	Autumn	13.0	11.26	13.16	1221.9	185.1	178.3	102.0	0.3	6.10
Supplementary	Anticipatory	12.04	10.87	9.19	1221.8	174.4	163.4	28.41	0.28	5.77
Supplementary	Spring	14.63	9.87	8.98	1221.8	143.6	143.8	6.55	0.24	4.74
Full	Autumn	13.19	9.84	6.57	1221.7	117.7	130.2	10.45	0.27	5.68
Full	Anticipatory	9.00	2.51	5.92	1221.6	115.3	118.7	16.02	0.53	7.03
Full	Spring	2.85	3.76	5.68	1221.6	101.4	31.53	0.00	0.66	1.27
Total average	-	14.13	9.12	11.31	1222.1	169.1	154.6	61.66	0.47	6.83

CA: caffeic acid, RA: rosmarinic acid, Ru: rutin, GA: gallic acid, ChA: chlorogenic acid, Co: coumaric acid, Qu: quercetin, CiA: cinnamic acid, A: apigenin

5-3-Nitric Oxide and Superoxide Radical Scavenging Activity

Nitrite has traditionally been used in meat products like sausages as a color fixative and preservative. However, nitrate from vegetables can be converted to nitrite through bacterial reactions in the human body, subsequently forming carcinogenic nitrosamines [40]. Therefore, compounds that can scavenge these precarcinogenic agents like nitrite may play a protective role against cancer. Nitric oxide (NO) and superoxide (O_2^-) play significant roles in physiological functions [41], but these free radicals can cause unlimited oxidation of cellular components and damage biological structures. An effective approach to neutralize free radicals is through antioxidant compounds that act as radical scavengers, highlighting the importance of antioxidants in protecting cells from oxidative damage, particularly under drought stress conditions [42]. Nitric oxide, as a free radical, contributes to inflammatory diseases by reacting with superoxide to form peroxynitrite ($ONOO^-$), a potent oxidant that causes various oxidative damages [43]. Our results on NO and superoxide radical scavenging activity are presented in Figure 3. Among irrigation treatments, the highest NO scavenging capacity was observed in non-irrigated (23.65%), supplemental irrigation (12.23%), and full irrigation (6.07%) treatments. Similarly, among planting dates, autumn cultivation (16.86%), delayed planting (14.23%), and spring cultivation (10.86%) showed the highest NO inhibition (Figure 3a). The superoxide scavenging capacity followed a similar trend, with the highest activity in non-irrigated (27.28%) and autumn cultivation (22.94%), while full irrigation (16.21%) and spring cultivation (19.09%) showed the lowest activity. Supplemental irrigation (19.64%) and delayed planting (21.1%) demonstrated intermediate values (Figure 3b). These results suggest that autumn planting and drought stress (non-irrigation) are optimal for enhancing the antioxidant capacity of *Cephalaria syriaca* L.under experimental conditions. Previous studies have reported that the antioxidant activity of *Thymus vulgaris* increases under drought stress [44]. Research on NO radical inhibition has shown that anthocyanins and phenolic compounds in berries, *Phyllanthus* species, and pine bark exhibit potential NO scavenging activity [45-46]. Experimental evidence indicates that NO derived from nitroprusside reacts with oxygen to form nitrite, and plant extracts can competitively inhibit nitrite formation through direct radical scavenging. Higher phenolic and flavonoid concentrations correlate with greater inhibitory effects [47]. Nikokhah et al. (2008) reported similar findings in studies on blackberry, mulberry, and strawberry extracts [29]. Analysis of polyphenolic compounds in *Cephalaria syriaca* L. (Table 2) revealed that non-irrigated and autumn cultivation treatments contained the highest levels of gallic acid, chlorogenic acid, coumaric acid, and quercetin. This likely explains the enhanced NO and superoxide inhibition observed under drought stress and autumn planting conditions.

