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Abstract

*Silybum marianum* (L.) Gaertn. (milk thistle), grown as a medicinal plant in several countries, is considered as a weed in pastures and cereal crops but also as an interesting plant for biomass production. As an additional contribution to the full exploitation of a such promising species, two Sardinian populations of *S. marianum* were investigated for chemical composition, bioactive compounds and antioxidant properties at vegetative and reproductive stages. Dry matter yield was affected by the phenogical stage and differed between populations, ranging from 148 to 246 g plant$^{-1}$. Chemical composition did not differ between populations. Antioxidant capacity detected by means of ABTS [(2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt)] and by DPPH (1,1-diphenyl-2-picrylhydrazyl) methods ranged from 3.45 to 5.42 and 3.83 to 6.32 mmol/100 g dry weight of Trolox equivalent antioxidant capacity, respectively. Differences in antioxidant capacity and bioactive compound contents in the different plant organs were found and also a significant linear correlation between antioxidant capacity and total phenolics and flavonoids, at flowering compared to vegetative stage. Research highlights antioxidant capacity in different organs of milk thistle and encourages the exploitation of biomass also as functional food, source of natural antioxidants and as a complementary fodder.

Keywords (separated by '-') Dry matter yield - Milk thistle - Plant organs - Phenological stages - Polyphenols - *Silybum marianum*

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RESEARCH ARTICLE

# Chemical and productive properties of two Sardinian milk thistle (*Silybum marianum* L. Gaertn.) populations as sources of nutrients and antioxidants

**Leonardo Sulas · Giovanni A. Re · Simonetta Bullitta · Giovanna Piluzza**



Received: 17 December 2014 / Accepted: 30 March 2015


**Abstract** *Silybum marianum* (L.) Gaertn. (milk thistle), grown as a medicinal plant in several countries, is considered as a weed in pastures and cereal crops but also as an interesting plant for biomass production. As an additional contribution to the full exploitation of a such promising species, two Sardinian populations of *S. marianum* were investigated for chemical composition, bioactive compounds and antioxidant properties at vegetative and reproductive stages. Dry matter yield was affected by the phenogical stage and differed between populations, ranging from 148 to 246 g plant$^{-1}$. Chemical composition did not differ between populations. Antioxidant capacity detected by means of ABTS [(2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt)] and by DPPH (1,1-diphenyl-2-picrylhydrazyl) methods ranged from 3.45 to 5.42 and 3.83 to 6.32 mmol/100 g dry weight of Trolox equivalent antioxidant capacity, respectively. Differences in antioxidant capacity and bioactive compound contents in the different plant organs were found and also a significant linear correlation between antioxidant capacity and total phenolics and flavonoids, at flowering compared to vegetative stage. Research highlights antioxidant capacity in different organs of milk thistle and encourages the exploitation of biomass also as functional food, source of natural antioxidants and as a complementary fodder.

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**Keywords** Dry matter yield · Milk thistle · Plant organs · Phenological stages · Polyphenols · *Silybum marianum*

## Introduction

*Silybum marianum* (L.) Gaertn., synonym *Carduus marianus* L. common name milk thistle, a member of the Compositae family, is an annual or biennial herbaceous plant, native to the Mediterranean basin, but now naturalized and widespread throughout the world (Kaur et al. 2012; Sidhu and Saini 2012). The role and uses of this species may be controversial, taken into account the different possible contexts. Its fruits (i.e. achenes), often referred to as seeds, have been valued for their medicinal properties (Gazák et al. 2007; Kroll et al. 2007), have been utilized as medicine for over 2000 years and were known for liver protecting properties since ancient Greek civilization (Alemardan et al. 2013). Milk thistle is also a traditional medicinal plant cultivated in Italy (Hammer et al. 1992). Currently, it is grown commercially as a medicinal plant in Europe, Egypt, China and Argentina (Veres and Tyr 2012). However, milk thistle is considered a weed in sowed annual legume pastures (Sulas et al. 2008), waste areas, cereal crops, decreasing wheat yields (Khan et al. 2009), and along roadsides (Karkanis et al. 2011). On the other hand, milk thistle is currently being regarded as an interesting crop for bioenergy production in Mediterranean environment (Sulas et al. 2008; Ledda et al. 2013), and as a source for biodiesel production (Ahmad et al. 2014); the biogas production from its biomass is under investigation (Andrzejewska J, unpublished). It is AQ2 grown also as an ornamental plant (Bhattacharya 2011) and as a tolerant species for soils polluted by heavy metals (Rio-Celestino et al. 2006; Perrino et al. 2014). In addition, is considered as a new source of plant rennet for aspartic peptidases present in its flowers (Vairo Cavalli et al. 2005). Probably due to the plurality of biological activities from its secondary metabolites, milk thistle is also the most studied plant for the treatment of liver disease and this is documented by the huge increase of papers on this topic, over 800 publications, in the last 5 years (Alemardan et al. 2013). Phytochemicals, pharmaceutical and clinical studies regarding milk thistle as well as the medicinal importance of the species have been recently reviewed by Abenavoli et al. (2010), Kaur et al. (2012) and by Sidhu and Saini (2012), respectively. Specific medicinal properties, against hepatotoxicity and acute and chronic liver diseases, are attributed to its main pharmacological active ingredient silymarin, a standard mixture of flavonolignans (Pereira et al. 2012).

