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ORIGINAL PAPER

Effect of Environment and Genotype Interactions on Blessed Thistle (*Cnicus Benedictus* L.) Agronomic, Total Phytochemical Content, Cnicin Concentration, Phenolic Profile, and Functional Biological Activity

Mehdi Ghiasy-Oskoee[1] · Majid AghaAlikhani[1] · Kalidas Shetty[2] · Harlene Hatterman-Valenti[2]

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Abstract

Purpose This study provides insights that blessed thistle (C*nicus benedictus* L.) ecotypes grown in different eco-edaphic conditions can be targeted as a potential source of health-relevant phytochemicals linked to their antioxidant and enzymatic inhibitory activities.

Methods Agronomic productivity, phytochemical contents as well as cnicin concentration and phenolic profile linked to the functional biological properties (α-amylase and α-glucosidase inhibitory and antioxidant activities) were assessed with three ecotypes (Tehran, Shiraz, Sari) grown in four various eco-edaphic sites (S1-S4) during two growing seasons.

Results The greatest biomass yield (12,005 kg DW ha^{-1}) was noted for plants grown in the arid-sandy soil condition (S1), while the lowest yield (10,357 kg DW ha^{-1}) was recorded for plants grown in the humid climate-clay soil condition (S4). The Tehran ecotype demonstrated richer phytochemicals in arid and semi-arid sites compared to others. The HPLC analysis showed that while chlorogenic acid, as the dominant phenolic compound, was higher in ecotypes grown in S1; quercetin and rutin, the minor compounds responsible for plant enzymatic inhibitory, were more abundant in the semi-arid (S2) plants.

Conclusions Cnicin and individual phenolic acids like chlorogenic acid and flavonoids, mainly quercetin and rutin, had a potential synergetic effect on the plant's amylase and α-glucosidase inhibition potential. The Tehran ecotype could be considered the most important therapeutically relevant ecotype for future plant variety developments. The high agronomic productivity of blessed thistle in marginal lands and its biological activity linked to certain phytochemicals make the plant a potential target for further developments and glycemic control therapeutic applications.

Keywords Eco-edaphic sites · Ecotypes · Chlorogenic acid · Flavonoids · Glycemic control potential and management of type 2 diabetes

1 Introduction

Considerable attention has been currently given to the ethnomedicinal and therapeutic values of medicinal plants, with an emphasis on identifying novel plant-based medicines including minimal side effects. Plant-derived bioactive compounds and their associated antioxidant phytochemicals are particularly interesting as potential novel therapeutic agents within human healthcare systems (Tlili and Sarikurkcu 2020; Foss et al. 2022; Dincheva et al. 2023). It is thus, important to identify herbs known as a rich source of phytochemicals that are grown abundantly in diverse ecologically adapted areas. The blessed thistle (*C. benedictus*), an Asteraceae family thistle-like herb, is a well-known ethnomedicinal plant used therapeutically to cure several types of diseases, especially liver and gastro-intestinal disorders (Ulbricht et al. 2008; Al-Snafi 2016). Recent clinical trials have shown the plant has versatile pharmaceutical activities, such as anti-inflammatory (Paun et al. 2019), antioxidant, antinociceptive, and anti-parasite

✉ Harlene Hatterman-Valenti
h.hatterman.valenti@ndsu.edu

Mehdi Ghiasy-Oskoee
mehdi.ghiasyoskoee@modares.ac.ir

[1] Department of Agronomy, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran

[2] Department of Plant Sciences, North Dakota State University, Fargo, ND 58108, USA

properties (Ahmadimoghaddam et al. 2020; Queiroz et al. 2021), as well as its potential for reducing skin inflammation (Sousa et al. 2021). It also has been tested as a novel plant-based drug for inhibiting cancer cells with promising results (Tiwana et al. 2021). The plant's potential therapeutic effects have been mainly attributed to its high content of sesquiterpene lactones, mainly cnicin and phenolic metabolites (Ghiasy-Oskoee et al. 2018; Paun et al. 2019; Queiroz et al. 2021). Several studies have reported cnicin-linked therapeutic effects of thistle-like medicinal plants, such as blessed thistle and *Centurea* species, including anti-inflammation (Paço et al. 2022; Rezig et al. 2024), antirheumatic, antipyretic (Fattaheian-Dehkordi et al. 2021), coupled with antiproliferative, anti-Alzheimer and antibiotic properties (Petropoulos et al. 2020; Rezig et al. 2024). Interestingly, cnicin also exhibited promising potential as an effective antitumor (Moura et al. 2018) and anti-SARS-CoV-2 plant-based drug (Alhadrami et al. 2022). In addition, the blessed thistle-enriched phenolic profile was known to be linked to biochemical and pharmacological effects, such as antioxidant and α-amylase and α-glucosidase inhibitory properties (Paun et al. 2019). Both α-amylase and α-glucosidase are associated with glucose metabolism, and inhibiting these enzymes helps in glycemic control for the management of type 2 diabetes (Tshiyoyo et al. 2022; Nina et al. 2023).

The current approach to cultivating medicinal plants has been focused on marginal lands not suited for growing crops, but the availability of valuable and versatile medicinal plants is often limited in these areas (Singh et al. 2022). Furthermore, plant genotype variations and eco-edaphic conditions and their interaction are essential factors influencing the abundance and profile of secondary metabolites in herbs and medicinal plants (Generalić Mekinić et al. 2019; Yaldiz et al. 2019; Pant et al. 2021). Thus, introducing high-value medicinal herbs successfully adapted to marginal lands would help to advance understanding of the effect of environmental conditions on yield and chemical constituents that would be the basic step towards establishing a successful farming model for the adaption of medicinal plants. In addition, relying on these plants could contribute to more agricultural land use and biodiversity in marginal areas as a promising approach to diversifying agricultural systems and making plant cultivation and industry viable in these regions. Our previous field studies have shown that blessed thistle adapted perfectly to stressed marginal lands (Ghiasy-Oskoee et al. 2018, 2020). This species appears to be a very flexible herb and responds strongly to different environmental conditions. Thus, the amount and composition of chemically active constituents of blessed thistle will vary across ecological regions, which inevitably will impact its pharmacological potential. Also, it has a broad ecological niche and many ecotypes grown across different environments that may have potentially different yields, chemical contents, and compositions. However, the potential of blessed thistle ecotypes as agronomically promising plants in different eco-edaphic environments, especially marginal agroecosystems, and their potential therapeutic values in the management of type-2 diabetes have not been evaluated so far. Therefore, this study aimed to investigate the effect of the various eco-edaphic conditions on agronomic productivity (biomass yield, plant height, LAI, and phenology) and phytochemicals (cnicin concentration, C/N ratio, total phenolic content, phenolic profile) linked to the biological activity (α-amylase and α-glucosidase inhibitory and antioxidant activities) of different ecotypes of blessed thistle.

2 Materials and methods

2.1 Site Description and Field Experiment

The field study of the research consisted of growing three blessed thistle ecotypes (Tehran, Shiraz, and Sari) in four different eco-edaphic conditions of Iran during the 2018–2019 and 2019–2020 growing years. The experimental fields were located in the central, north-central, western, and northern regions of Iran, each representing distinct climate conditions of arid, semi-arid, moderate, and humid climatic conditions, respectively (Peel et al. 2007). These regions are quite typical representations of the country's overall climate conditions. The seeds for each ecotype were collected from wild populations in the Tehran, Shiraz, and Sari regions of Iran.

The central site field (S1) was located at the research farm of the Iranian National Salinity Research Center (32° 03' N, 54° 14' E, 1136 m above sea level), Yazd, Iran. Based on the Köppen climate classification (Peel et al. 2007), this site has a hot desert climate with only an annual precipitation of 49 mm which primarily occurs during the winter season. This area is characterized by alkaline sandy soil and low organic matter levels. The north-central site field (S2) was located at the research farm of Tarbiat Modares University (35° 44' N, 51° 09' E, 1265 m above sea level), Tehran, Iran. This area is characterized by a semi-arid climate with a long-term mean annual precipitation and temperature of 232 mm and 22°C, respectively. The site has sandy loam soil with low amounts of nitrogen content and high phosphorous content. The western site field (S3) was at an experimental farm in the Dehloran region (31° 58' N, 45° 24' E, 1174 m above sea level), Ilam, Iran. This area has a continental moderate climate with hot summers and mild winters. The long-term mean annual precipitation in this area is 560 mm, evenly distributed throughout the year. This region could be considered to be one of the areas with great plant diversity

and natural ecosystem value in the entire Middle East (Rostami et al. 2022). The northern site field (S4) was situated in the Rasht region (37° 17' N, 49° 36' E, 7 m above sea level), Guilan, Iran, a coastal area with a humid subtropical climatic condition (Daemei et al. 2019). The average annual rainfall and air temperature are 1506 mm and 15 °C, respectively. The site is characterized by calcareous and clay soil and a good level of organic carbon. For each study site and growing season, major monthly meteorological data (temperature, rainfall, relative humidity, and daylight hours) were recorded by weather stations placed nearby the trial fields (Table 1). Soil physicochemical properties were tested two weeks before the experiments by collecting soil samples at 0.3 m depth in each site (Table 2). Accordingly, sites 1 and 2 were representative of arid and semi-arid marginal lands, respectively, while sites 3 and 4 were regarded as medium and optimum environments, respectively.