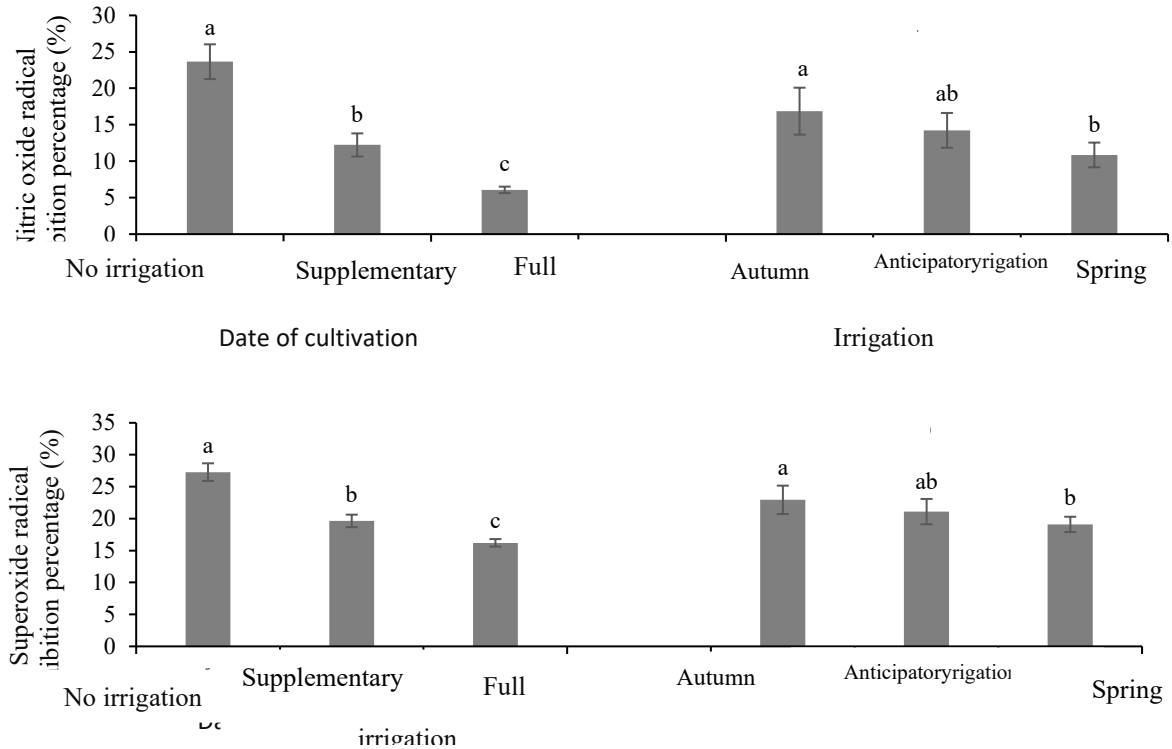

Fig 3. The results of comparisons of the simple effects of irrigation regimes and cultivation date on the scavenging capacity of nitric oxide radical (a) and superoxide radical (b) in *Cephalaria syriaca* L.

6-3-Organoleptic Evaluation

Following the determination of the optimal treatment (autumn cultivation under drought stress) based on phenolic, antioxidant, and radical-scavenging properties, varying concentrations of the dried extract (0.1, 0.2, and 0.3 mg) were incorporated into 100 mL of kefir. After homogenization and refrigeration, a sensory evaluation was conducted by an expert panel assessing color, extract powder dispersibility, beverage homogeneity, fresh taste, desirable aroma, and overall acceptability. The 0.2 mg/100 mL kefir formulation is recommended as the optimal dosage, effectively balancing functional properties (e.g., antioxidant activity) with minimal adverse effects on sensory attributes. This concentration achieved the highest consumer preference while preserving the product's organoleptic quality.

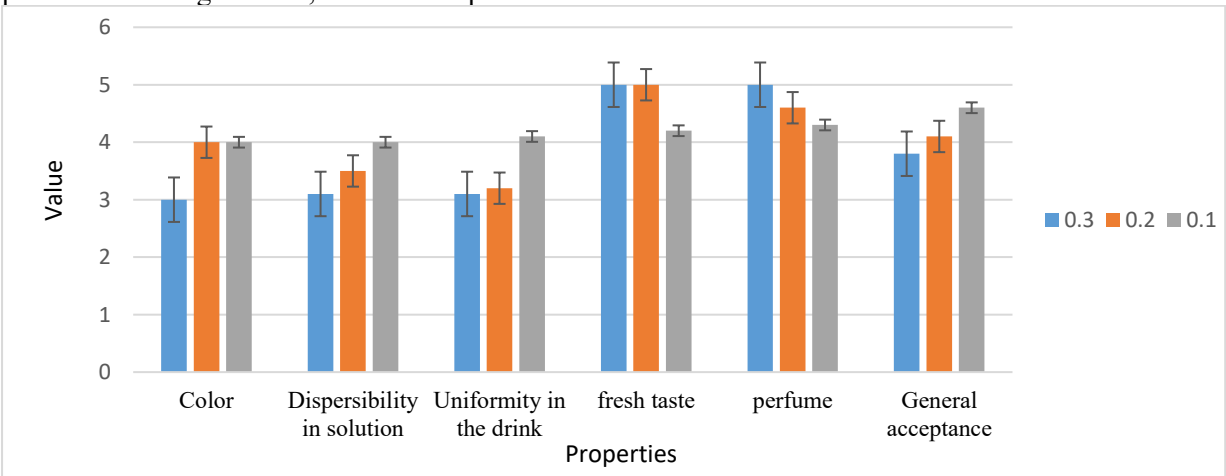

Fig 4. The results of the organoleptic test of kefir enriched with *Cephalaria syriaca* L. plant extract powder