From ethnobotanical studies, it has been documented the alimentary and/or the therapeutic uses of non-cultivated milk thistle plant organs (except seeds) in different countries. The use of young stems, (fresh, boiled or fried), and leaves of wild milk thistle for human consumption has been reported by Vaknin et al. (2008) for the Arab sector in Israel, by Lancioni et al. (2007) and Atzei (2003) for Sardinia, by Pieroni et al. (2002) and Passalacqua et al. (2006) for Italy, by Mattalia et al. (2013) for the western Italian Alps and by Tardío et al. (2006) and Sanchez-Mata et al. (2012) for Spain. So, milk thistle has an increasing interest also for nutritional scientists.

According to Carpino et al. (2003), milk thistle, as spontaneous weed, is scarcely consumed by large and small ruminants grazing on Mediterranean pastures but an increased animal preference has been observed in Sardinia by local farmers when milk thistle is harvested as silage or hay (Sanna S, pers. comm.). In order to reduce milk thistle biomass, grazing by goat has been suggested for non-crop areas (Khan et al. 2009). Moreover, silage production from its biomass, fruit expeller and also silymarin extracts for animal feeding have been evaluated (Grabowicz et al. 2001; Tedesco et al. 2004; Křížová et al. 2011). In addition, residues of fruit and vegetable food industries, up to now scarcely employed due to their high pectin contents, were blended with milk thistle biomass to study the possibility to convert them, via microbial fermentation, in a balanced product for ruminants (Tagliapietra et al. 2014).

The leaves have been scarcely investigated for bioactive compounds so far. Omar et al. (2012) studied the silymarin components in leaves and seeds of *S. marianum* during different growth stages in Egypt, and found that each kilogram of leaves collected during the pre-flowering stage yielded 5.82 g of the total flavolignans and 3.42 g of taxifolin which was found to be considerably higher than the concentrations obtained in similar studies during both flowering and fruiting stages. The same authors pointed out the possibility of using the leaves during the pre-flowering stage as a major source for the production of silymarin, taking the advantages of the huge weight of the leaves, the short period of cultivation time, and the good yield of silymarin. Balian et al. (2006) showed that methanolic extracts of leaves exert anti-inflammatory effects.

In the frame of a general activity aimed at the exploitation of Sardinian herbaceous plant germplasm for multiple uses, our specific objective was to deepen the knowledge about chemical composition, antioxidant properties and bioactive compounds of *S. marianum* plant and organs at different phenological stages to contribute to the full exploitation of this promising species.

## Materials and methods

Two *S. marianum* populations 001S and 002P previously collected in Sardinia (Italy) were sown in October 2010 in North-Sardinia (41°N, 8°E, 81 m a.s.l.). Soil is a flat sandy-clay-loam overlaid on limestone (Xerochrepts), with low organic carbon (12 g $kg^{-1}$) and N (0.8 g $kg^{-1}$), pH 7.5, low $P_2O_5$ content and adequate $K_2O$ content. The climate is typically Mediterranean, with a long-term average annual rainfall of 554 mm and a mean annual air



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temperature of 16.2 °C; rainfall and temperature data for the experimental period did not substantially differ from such values.

In autumn 2010, plots sized 20 $m^2$ of the two Sardinian populations of milk thistle were manually sown, 50 cm between rows and 40 cm apart within rows, under a randomised block design with three replicates. At sowing, fertilisation was applied with 36 kg $ha^{-1}$ of N and 90 kg $ha^{-1}$ of $P_2O_5$; no herbicide application or irrigation were necessary.