Each blessed thistle ecotype was sown in plots that were 10 m × 5 m (20 rows, 50 cm spacing between rows) arranged in a randomized complete blocks design with four replicates per ecotype at each studied site. Sowing took place in early October on all sites, at depths of 4–5 cm and a density of 20 seeds m^{-2}. Three seeds per hole were hand-sown in rows in each plot with 0.5 m spacing between the rows and 0.22 m between the holes to achieve the desired density. Thinning to a single plant per each row was done once seedlings produced four leaves.

In the second year, however, the abundant rainfall at the beginning of October delayed the sowing date at the S4 site. Prior crops grown in sites were summer corn (*Zea mays*) at the S2 and S3 sites as well as fallow at the S1 and S4 sites. Agronomic practices were conducted according to the specific soil needs of each site. Field experiments were implemented under low-input farming conditions, which were adopted by applying low amounts of mineral fertilizers and chemical inputs. Only at the Yazd site (S1 arid region), light irrigation (total 150 mm) was applied by drip irrigation each year after sowing and during the seedling stage to ensure germination and uniform stand. The irrigation volume was controlled using a water meter. Approximately 50 kg of urea (46% N) per hectare was manually applied (50% before sowing and the remaining 30 days after sowing) to meet the required nitrogen of ecotypes at the Yazd (S1) and Tehran (S2) sites. No nitrogen or other mineral fertilizers were used at the Ilam and Rasht sites (S3 and S4) as the soil analysis demonstrated sufficient nutrient levels in the soil of these sites (Table 2). There were no notable occurrences of insect pests or diseases during plant growth. Weeds were controlled by hand-weeding as necessary.

2.2 Agronomic Productivity

At each study site, 5 m^2 per replicate area was randomly sampled once the blessed thistle ecotypes reached the full flowering stage (BBCH-69; Lancashire et al. 1991). The aerial parts (leaf + stem + flower) of the plants were hand-harvested from ground level to assess the biomass yield components. Within each sampling area, plant height (cm), biomass yield (kg ha^{-1}), and leaf area index (LAI) data were collected to estimate the agronomic productivity of the ecotypes at each site. Aboveground biomass dry matters (DM) were assessed after oven drying at 75 °C to a constant weight. Leaf area per plant was measured by a leaf area meter (Delta-T Devices LTD, Cambridge, UK), and then LAI was calculated on a ground area basis. For each site and growing season, growth-cycle length was calculated as the number of days from sowing to 100% flowering stage, and the accumulated growing degree days (GDD) were measured as follows:

$$GDD = \sum[(T_{max}+T_{min})/2 - T_{base}]$$

Where T_{max} and T_{min} referred to the maximum and minimum air temperatures, respectively, and T_{base} was the minimum temperature at which the crop can grow, defined as 5 °C for similar Asteraceae thistle-like plants (Parmoon et al. 2018).

2.3 Phytochemicals

Blessed thistle samples were ground to a fine powder using a mortar and pestle. Afterward, 20 mg of the tissue was extracted twice with 10 mL of 60% ethanol under sonication (Elma Ultrasonics, Singen, Germany) at 20 °C for 20 min and then centrifuged at 15,000 × g for 10 min. The supernatant was filtered through a 0.2 µm filter (Orange Scientific, Belgium), and the solvent was evaporated by a rotary evaporator (Buchi Rotavapor R-114, Flawil, Switzerland) at 20 °C over a period of 30 min. Extracts were dissolved in 1 mL of HPLC-grade methanol for further analysis.

2.3.1 Total Soluble Phenolic and Flavonoid Contents

The total soluble phenolic of samples was assessed using the Folin-Ciocalteu spectrophotometric-based method (Slinkard and Singleton 1977); Briefly, 1 mL of each extract was mixed with 1 mL of pure ethanol and 5 mL of distilled water in a test tube. To each sample, an equal volume of Folin-Ciocalteu reagent (10%) and 0.8 mL of Na_2CO_3 (7.5%) was added and mixed. After incubation for 30 min at ambient temperature in the dark, the absorbance was determined at 765 nm using a UV-1800 spectrophotometer (Shimadzu UV–VIS 1601, Kyoto, Japan). Measurements were performed in triplicate. Gallic acid (GAE) was used as a

Table 1 Monthly weather data at studied sites (S1-S4) during the 2018–2019 and 2019–2020 growing seasons

	2018–2019															
	S1[a]				S2				S3				S4			
Months	**Avg temp (^{O}C)**	**Rainfall (mm)**	**RH (%)**	**Sunshine (h)**	**Temp (^{O}C)**	**Rainfall (mm)**	**RH (%)**	**Sunshine (h)**	**Temp (^{O}C)**	**Rainfall (mm)**	**RH (%)**	**Sunshine (h)**	**Temp (^{O}C)**	**Rainfall (mm)**	**RH (%)**	**Sunshine (h)**
Oct	17.7	0.0	14.8	348.5	10.5	1.7	25.7	269.7	26.5	5.8	29.0	245.5	20.2	620.0	86.4	158.5
Nov	13.5	16.5	33.5	228.7	11.8	21.5	52.5	156.7	17.8	31.3	50.3	185.6	16.8	490.7	83.5	110.5
Dec	10.7	6.7	40.5	214.0	3.7	54.2	55.5	161.3	9.4	19.0	48.5	159.5	12.0	358.7	84.5	100.0
Jan	7.5	18.2	44.0	230.3	2.7	10.0	45.2	187.0	2.7	28.5	45.4	134.5	7.3	272.0	80.3	90.8
Feb	7.7	5.7	26.4	280.5	6.4	22.7	47.2	220.7	1.0	49.5	44.5	170.8	8.5	308.7	75.5	88.0
Mar	14.8	6.5	22.9	288.9	7.3	14.1	39.5	218.0	1.4	44.8	39.7	149.5	8.4	290.0	75.0	70.5
Apr	21.7	8.2	19.0	305.0	14.4	1.9	32.1	296.3	8.2	18.6	40.2	288.4	15.5	140.7	80.5	140.0
May	29.2	0.0	12.7	348.3	20.5	22.0	30.3	315.0	21.1	7.5	35.7	304.0	29.4	120.8	68.3	158.9
Jun	30.3	0.4	15.5	355.8	28.5	8.5	25.2	370.4	22.4	12.2	33.0	338.5	30.5	88.2	67.8	160.9
Jul	29.7	0.0	14.3	368.5	30.7	2.4	20.5	344.4	26.9	5.5	24.4	322.6	28.8	62.7	66.5	197.8
Aug	26.5	0.0	10.0	350.4	27.3	1.0	22.5	298.5	25.5	2.5	28.5	268.4	26.4	77.2	70.2	197.3
Total GDD		2110			1890				1770				1690			
	2019–2020															
Months	Avg temp (^{O}C)	Rainfall (mm)	RH (%)	Sunshine (h)	Temp (^{O}C)	Rainfall (mm)	RH (%)	Sunshine (h)	Temp (^{O}C)	Rainfall (mm)	RH (%)	Sunshine (h)	Temp (^{O}C)	Rainfall (mm)	RH (%)	Sunshine (h)
Oct	18.3	0.80	17.5	344.0	11.0	2.1	24.4	270.7	27.2	9.4	27.4	249.7	20.9	715.0	86.0	155.2
Nov	14.7	12.4	30.0	239.0	11.5	22.4	51.4	159.3	18.2	29.0	52.9	198.5	16. 8	450.5	85.0	119.8
Dec	10.9	2.5	37.1	210.0	3.4	44.7	50.2	161.5	8.5	20.5	51.3	160.7	12.0	390.7	84.0	111.0
Jan	7.8	14.2	48.0	230.3	3.6	12.0	45.2	199.7	2.8	29.0	44.8	147.5	9.3	242.0	85.3	98.8
Feb	6.0	6.8	25.0	284.0	5.2	19.7	35.2	239.7	1.8	45.4	41.7	180.5	8.1	195.7	80.5	88.0
Mar	13.5	7.5	22.5	295.0	7.4	15.5	40.5	220.0	2.5	39.5	39.2	152.8	7.3	240.0	78.2	78. 5
Apr	22.2	9.0	20.4	302.5	13.2	0.9	28.2	290.3	9.1	17.5	37.6	210.7	16.6	230.7	74.5	144.0
May	30.5	0.5	14.1	346.5	21.9	14.8	31.3	320.3	19.5	9.8	35.7	288.0	30.4	112.2	75.3	160.4
Jun	31.0	0.0	12.7	368.3	28.0	7.5	20.2	378.7	23.0	17.2	35.0	338.5	31.0	90.2	68.8	168.5
Jul	30.0	0.0	12.5	360.0	31.2	0.4	15.5	345.7	27.5	3.5	25.5	334.7	28.4	44.5	63.5	201.8
Aug	27.5	0.20	12.3	344.8	26.0	1.5	20.5	309.5	23.7	4.3	31.5	272.5	27.5	50.2	75.2	205.3
Total GDD	2111				2107				1792				1745			