4-Conclusion

The present study demonstrated that the extract derived from *Cephalaria syriaca* L. subjected to non-irrigated (drought stress) and autumn cultivation exhibited a significant increase ($p < 0.05$) in total phenolic content, flavonoid concentration, and radical-scavenging activity (DPPH, nitric oxide, and superoxide radicals) compared to other irrigation regimes. HPLC analysis identified gallic acid, chlorogenic acid, coumaric acid, and crocin as the dominant polyphenolic compounds, with their concentrations positively correlated with drought stress intensity. This suggests that controlled water deficit enhances bioactive compound biosynthesis in *Cephalaria syriaca* L.. Furthermore, organoleptic evaluation of kefir fortified with the optimized extract revealed that the 0.2 mg/100 mL dosage achieved the highest acceptability, balancing functional efficacy with minimal sensory alterations. These findings position *Cephalaria syriaca* L.as a promising natural antioxidant source for functional food applications, particularly in dairy products.

5-References

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مقاله علمی_پژوهشی

تاثیر عصاره دانه سفالاریای سوری (*Cephalaria syriaca* L.)، تولید شده در تاریخ‌های کاشت و شرایط آبیاری مختلف بر کیفیت نوشیدنی کفیر

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چکیده

سفالاریای سوری (*Cephalaria syriaca* L.) از جمله گیاهان دارویی یک‌ساله می‌باشد که تاکنون تحقیقات اندکی در مورد آن گزارش شده است. در پژوهش حاضر، ترکیبات فنولی و فلاونوئیدی کل، فعالیت ضداکسیدانی و ضدرادیکالی سفالاریای سوری تحت شرایط اکولوژیک ارومیه (زمان‌های کشت بهاره، انتظاری، پاییزه و رژیم آبیاری کامل، آبیاری تکمیلی، دیم) مورد مطالعه و مقایسه قرار گرفت. در این پژوهش، ابتدا ۱ گرم از پودر دانه گیاهی با ۱۰ میلی‌لیتر حلال اتانول-آب (۷۰:۳۰) ترکیب سپس به مدت ۳ ساعت بر روی شیک پلیت با دور ۱۰۰۰rpm و در نهایت به مدت ۱۵ دقیقه در دمای ٤۰ درجه سانتی‌گراد در حمام فرا صوت قرار داده شد. شناسایی و اندازه‌گیری مقادیر فنولی تام و فلاونوئیدها توسط کروماتوگرافی مایع با کارایی بالا، و اندازه‌گیری فعالیت ضداکسیدانی و ضدرادیکالی توسط دستگاه اسپکتروفوتومتر اندازه‌گیری شد. تجزیه و تحلیل نتایج با استفاده از نرم‌افزار SAS و روش آزمون آنالیز واریانس انجام شد. نتایج نشان داد که عصاره استخراج شده مربوط به تیمار بدون آبیاری و کشت پاییزی، نسبت به دو رژیم دیگر آبیاری، افزایش معنی‌داری در میزان فنول کل ($mg\ QUEg^{-1}$ ۲۵/۳۰۸)، فلاونوئید ($mg\ QUEg^{-1}$ ۲/۲۰۲) و درصد مهارکنندگی رادیکال DPPH (۳۷/۱٤۹)، درصد مهار رادیکال‌های نیتریک اکسید (۳۵/۵٦۳) و سوپر اکسید (۲۷/۲۹۲) داشت. نتایج شناسایی ترکیبات فنولی نشان داد گالیک اسید، کلروژنیک اسید، کوماریک اسید و کروستین به‌عنوان ترکیبات اصلی پلی‌فنل سفالاریای سوری شناسایی شدند که این ترکیبات با افزایش تنش خشکی بر محتوای آنها در گیاه افزوده شد به طوری که در شرایط یکسان، گالیک اسید ($mg\ QUEg^{-1}$ ۱۲۲۲/۱۰۵۹۵۲) و سینامیک اسید ($mg\ QUEg^{-1}$ ۰/۸٦) به ترتیب بالاترین و کمترین مقدار را داشتند. نتایج ارگانولپتیک کفیر غنی‌سازی شده نشان داد که تیمار ۰/۲ میلی‌گرم پودر عصاره در ۱۰۰ سی‌سی کفیر بهترین نتایج را داشت. نتایج نهایی نشان داد می‌توان از خواص فراسودمند گیاه سفالاریا سوری در محصولات دارویی و غذایی بهره‌مند شد.