### Plant biometric parameters, dry matter yield (DMY) and chemical composition

Milk thistle plants were harvested for yield and quality determinations when plants were at vegetative (early spring) and reproductive (late spring) stage, respectively corresponding to the BBCH stages of 3 and 6 described by Martinelli et al. (2014). Harvested plants were immediately weighted to determine fresh weight, the contributions of each plant component (stems, leaves, and heads) to the above ground biomass were also determined.

Phytomass sub-samples were oven dried at 65 °C for 48 h, then ground to 1 mm screen to be analyzed for quality traits. Total N was determined using Kjeldahl method and crude protein was calculated by multiplying the N content by 6.25. Neutral and acid detergent fibres (NDF and ADF) and acid detergent lignin (ADL) were determined by using the procedure of Van Soest et al. (1991) and fat using Soxhlet extraction.

### Antioxidant capacity and bioactive compounds

Harvested plant samples were kept on ice, freeze dried and ground to a fine powder for chemical analysis. The powdered material was then used for extract preparation as reported by Piluzza et al. (2014).

Antioxidant capacity was determined by means of the improved ABTS [(2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt)] and by DPPH (1,1-diphenyl-2-picrylhydrazyl) assays with some modifications (Surveswaran et al. 2007; Piluzza and Bullitta 2011). Trolox, a water-soluble analogue of vitamin E was used as the reference standard. The results were expressed in terms of Trolox Equivalent Antioxidant Capacity (TEAC), as mmol Trolox equivalents per 100 g dry weight of plant material (mmol TEAC/100 g DW).

Total phenolics (TotP), non-tannic phenolics (NTP) and tannic phenols (TP) were determined using the Folin-Ciocalteu colorimetric assay according to procedures previously described by Piluzza and Bullitta (2010). Results were expressed as g gallic acid equivalent (GAE) $kg^{-1}$ dry weight of plant material (g GAE $kg^{-1}$ DW).

The butanol assay was used for quantification of the extractable condensed tannin content from samples, expressed as g delphinidin equivalent per $kg^{-1}$ dry matter (g DE $kg^{-1}$ DM) (Piluzza and Bullitta 2010).

Total flavonoids (TotF) were quantified by colorimetric assay using Aluminium trichloride, following procedures previously reported (Piluzza and Bullitta 2011). Catechin was used as a standard and the flavonoid content was expressed as g catechin equivalent $kg^{-1}$ dry weight of plant material (g CE $kg^{-1}$ DW).

### Statistical analysis

For all determinations three samples (n = 3) were analysed and all the assays were performed in triplicate. The results are expressed as mean values and standard deviation. The regression analysis between polyphenols, fibre fractions and antioxidant capacity were calculated using Microsoft Excel 2000.

## Results

### Biometric parameters, DMY and chemical composition

Plant biometric parameters and DMY differed between populations and were affected by the phenogical stage (Table 1). The population 002P showed AQ3 higher DM production at both stages. In addition, 002P showed highest height per plant and a highest number of lateral ramifications and capitula per plant, resulting also in a relative higher contribution of stems (Fig. 1). However, chemical composition of milk thistle plants did not significantly differ between populations, even if marked variations were observed between stages (Table 2). In fact, CP concentration decreased from 215 in early spring to about 50 g $kg^{-1}$ DM in late spring. As it was expected, NDF and ADF





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**Table 1** Biometric parameters and dry matter yield of milk thistle (means and standard deviations)

| Populations | Plant phenological stage | Plant height (cm) | Stems (no. plant$^{-1}$) | Heads (no. plant$^{-1}$) | Dry matter (g plant$^{-1}$) |
|---|---|---|---|---|---|
| 001S | Vegetative | 34 ± 5.0 | – | – | 44.6 ± 8.1 |
| 002P | Vegetative | 41 ± 7.0 | – | – | 74.1 ± 11.1 |
| 001S | Flowering | 176 ± 14.2 | 7 ± 1.0 | 21 ± 3.1 | 148.2 ± 24.0 |
| 002P | Flowering | 207 ± 9.3 | 10 ± 1.9 | 37 ± 9.5 | 245.8 ± 38.1 |