S1[a] (Yazd site:): arid climate-sandy soil condition, S2 (Tehran site): semiarid climate -sandy soil condition, S3 (Ilam site): moderate climate-loamy soil condition, S4 (Rasht site): humid climate-clay soil condition. GDD, growing degree days; Avg temp, average temperature; RH, relative humidity

Table 2 Physico-chemical properties of the soil sites (0–30 layer) before experiment outset in 2018–2019 and 2019–2020

		2018–2019				2019–2020			
		S1[a] (sandy loam)	S2 (sandy loam)	S3 (loamy sand)	S4 (clay)	S1 (sandy loam)	S2 (sandy loam)	S3 (loamy sand)	S4 (clay)
pH	-	8.0	7.4	7.2	6.8	7.5	8.0	7.5	7.0
Salinity	dS m^{-1}	2.8	0.6	0.5	0.5	1.2	1.5	0.9	0.5
Sand	%	74.0	61.2	51.4	9.6	61.2	74.0	51.3	9.6
Silt		17.8	21.9	41.3	27.4	21.9	17.8	41.2	27.3
Clay		8.2	16.8	7.4	63.0	16.8	8.2	7.4	63.0
OM		0.7	0.9	1.8	2.4	0.6	0.4	1.5	2.4
N		0.1	0.2	0.7	1.5	0.1	0.1	0.9	1.5
FC		15.7	33.3	38.5	40.6	27.0	14.3	33.6	40.6
WP		10.5	13.8	22.2	28.9	12.0	6.8	20.5	28. 9
AW		15.6	17.1	27.2	42.2	14.0	9.5	24.6	33.3
P	mg kg^{-1}	30.5	25.0	50.9	18.8	29.0	30.5	55.9	18.8
K		412.7	285.4	375.8	243.7	350.0	312.5	420.5	243.7
Fe		3.6	8.4	9.4	9.4	6.5	4.3	8.2	9.4
Zn		1.1	2.8	3.2	3.8	2.8	0.9	2.9	3.8
Mn		0.6	1.8	1.7	1.9	1.2	0.5	1.6	1.9

S1[a] (Yazd site:): arid climate condition, S2 (Tehran site): semiarid climate condition, S3 (Ilam site): moderate climate condition, S4 (Rasht site): humid climate condition. OM, organic matter; FC, field capacity; WP, wilting point; AW, available water

standard. Total phenolic was expressed as mg GAE per g dry weight (mg GAE g^{-1}). The total flavonoid content was also assessed according to the flavonoid-aluminum complex, as described by Ordonez et al. (2006). The absorbance was measured at 420 nm with quercetin as a standard. Total flavonoid content was expressed as mg quercetin equivalents (QE) per g of dry weight (mg QE g^{-1}).

2.3.2 Phenolic Compositions

The identification and quantification of phenolic acids of blessed thistle extracts were conducted using HPLC (Knauer, Berlin, Germany) equipped with a smart line 1000 solvent pump unit, a Rheodyne 7725 injector of 20 µl loop volume (Cotati, CA, USA), and a K-2800 photodiode array detector (PDA). The column used for the separations was a stainless steel reversed-phase C18 column (150 mm × 4.6 mm, 5 µm particle size, Shim-Pack, Shinwa Chemicals, Kyoto, Japan). The column was operated at an ambient temperature, and the chromatographic data were monitored by a software program (Shimadzu Scientific Instruments, Inc. Columbia, MD). The flow rate was 0.7 mL min^{-1}. The mobile phase was HPLC-grade water (A) and methanol (B) both with 0.02% trifluoroacetic acid. The gradient elution condition involved a linear gradient as follows: 0–5 min, 5% solvent B which reaches 15% within 20 min; 20–25 min, 35% solvent B; 25–45 min, 55% solvent B, and at 45–55 min solvent B reaches 100%. The UV detection was set at four different wavelengths (220, 280, 320, and 360 nm). Identification was carried out by comparing the retention times and spectral data with those of standards, while quantification was performed using the corresponding calibration curves ($r=0.99$) from standards diluted in methanol and results were expressed as mg g^{-1} dry weight.

2.3.3 Cnicin Content and C/N Ratio

Cnicin was analyzed using high-performance liquid chromatography (HPLC), as described above, and it was separated on a reversed-phase Hypersil BDS-C18 column (250 mm × 4 mm, 5 µm particle size, Agilent, Santa Clara, CA, USA). The solvent gradient of 10–25% acetonitrile (ACN) in water was used for the first 20 min, 25% ACN the next three minutes, 25–40% ACN in three minutes, and three minutes more kept in 40% ACN with a flow rate of one milliliter/minute. Cnicin was diluted in methanol (HPLC grade) to reach a final concentration of 2 mg mL^{-1}. The solution was filtered through 0.45 µm membrane filters and degassed before usage, injecting a volume of 15 µL. Detection was set at 215 nm. The cnicin standard curve was obtained by plotting standard concentrations (0.05, 0.1, 0.2, 0.3, and 0.4 mg mL^{-1}) as a function of peak area in HPLC chromatograms. High linearity ($R^2>0.99$) was obtained for each calibration curve. Each sample was analyzed twice. Cnicin contents were expressed in mg g^{-1} dry weight. Cnicin yield (kg ha^{-1}) was calculated by multiplying cnicin content and biomass yield. All related reagents, chemicals, and standards were obtained from Sigma-Aldrich Chemie GmbH (Munich, Germany) and Merck (Darmstadt, Germany). The total carbon and nitrogen contents of the blessed thistle were measured by a dry combustion method using a CR-612 carbon analyzer (LECO, Corporation, St. Joseph, MI, USA). This instrument used a combustion method with infrared

detection for the simultaneous measurement of total carbon and nitrogen contents in organic samples.

2.4 Biological Activity

2.4.1 α-Amylase Inhibitory Activity

The α-amylase inhibitory activity assay was measured based on the method explained by Ramakrishna et al. (2017) with slight modifications. A volume of 500 µL of blessed thistle sample extract and 500 mL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing alpha-amylase solution (0.5 mg mL^{-1}) were incubated at 25 °C for 10 min. Following this, 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube at timed intervals. The reaction was stopped with 1.0 mL of dinitrosalicylic acid color reagent (1% 3, 5-dinitro salicylic acid, 0.2% phenol, 0.05% Na_2SO_3, and 1% NaOH in aqueous solution). The test tubes were then incubated in a boiling water bath for 10 min and cooled to room temperature. The mixture was then diluted using distilled water, and the mixture was read at 540 nm using a spectrophotometer (UV-vis Multiskan Spectrum, Thermo Scientific, Waltham, MA, USA). The absorbance of sample blanks (buffer instead of enzyme solution) and a control (buffer in place of sample extract) were also recorded. The α-amylase inhibitory activity was calculated as percentage (%) inhibition using the following equation:

$$\text{Inhibition (\%)} = \frac{Abs_{control} - (Abs_{sample\ extract} - Abs_{sample\ blank})}{Abs_{control}} \times 100$$

Where $Abs_{control}$ was the absorbance without sample, $Abs_{sample\ extract}$ was the absorbance of sample extract and $Abs_{sample\ blank}$ was the absorbance of the extract with reagent.