– Unavailable at vegetative stage

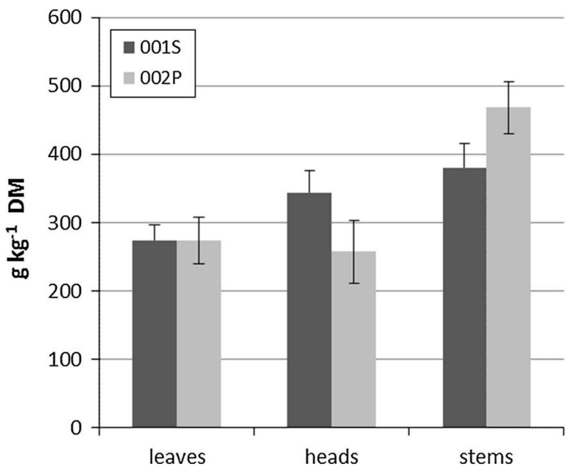


**Fig. 1** Contributions (g kg$^{-1}$ DM) of leaves, heads and stems to shoot dry matter yields in milk thistle populations (*vertical bars* indicate standard deviations of means)

increased in meantime whereas ADL content decreased. Ash content reduction is related to the relative lower contribution of leaves to the total DM in mature plants compared to young plants.

At the late stage, the chemical analysis of single plant components (Fig. 2a, b) did not show substantial differences between populations except for NDF and ADF values in leaves that were higher in 001S (400 and 300 g kg$^{-1}$ DM) compared to 002P (300 and 230 g kg$^{-1}$ DM). On the average of both populations, NDF values were about 600 g kg$^{-1}$ DM in stems and progressively decreased in heads and leaves; CP contents decreased from heads (75) to leaves (60) and stems (45 g kg$^{-1}$ DM). Finally, ash content increased from heads (75) to stems (90) reaching about 180 g kg$^{-1}$ DM in leaves.

The above mentioned variations found in plant portions and their relative contributions to the total plant DM (Fig. 1) affected the chemical composition of whole mature plants.

## TEAC and phenolic contents

The content of phenolics and the antioxidant activities detected by means of the two in vitro assays (ABTS, DPPH) on the milk thistle natural populations at vegetative and reproductive phenological stages are shown in Table 3. ABTS assay exhibited a variation of antioxidant capacities from 3.45 (001S, flowering) to 5.42 (002P, vegetative) mmol TEAC/100 g DW. The total antioxidant capacity determined through the DPPH assay also showed a variation from 3.83 (001S, flowering) to 6.32 (002P, vegetative) mmol TEAC/100 g DW.

Total phenolics (TotP) ranged from 10.02 to 13.27 g GAE kg$^{-1}$ DW, in 002P at flowering and 002P at vegetative stage (Table 3), respectively. High TEAC values corresponded to high TotP contents, and low TEAC values to lower TotP contents. However, the two natural populations did not show substantial

**Table 2** Plant chemical composition (g kg$^{-1}$ DM) in milk thistle: crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), fat and ash (means ± standard deviations)

| Populations | Plant phenological stage | CP (g kg$^{-1}$ DM) | NDF (g kg$^{-1}$ DM) | ADF (g kg$^{-1}$ DM) | ADL (g kg$^{-1}$ DM) | Fat (g kg$^{-1}$ DM) | Ash (g kg$^{-1}$ DM) |
|---|---|---|---|---|---|---|---|
| 001S | Vegetative | 214 ± 1 | 443 ± 17 | 296 ± 12 | 152 ± 6 | 27 ± 13 | 191 ± 12 |
| 002P | Vegetative | 215 ± 1 | 430 ± 20 | 280 ± 10 | 145 ± 5 | 28 ± 13 | 193 ± 9 |
| 001S | Flowering | 47 ± 3 | 504 ± 47 | 371 ± 34 | 76 ± 13 | 24 ± 8 | 128 ± 10 |
| 002P | Flowering | 55 ± 5 | 479 ± 52 | 364 ± 43 | 85 ± 20 | 34 ± 17 | 126 ± 23 |