2.4.2 α-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory activity was determined based on details in Worthington's manual (1993), with some modifications (Ramakrishna et al. 2017). A 50 µL extract sample and 100 µL of 0.1 M phosphate buffer (pH 6.9) containing a-glucosidase solution (1-unit mL^{-1}) were incubated in 96 well microplates at 25 °C for 10 min. Following this, 50 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25 °C for 5 min. Absorbance values were recorded at 405 nm before and after incubation using a microplate reader (340, Bio-Tek Instruments, Winooski, VT, USA) and compared with a control containing 50 µL of buffer solution versus sample extract. The α-glucosidase inhibitory activity was expressed as percentage (%) inhibition and was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\Delta\ Abs_{control} - \Delta\ Abs_{sample}}{\Delta\ Abs_{control}}$$

Where $Abs_{control}$ was the absorbance without sample, Abs_{sample} was the absorbance of sample extract.

2.4.3 Antioxidant Activity

The antioxidant potential of the blessed thistle extracts was assayed by a modified DPPH (1,1- diphenyl-2-picryl-hydrazyl, Sigma Chemical Co, St Louis, MO, USA) free radical scavenging method (Kwon et al. 2006). A volume of 250 µL of the blessed thistle sample extract was mixed with 1250 µL of DPPH stock solution (60 µM in ethanol). The absorbance was recorded after 5 min at 517 nm using a spectrophotometer (UV-vis Multiskan Spectrum, Thermo Scientific, Waltham, MA, USA). The readings were compared with the controls, containing 95% ethanol instead of sample extract. The antioxidant potential of the sample extracts was expressed as percentage (%) inhibition of DPPH free radical formation and was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\Delta\ Abs_{control} - \Delta\ Abs_{sample}}{\Delta\ Abs_{control}}$$

where $Abs_{control}$ is the absorbance of DPPH radical + methanol; Abs_{sample} is the absorbance of DPPH radical + sample extract/standard.

2.5 Statistical Analysis

A standard analysis of variance procedure was performed on the data with year, genotype (ecotype), and environment (site) modeled as fixed effects, and replication as a random effect using SAS software (9.3, Cary, USA). Differences among means were assessed utilizing the least significant difference test (LSD) at the 0.05 probability level where appropriate. Bartlett's test was applied to assay the homogeneity of the means square error between the two years. Due to the non-homogeneous error in Bartlett's test, parameters were analyzed separately for each year.

3 Results

3.1 Agronomic Productivity

Significant differences in agronomic parameters were observed across growing regions during the two growing seasons (Table 3). Nonetheless, the effect of genotype on the agronomic parameters was not statistically significant (data not shown). The greatest biomass production (12,005 kg DW ha^{-1}) was recorded in blessed thistles grown during the

Table 3 Agronomic parameters and phenology of *C. benedictus* as affected by site environmental conditions in the 2018–2019 and 2019–2020 growing seasons

Traits		Height (cm)	Biomass yield (kg DW ha^{-1})	LAI	Phenology (Day to 50% flowering)
2018–2019	S1[a]	22.3 b[b]	11,327 ab	3.6	178.8 d
	S2	28.7 a	12,005 a	4.1	208.7 c
	S3	29.4 a	11,986 a	3.9	222.7 b
	S4	22.3 b	10,357 b	3.7	234.7 a
	F test	**	*	ns	**
2019–2020	S1	26.1 a	8412	3.1 ab	85.0 c
	S2	18.7 b	7458	2.3 c	71.0 d
	S3	26.5 a	8500	3.3 a	94.7 b
	S4	20.4 b	8215	2.8 b	104.4 a
	F test	**	ns	**	**

S1[a] (Yazd site:): arid-sandy soil condition, S2 (Tehran site): semiarid-sandy soil condition, S3 (Ilam site): moderate-loamy soil condition, S4 (Rasht site): humid-clay soil condition. [b] Means in each column and growing season followed by a similar letter are not significantly different at a 5% probability level using the least significant difference (LSD) test. ns: non-significant; *significant at the 0.05 probability levels; **significant at the 0.01 probability levels. LAI, leaf area index

2018–2019 season at the S2 site. However, this yield was only greater than the biomass yield (10,357 kg DW ha^{-1}) recorded at the S4 site (Table 3). The biomass yield did not differ among sites for the 2019–2020 growing season which was attributed to the variations in rainfall and temperature observed between the two growing years (Table 1).

Plant height followed a similar trend as plant biomass during the 2018–2019 season while blessed thistle leaf area index (LAI) did not differ among sites (Table 3). During the 2019–2020 season, plants at sites S1 and S3 were taller than plants at sites S2 and S4. Blessed thistle plant LAI followed a similar trend as height with no differences due to site during 2018–2019 while plant LAI from S1 and S3 were greater than plant LAI from S2 and S4 with the exception that plant LAI from S1 and S4 were similar.

The blessed thistle plant completed its growth cycle in approximately 179 days during the first year and 85 days during the second year at the S1 arid site, while this duration was significantly longer at the S4 humid site, approximately 234 days in the first and 104 days in the second year (Table 3).

3.2 Phytochemicals

3.2.1 Total Soluble Phenolic and Flavonoid Acids

Although agronomic productivity of the blessed thistle ecotypes remained relatively constant across ecotypes and growing regions depending on the year, phytochemical contents substantially varied in studied growing sites, among ecotypes, and by growing season (Fig. 1a-b). The ecotype Tehran grown in the S1 arid site showed the highest total soluble phenolic contents in both growing seasons. In contrast, the lowest levels were observed in the S4 humid region with the ecotype Sari having the highest total soluble phenolic during 2018–2019, while both Tehran and Sari ecotypes had the highest contents during 2019–2020 (Fig. 1a). The trend for total flavonoid content was similar to the trend observed for total soluble phenolic contents, with the ecotype Tehran having the greatest total flavonoid at S1 and ecotype Sari having the greatest total flavonoid at S4 during 2018–2019 (Fig. 1b). Even though there were generally more total flavonoid contents for each ecotype during 2019–2020 compared to 2018–2019 season, Tehran ecotype had the greatest total flavonoid at S1 in both growing seasons while Sari ecotype had the highest total flavonoid contents at S4 in both years (Fig. 1a-b).

3.2.2 Phenolic Composition

The phenolic compositions of the studied blessed thistle ecotypes grown across the studied sites indicated that only two (caffeic and apigenin) of the nine phenolic compounds evaluated were not influenced by the blessed thistle ecotype and specific site (Table 4). Chlorogenic acid was the dominant phenolic compound of blessed thistle and had the highest concentration in the Tehran ecotype regardless of the site where the plants were grown. In addition, the Tehran ecotype grown in the S1 arid site with the lowest rainfall and highest temperature conditions had the maximum levels of chlorogenic acid.

The P-coumaric acid concentration differed within ecotypes only at the S1 site with Tehran and Sari having a higher concentration than Shiraz (Table 4). In contrast, luteolin concentration differed within ecotypes only at the S1 site with Tehran and Sari having a lower concentration than Shiraz. On the other hand, certain minor compounds like quercetin and rutin, belonging to the flavonol class, were found to be more abundant in the ecotypes grown in the S2 semi-arid region compared to those from the S1 arid region or others.