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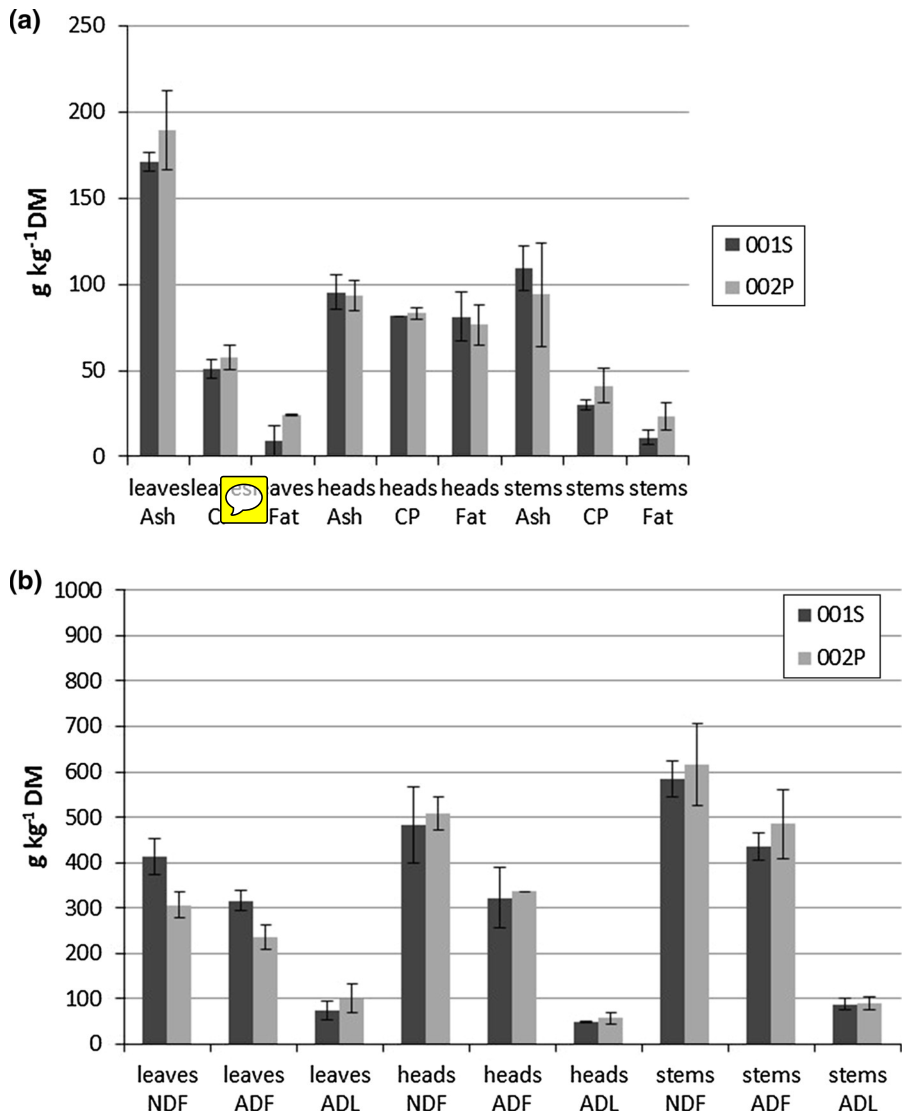


**Fig. 2** **a** Concentrations (g $kg^{-1}$) of ash, crude protein (CP) and fat in plant organs of milk thistle populations (*vertical bars* indicate standard deviations of means). **b** Concentrations (g $kg^{-1}$ DM) of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) in plant organs of milk thistle populations (*vertical bars* indicate standard deviations of means)

**Table 3** Trolox Equivalent Antioxidant Capacity (TEAC) by ABTS and DPPH methods, total phenolics (TotP), non tannic phenolics (NTP), tannic phenolics (TP), total flavonoids (TotF) of milk thistle

| Populations | Plant phenological stage | TEAC (mmol/100 g DW) | | TotP (g GAE $kg^{-1}$ DW) | NTP (g GAE $kg^{-1}$ DW) | TP (g GAE $kg^{-1}$ DW) | TotF (g CE $kg^{-1}$ DW) |
|---|---|---|---|---|---|---|---|
| | | ABTS | DPPH | | | | |
| 001S | Vegetative | 4.77 ± 1.11 | 5.44 ± 1.27 | 12.97 ± 1.48 | 10.09 ± 1.96 | 2.88 ± 0.88 | 8.05 ± 1.41 |
| 002P | Vegetative | 5.42 ± 0.88 | 6.32 ± 0.43 | 13.27 ± 1.51 | 10.49 ± 0.88 | 2.78 ± 1.02 | 9.47 ± 1.18 |
| 001S | Flowering | 3.45 ± 0.31 | 3.83 ± 0.33 | 10.66 ± 0.33 | 7.33 ± 0.56 | 3.33 ± 0.74 | 5.75 ± 0.62 |
| 002P | Flowering | 3.61 ± 0.21 | 4.08 ± 0.34 | 10.02 ± 0.49 | 7.42 ± 0.50 | 2.6 ± 0.15 | 6.46 ± 0.79 |

differences for the contents of Non tannic phenolics (NTP), Tannic phenolics (TP), Total flavonoids (TotF) (Table 3). No condensed tannins were detected in the two natural populations under study.