3.2.3 Cnicin Content, Yield and C/N Ratio

Cnicin content and cnicin yield significantly differed in ecotypes and growing regions (Fig. 2a-b). In general, the Tehran ecotype, especially when grown in the arid site during the growing season of 2019–2020, contained higher levels of cnicin (11.30 mg g^{-1}) compared to those in moderate and humid regions (Fig. 2a). In contrast, Shiraz ecotype grown in the humid site during the growing season of 2018–2019

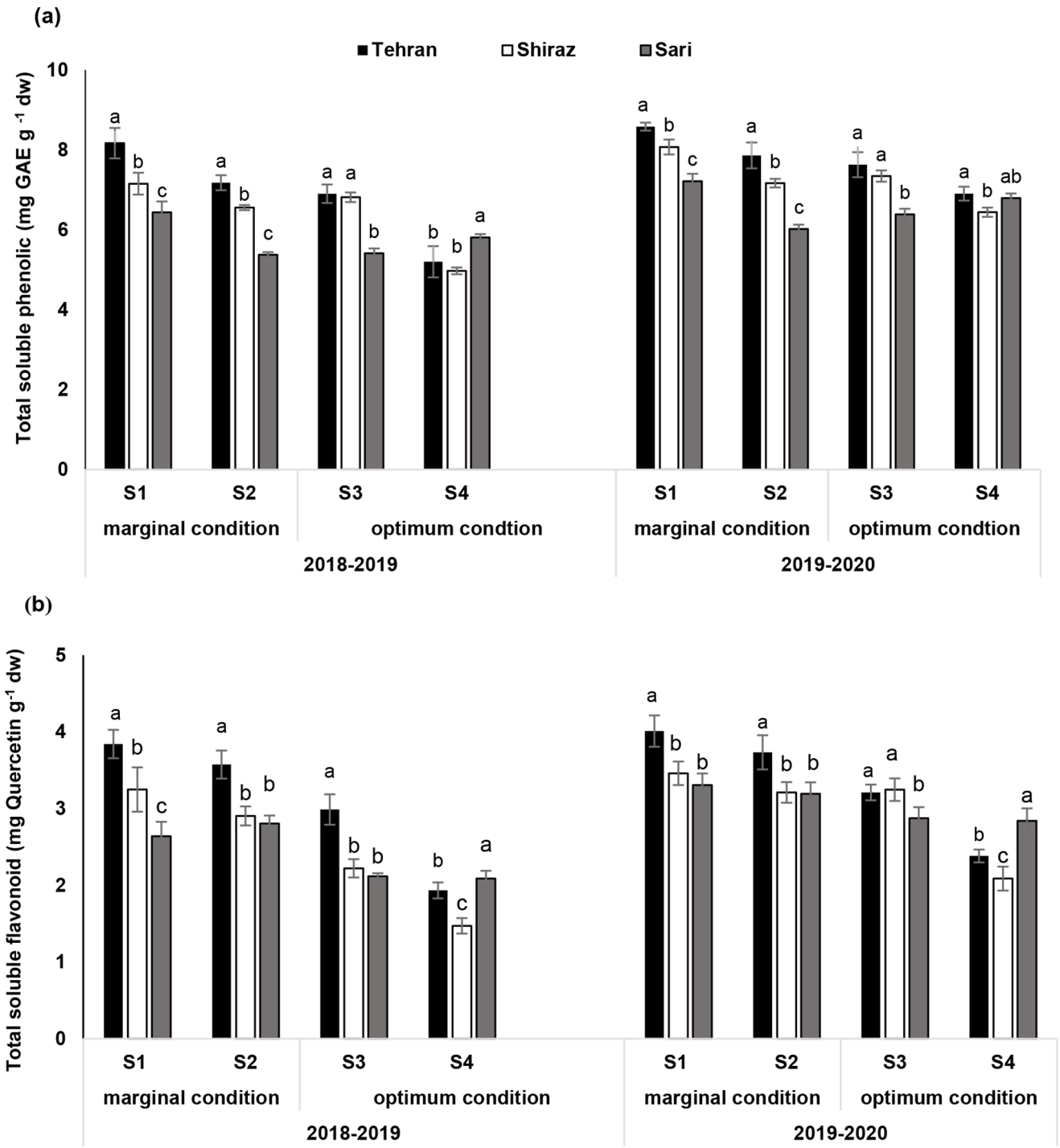


Fig. 1 **a** Total soluble phenolic. **b** Total soluble flavonoid content of *C.benedictus* as affected by the environment (sites, S1-S4) and ecotypes (Tehran, Shiraz, Sari) interactions in the first (2018-2019) and the second (2019-2020) growing seasons. S1 (Yazd site): arid-sandy soil condition, S2 (Tehran site): semiarid-sandy soil condition, S3 (Ilam site): moderate-loamy soil condition, S4 (Rasht site): humid-clay soil condition. Means followed by similar letters for each trait in bars are not significantly different at the 5% probability level by the LSD test. Error bars represent standard error of the means ($n=4$)

showed the lowest average cnicin production (5.88 mg g^{-1}), although there was no significant difference among ecotypes at the humid site in both years. Furthermore, no significant variations were found in the cnicin content of plants grown at the humid S4 site with those grown at the moderate S3 site during both years (Fig. 2a).

Considering both the quantitative and qualitative aspects of blessed thistle, cnicin yield can be considered the most

Table 4 Phenolic acid composition of *C. benedictus* ecotypes grown in various site environmental conditions (S1-S4)

		Phenolic acids (mg g^{-1} dry weight)								
Treatments		Chlorogenic acid	P-coumaric acid	Caffeic acid	Sinapic acid	Quercetin	Apigenin	Rosmaric acid	Luteolin	Rutin
	Teheran	3.4 a[b]	0.09 a	0.07	0.81 a	0.28 a	0.01	0.12 a	0.01b	0.24 a
S1[a]	Shiraz	2.7 b	0.08 b	0.07	0.55 b	0.29 ab	0.01	0.08 b	0.02a	0.17 b
	Sari	2.9 c	0.09 a	0.06	0.54 b	0.25 b	0.01	0.00 c	0.01b	0.15 b
	F test	**	ns	ns	*	**	ns	*	*	**
S2	Teheran	2.8 a	0.07	0.05	0.77 a	0.56 a	0.06	0.09 a	0.02	0.45 a
	Shiraz	2.5 b	0.06	0.04	0.65 b	0.49 b	0.05	0.08 ab	0.02	0.30 b
	Sari	2.2 c	0.07	0.05	0.58 c	0.50 b	0.05	0.07 b	0.01	0.38 b
	F test	**	ns	*	*	*	ns	*	ns	**
S3	Teheran	2.5 a	0.06	0.07	0.69 a	0.35 b	0.04	0.07	0.01	0.25 a
	Shiraz	2.2 ab	0.06	0.07	0.53 b	0.33 b	0.05	0.09	0.00	0.20 b
	Sari	1.8 b	0.05	0.06	0.49 b	0.41 a	0.05	0.07	0.00	0.20 b
	F test	*	ns	ns	**	*	ns	ns	ns	*
S4	Teheran	1.5 ab	0.06	0.04	0.81	0.28 c	0.0	0.01	0.00	0.18 a
	Shiraz	0.9 b	0.07	0.04	0.55	0.35 b	0.01	0.01	0.00	0.11 b
	Sari	1.9 a	0.08	0.06	0.54	0.40 a	0.01	0.02	0.01	0.19 a
	F test	*	ns	ns	ns	*	ns	ns	ns	ns

S1[a] (Yazd site:): arid-sandy soil condition, S2 (Tehran site): semiarid-sandy soil condition, S3 (Ilam site): moderate-loamy soil condition, S4 (Rasht site) humid-clay soil condition. [b] Means in each column and site followed by a similar letter are not significantly different at a 5% probability level using the least significant difference (LSD) test. ns: non-significant; *significant at the 0.05 probability levels; **significant at the 0.01 probability levels. Means are averaged over two growing seasons

important attribute to compare the productive performance of blessed thistle ecotypes grown across different regions. Despite the higher average cnicin concentrations in plants grown in the S1 arid region during the second year (9.50 mg g^{-1}), the highest average cnicin yield (95.21 kg ha^{-1}) was from plants grown in the S2 semi-arid region during the first year (Fig. 2b). In fact, even though plants grown in an arid region contained a comparatively higher cnicin content than in the semi-arid region, the relatively higher biomass yield of plants in the semi-arid region compensated for their lower cnicin contents.

3.3 Biological Activity (Antidiabetic and Antioxidant Activities)

Similar to the levels of phytochemical compounds, the biological activity of each ecotype generally varied across growing regions (Fig. 3a-c). The Tehran ecotype grown in the S1 arid or S2 semi-arid sites had superior enzyme inhibitory and antioxidant activity during both growing seasons even though biological activity during 2019–2020 was higher than in 2018–2019. Upon comparing the degree of inhibition against α-glucosidase and α-amylase enzymes, all genotypes inhibited the α-glucosidase enzyme more effectively than the α-amylase enzyme (Fig. 3a-b).

4 Discussion

Three blessed thistle ecotypes (Tehran, Shiraz, and Sari) were grown in four regions (sites) with distinctive climate conditions for two growing seasons (2018–2019 and 2019–2020). The sites represented a wide range of climate conditions, from arid to humid climates, and soil types, from sandy to clay soils (Tables 1 and 2). The variations in the rainfall patterns within sites and growing years were relevant, as on average, the first year had greater precipitation than the second year in arid and semi-arid sites (S1 and S2), but an opposite pattern was observed in moderate and humid sites (S3 and S4). Differences in temperatures and relative humidity remained constant in the two years and were more pronounced in the S1 arid site than in the S3 moderate and S4 humid sites. In S1 arid and S2 semi-arid sites, the total annual rainfall during the first was only 62 and 148 mm, respectively. This amount was significantly lower when compared to the S3 moderate climate region where the total annual rainfall reached 205 mm. The S4 humid site was characterized as a humid climatic region with high rainfall (average 2500 mm) well distributed throughout the plant-growing season of each year. The S1 and S4 sites had the lowest and highest mean air temperatures (9.6 and 14.7 °C), respectively, during the plant-growing season of each growing year. Interestingly, despite these differences in the growth period, negligible variations were observed in Growing Degree Days (GDD) accumulated throughout the plant's growing season across the studied sites (Table 1). Therefore, the difference in growing cycle duration can be

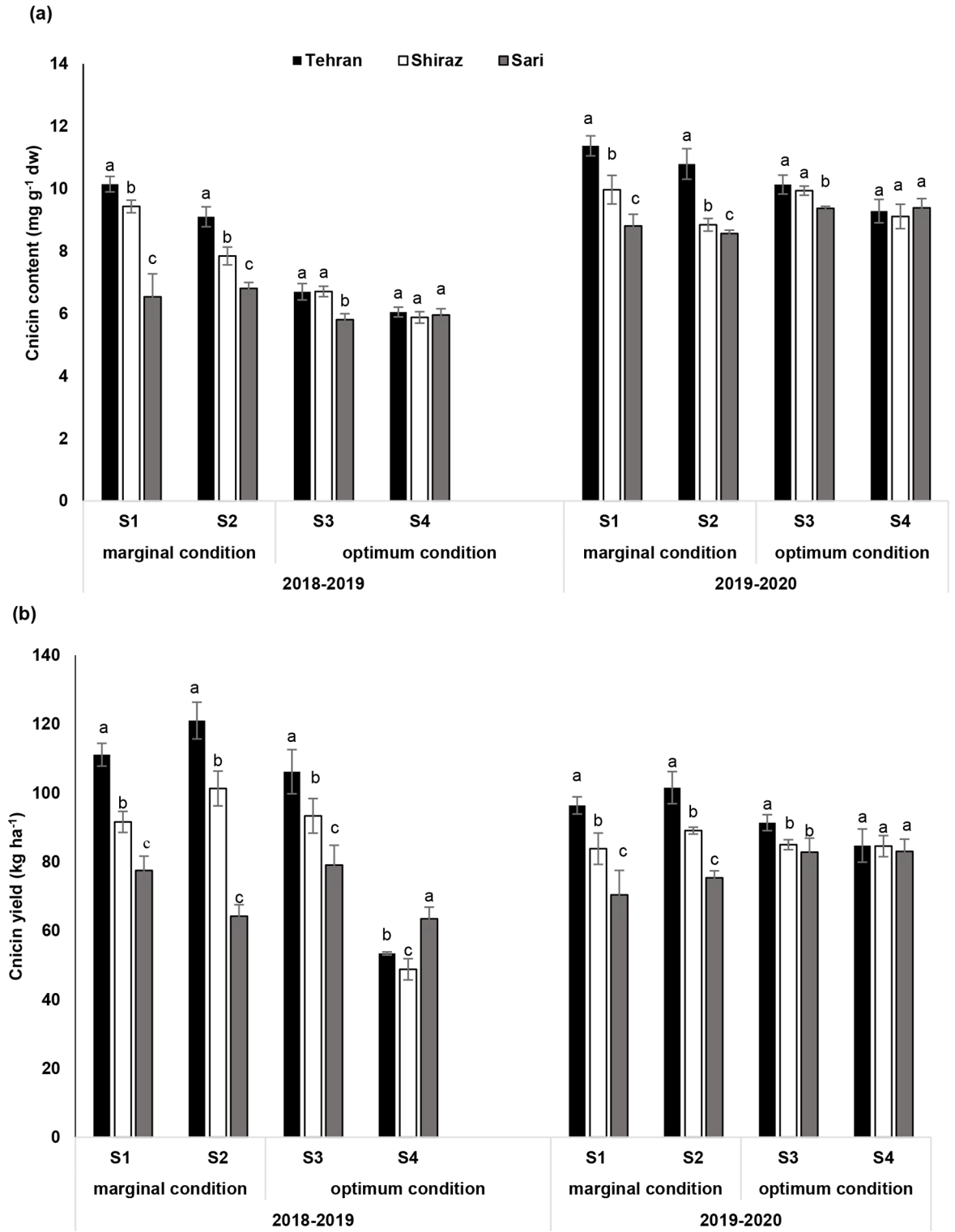


Fig. 2 **a** Cnicin content. **b** Cnicin yield of *C. benedictus* as affected by the environment (sites, S1-S4) and ecotype (Tehran, Shiraz, Sari) interactions in the first (2018-2019) and the second (2019-2020) growing seasons. S1 (Yazd site:): arid-sandy soil condition, S2 (Tehran site): semiarid-sandy soil condition; S3 (Ilam site): moderate-loamy soil condition, S4 (Rasht site): humid-clay soil condition. Means followed by similar letters for each trait in bars are not significantly different at the 5% probability level by the LSD test. Error bars represent standard error of the means ($n=4$)

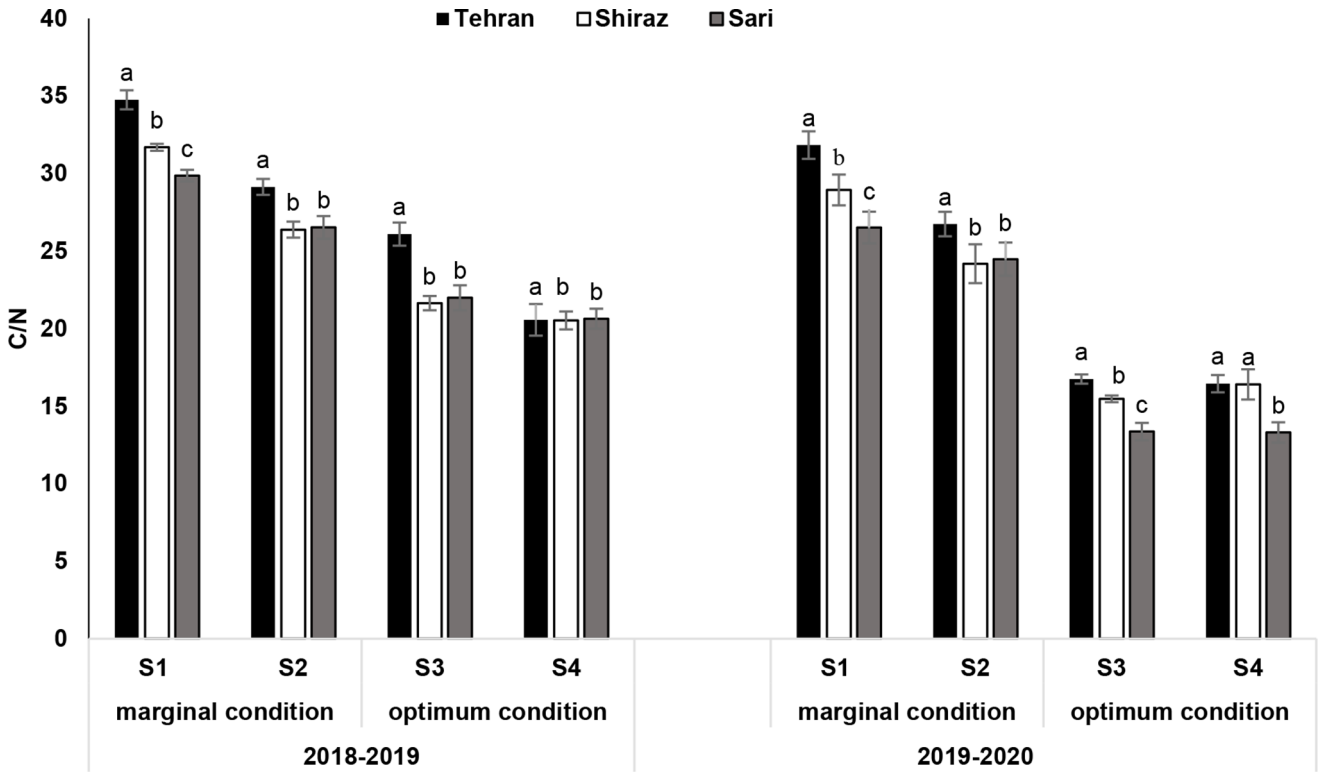


Fig. 3 *C. benedictus* C/N affected by the environment (sites, S1-S4) and ecotype (Tehran, Shiraz, Sari) interactions in the first (2018-2019) and the second (2019-2020) growing seasons. S1 (Yazd site:): arid-sandy soil condition, S2 (Tehran site): semiarid-sandy soil condition; S3 (Ilam site): moderate-loamy soil condition, S4 (Rasht site): humid-clay soil condition. Means followed by similar letters for each trait in bars are not significantly different at the 5% probability level by the LSD test. Error bars represent standard error of the means ($n=4$)

directly attributed to the variations in air temperatures and cumulative precipitations observed across the studied sites.