The correlations ($R^2$ and equation) between the antioxidant activity revealed by the two assays (ABTS and DPPH), and TotP, NTP, TP and TotF are reported in Table 4. TotP ($R^2 = 0.8419$; 0.7759), NTP



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($R^2 = 0.9291$; 0.9062), ToTF ($R^2 = 0.9479$; 0.9131) showed highly significant linear correlation with antioxidant activity at flowering, but no significant correlations were found among the antioxidant activity and TP at vegetative stage in both assays.

The antioxidant capacity in leaves, heads and stems of the two natural populations of *S. marianum* is shown in Fig. 3. Both ABTS and DPPH assays evidenced a high TEAC value in leaves compared to heads and stems.

Figure 4 shows the average concentrations of TotP, NTP and TP for each plant organ (leaves, heads, stems) at flowering in the natural populations. TotP, NTP and TP concentration in leaves was higher than in the other examined plant parts. Higher TotP contents in 001S leaves compared to 002P were found. The average flavonoid contents for each plant organ (Fig. 5) indicate higher contents in leaves compared to heads and stems.

The correlations ($R^2$ and equation) between the antioxidant activity, by the two assays (ABTS and DPPH) and NDF, ADF and ADL at vegetative and flowering stages are shown in Table 5. NDF ($R^2 = 0.7222$; 0.6462), and ADF ($R^2 = 0.5787$; 0.4866) showed a highly significant linear correlation with antioxidant activity at flowering, while no significant correlation was found among the antioxidant activity at vegetative stage. Moreover NDF and ADF showed highly significant correlation with ToTP ($R^2 = 0.6323$), NTP ($R^2 = 0.7001$) and ToTF ($R^2 = 0.7343$) at flowering.

## Discussion

*Silybum marianum* is an interesting multipurpose annual crop for rainfed Mediterranean environments. Regarding the chemical composition of biomass, the scarcity of available information, to our knowledge, limits comparisons that would be very useful to elucidate the possible forage potential of milk thistle. Tagliapietra et al. (2014) reported similar values for CP, [illegible]nd lignin but higher values in NDF and ADF for the same (001P) Sardinian genotype under study, on plants harvested at a later stage than in the current experiment. We tried to compare our results with other close species such as cynara (*Cynara cardunculus* [illegible] *altilis*). The nutritive value of cynara green fo[illegible] and crop by-products were studied for chemical

**Table 4** Correlations ($R^2$ and equation) established between total phenolics (ToTP), non tannic phenolics (NTP), tannic phenolics (TP), flavonoids (ToTF) and antioxidant capacity (ABTS, DPPH) in milk thistle at vegetative and flowering stages

| | ABTS | | | | DPPH | | | |
|---|---|---|---|---|---|---|---|---|
| | Vegetative | | Flowering | | Vegetative | | Flowering | |
| ToTP | 0.7331* | Y = 1.1936x + 7.031 | 0.8419*** | Y = 2.4737x + 1.7055 | 0.6661* | Y = 0.5935x − 1.9077 | 0.7759*** | Y = 0.4593x − 0.7359 |
| NTP | 0.2789ns | Y = 0.753x + 6.4487 | 0.9291*** | Y = 1.5694x + 1.9053 | 0.8026* | Y = 1.2599x + 2.8824 | 0.9062*** | Y = 1.1026x + 3.0784 |
| TP | 0.248ns | Y = 0.5[illegible]x + 3.5072 | 0.4774* | Y = 0.528x + 2.128 | 0.0249ns | Y = −0.1807x + 6.3896 | 0.3972* | Y = 0.6769x + 2.2108 |
| ToTF | 0.6603* | Y = 1.1747x + 2.7686 | 0.9479*** | Y = 2.2719x − 1.9766 | 0.8994* | Y = 1.3523x + 0.8097 | 0.9131*** | Y = 1.5862x − 0.2336 |