Agronomic results reinforce previous studies that demonstrated dry matter accumulation was strongly correlated with both LAI and plant height, as taller plants and larger LAI resulted in greater photosynthetic activity and ultimately more biomass production in different plants (Wang et al. 2020). Although pronounced climatic and soil differences existed among the experimental sites, the eco-edaphic site conditions and weather patterns during the plant growth appeared favorable for blessed thistle growth and development. Blessed thistle produced significant aboveground biomass, on average 11,400 kg DM ha^{-1} in all sites, which was even higher in marginal conditions (semi-arid–poor sandy soils) than in optimum conditions (humid-well nutrient soils). The results confirmed the blessed thistle's wide ecological plasticity and propriety to be grown as a versatile plant in diverse eco-climatic conditions, and preferably in semi-dry habitats. Previous studies have shown the agronomic stability and productivity of the blessed thistle as a resilient Asteraceae species across different environments and soil types (Horn et al. 2015; Ghiasy-Oskoee et al. 2020). Nevertheless, the present study revealed noticeable productivity differences across the four growing regions and the two consecutive years to confirm that eco-edaphic variations can have a substantial impact on the agronomic productivity of this species. The relatively low dry matter accumulation in a humid climate condition with heavy soil (high amount of clay) can be related to this plant's adaptation to semi-arid environments and well-drained coarse soils, as it originated from regions with low precipitation and sandy soils. Despite differences in plant growth periods, the total GDD remained relatively consistent across sites, suggesting that variations in air temperatures and precipitation are the primary causes of differences in plant growth cycles. The impact of environmental parameters such as air temperature and rainfall on plant phenology has been widely acknowledged in the literature, documenting that plants compress their life cycle in dry areas to alleviate the risk of abiotic stress during the critical reproductive stage (Tang et al. 2016; Piao et al. 2019). In this context, blessed thistle seems to adopt compensatory plastic responses in morphology, biomass, and phenology in response to environmental factors in arid ecosystems, advancing their phenology by accumulating degree days, due to its adaptability to stressed marginal lands. This phenotypic adaptive response would allow the survival and dispersal of this species in arid ecosystems.

Although the blessed thistle exhibits consistent agronomic productivity, the plant phytochemical contents exhibited significant variability among different growing sites, ecotypes, and seasons (Fig. 1a-b). In this regard, the Tehran ecotype demonstrated richer total soluble phenolic and flavonoid amounts in arid and semi-arid sites compared to other ecotypes, while the highest amounts in moderate and humid sites were noted in the Sari ecotype (Fig. 1a-b). The range of variation in phytochemicals also depended on the variations in growing years, as the blessed ecotypes accumulated more phytochemicals in the second year than in the first year across all studied sites (Fig. 1a-b). Previous studies have demonstrated genetic variability and environmental parameters such as air temperature, water availability, and soil status, which vary across growing regions and years, can significantly influence the biosynthesis and accumulation of phytochemicals in medicinal herbs (Generalić Mekinić et al. 2019). For instance, studies on medicinal plants *Achillea pachycephala* (Gharibi et al. 2019), *Rosa damascene* (Hessini et al. 2022), and *Ocimum basilicumm* (Celikcan et al. 2021) have shown that herbs grown under stress conditions such as drought or poor soils tended to produce higher concentrations of phytochemicals, mainly phenolic compounds, as a protective defense mechanism. This mechanism is widely recognized as a potent adaptation strategy in plants grown in stressful conditions by which plants improve their overall resilience to harsh growing conditions (Caser et al. 2019; Li et al. 2020; Laoué et al. 2022).

Environmental conditions and genetic variability among ecotypes of the same species can affect the phenolic composition of medicinal plants, suggesting that both intrinsic and extrinsic factors play crucial roles in shaping the phenolic profile of plants (Li et al. 2020; Kołton et al. 2022). Chlorogenic acid was the most abundant phenolic compound in blessed thistle ecotypes, particularly in the Tehran ecotype, which reached maximum levels in the S1 arid site. Previous studies have also identified chlorogenic acid as a prominent phenolic acid within the Asteraceae family thistle-like species, such as *Arctium lappa* (Petkova et al. 2022), *Cynara cardunculus* (Shallan et al. 2020) and *Cirsium japonicum* (Jiang et al. 2018) as well as blessed thistle (Paun et al. 2019; Foss et al. 2022) plants. Also, other phenolics such as quercetin and rutin flavones were found to vary across studied sites and ecotypes, with higher levels in ecotypes from the S2 semi-arid site. These findings could suggest that environmental differences across studied sites, such as rainfall and temperature, can influence the accumulation of specific phenolic compounds within different blessed thistle ecotypes. The variability of the phenolic profile with reference to the genotype and location differences has been highlighted by several studies (Yaldiz et al. 2019; Yang et al. 2021). Furthermore, it has been reported that environmental stress can increase the concentration of chlorogenic acid and other beneficial phenolics in various plant species. Likewise, studies on *Taraxacum antungensen* (Liu et al.), *Achillea pachycephala* (Gharibi et al. 2019), and *Dracocephalum moldavica* (Kamalizadeh et al. 2019) showed that exposure to drought stress resulted in an elevation of total phenolic content as well as specific phenolic acids such as chlorogenic acid. This effect might be linked to changes in expression levels of genes involved in the biosynthesis pathway for these compounds, potentially contributing to improved plant defense mechanisms under stressful conditions (Soviguidi et al. 2022; Nina et al. 2023).

Tehran ecotype, originating from a semi-arid condition, showed higher total soluble phenolic content and chlorogenic acid than other ecotypes, especially in arid and semi-arid poor soil sites (Table 4). This refers to the fact that geographic differences can lead to the emergence of diverse local ecotypes within a given species with unique genetic structures and, therefore, phytochemicals that differ significantly from each other (Sarrou et al. 2022). This response is common in widespread species, such as blessed thistle, which are often characterized by both high phenotypic plasticity and high genetic variability due to geographic isolation and adaptation to varying climatic conditions.

Cnicin has been widely considered the primary bioactive compound of the blessed thistle, responsible for its various therapeutic properties (Queiroz et al. 2021).

The ecotypes, particularly Tehran ecotype, contained the highest levels of cnicin when grown in the arid site (S1), while the greatest average cnicin yield was found in plants grown in the semi-arid region (S2), despite lower cnicin content, due to the relatively higher biomass yield. Numerous studies have also mentioned that genotypes grown in dry and poor nutrient soils often exhibit higher levels of phytochemicals, while their growth and ultimately biomass yield tend to be lower under these conditions (Celikcan et al. 2021; Rahman et al. 2023). This approach can be explained by the concept of the growth-differentiation and carbon/nutrient balance hypotheses, which suggest that there is a trade-off between growth and secondary metabolite production. Accordingly, the soil's nutrient status could significantly affect the carbon/nutrient ratio of plants, which in turn affects their ability to produce phytochemicals (Nunes-Nesi et al. 2010). Ecotypes grown in sandy nutrient-poor soils at the S1 and S2 sites exhibited higher C/N ratios compared to those grown in loamy or clay nitrogen-rich soils of the S3 and S4 sites, which had higher amounts of nitrogen availability (Fig. 4). In addition, the Tehran ecotype contained a high level of phytochemicals and exhibited a greater C/N ratio than other ecotypes in both arid and semi-arid sites during both years. Therefore, it is plausible to state that the production of carbon-based phytochemicals such as phenols and

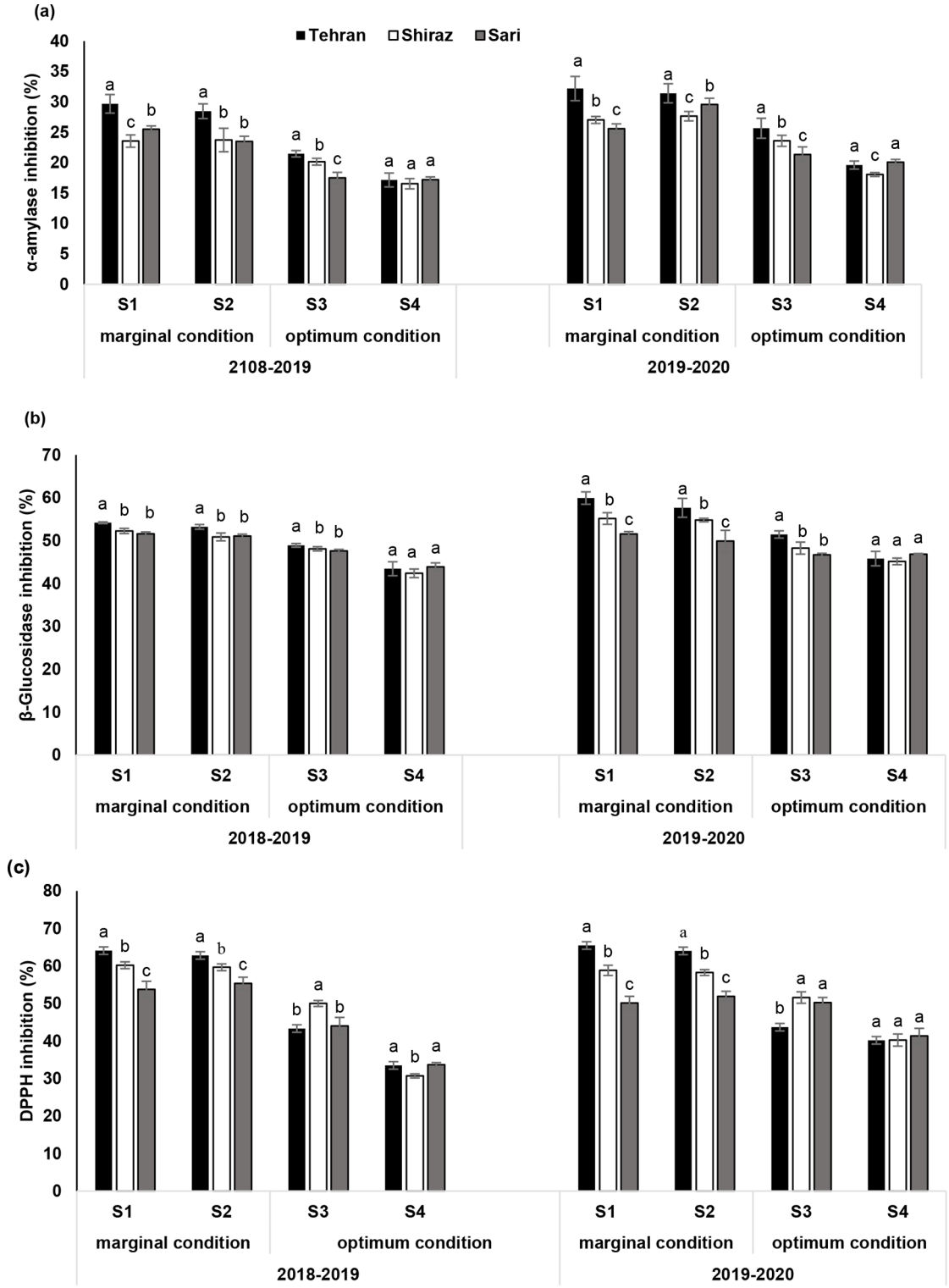


Fig. 4 **a** α-amylase. **b** α-glucosidase inhibitory. **c** Antioxidant activity of *C. benedictus* as affected by the environment (sites, S1-S4) and ecotype (Tehran, Shiraz, Sari) interactions in the first (2018-2019) and the second (2019-2020) growing seasons. S1 (Yazd site:): arid-sandy soil condition, S2 (Tehran site): semiarid-sandy soil condition; S3 (Ilam site): moderate-loamy soil condition, S4 (Rasht site): humid-clay soil condition. Means followed by similar letters for each trait in bars are not significantly different at the 5% probability level by the LSD test. Error bars represent standard error of the means ($n=4$)

cnicin can be influenced by the levels of carbon metabolic flow relative to nitrogen in plants, which are closely associated with soil nutrient status (Bustamante et al. 2020). In this regard, our previous study has demonstrated that growing blessed thistle in nitrogen-limited conditions resulted in a higher plant C/N ratio and was directly linked to enhancing the plant's carbon-based secondary metabolites, along with a decrease in its biomass yield (Ghiasy-Oskoee et al. 2018). These findings could emphasize the importance of soil properties in determining the phytochemical contents of medicinal plants and highlight how manipulating growing conditions can be used to enhance their phytochemicals.

The variation in the biological activity of blessed thistle ecotypes grown across different climatic regions was reflected in the differences in the content of the active phytochemicals of each species across growing regions. Indeed, various eco-edaphic conditions of the growing regions and genetic variability among ecotypes significantly affected total phenolic content and consequently the biological activity of the plants. This is in line with previous reports that demonstrated a positive correlation between total bioactive compounds and biological activities (Sarkar et al. 2016; Larit et al. 2019; Tlili and Sarikurkcu 2020).

Our results also showed that differences in biological activity can arise not only from variations in the total amount of soluble phenolics but also from variations in the levels of individual phenolics. In this regard, although chlorogenic acid was the dominant phenolic compound found in blessed thistle, the main biological activity of the plant may also have been attributed to the presence of flavones like rutin and quercetin. As observed, semi-arid plants with more rutin and quercetin proportions exhibited equal or even greater inhibitory activity than arid plants with more total phytochemical content and chlorogenic acid concentration. This finding could confirm the contribution of these flavonoids, rutin and quercetin, to the biological effect of blessed thistle. In vivo and in vitro studies have highlighted the remarkable potential of flavonoid compounds, specifically rutin and quercetin, contributing to the prevention and potential management of glycemic control associated with type 2 diabetes and associated metabolic disorders (Jucá et al. 2020; Sok Yen et al. 2021; Shen et al. 2023). These findings could support the fact that flavonoids may possess higher therapeutic potential compared with other phytochemicals, as the presence of specific functional groups in their molecules enables them to exhibit diverse biological activities that can benefit human health (Marella 2017; Jucá et al. 2020; Yuan et al. 2022).

The selective and weak inhibitory against α-amylase and strong α- glucosidase inhibitory is therapeutically important for the efficient treatment of diabetes since inhibition of both enzymes may have side gastrointestinal impacts. Therefore, the effective and selective biological activity of the blessed thistle ecotypes linked to their certain phytochemicals makes the plant a potential target for novel therapeutic applications and developments.

5 Conclusions

Herein, for the first time, a comprehensive evaluation of the agronomic productivity and phenolic phytochemicals linked to the glycemic control-related biological activity of three blessed thistle ecotypes grown in four distinct growing sites was conducted. From an agronomic point of view, blessed thistle genotypes showed wide environmental plasticity and suitability to be cultivated as versatile plants in different eco-edaphic conditions, preferably in semi-arid habitats. Among the key findings, the analysis demonstrated a wide range of variations in the phytochemical content and levels of individual phenolic compounds across the blessed thistle genotypes, with the ecotype from Tehran exhibiting superior attributes. Notably, chlorogenic acid emerged as the most abundant phenolic compound, with flavonoids like rutin and quercetin also playing crucial roles in the observed glycemic control properties of the blessed thistle plants. When considering both agronomic and therapeutic potentials, the Tehran ecotype grown in a semi-arid sandy area had the highest agronomic productivity and individual flavonoids potentially associated with enzymatic inhibitory and antioxidant activity. This underscores its potential as one of the most therapeutically important blessed thistle genotypes for future breeding programs and developments, although all ecotypes were active in all the assays. The findings presented may open novel approaches to valorize blessed thistle as a rich source of glycemic control relevant bioactive compounds targeting the management of early stages of type 2 diabetes and a productive plant for revitalizing marginal lands.

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Declarations

Conflict of interest The authors declare no competing interests.

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