* Significance level at $P \leq 0.05$

** Significance level at $P \leq 0.001$

*** Significance level at $P \leq 0.0001$





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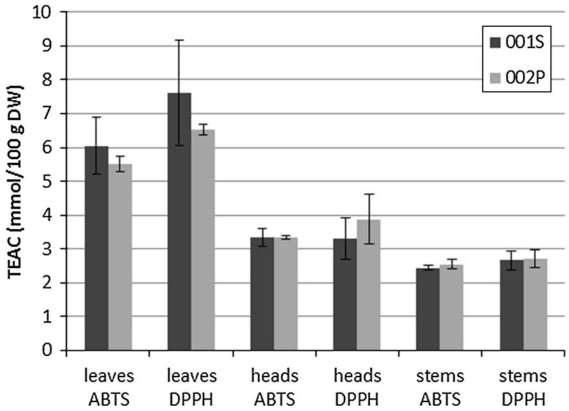



**Fig. 3** Antioxidant capacity in plant organs of milk thistle populations by ABTS and DPPH assays (*vertical bars* indicate standard deviations of means)

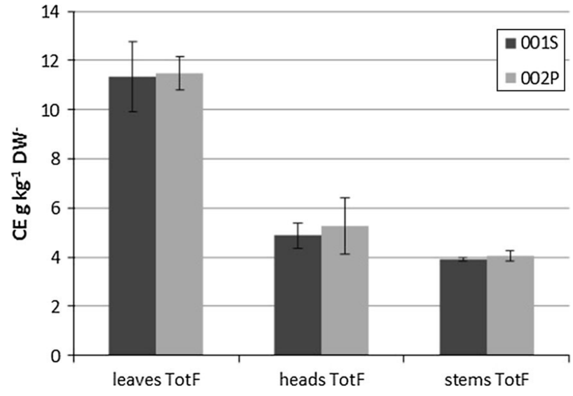
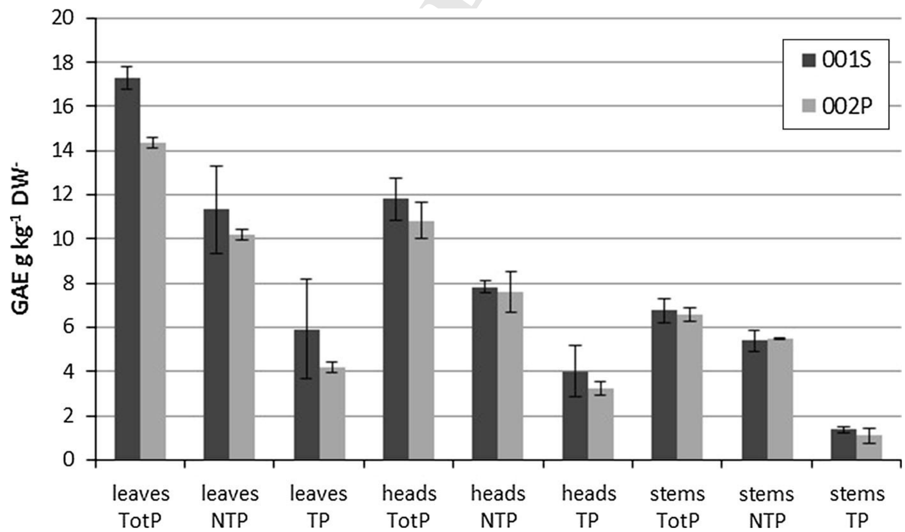



**Fig. 5** Total flavonoid (TotF) contents in plant organs of milk thistle populations (*vertical bars* indicate standard deviations of means)

**Fig. 4** Total phenolics (TotP), non-tannic phenolics (NTP) and tannic phenols (TP) in plant organs of milk thistle populations (*vertical bars* indicate standard deviations of means)




composition analysis by Cajarville et al. (1999), who showed that the composition of cynara green forage is adequate for fodder and silage, due to the low level of fibre and lignin. Compared to cynara, the crude protein and fibre concentrations of milk thistle were in a similar range, except for ADL, indicating a possible forage exploitation for the species. However, milk thistle is a spiny species and this need to be taken into account. Attempts to obtain spineless mutants using radiation were performed (Khan et al. 1988) and are still in progress (authors pers. comm.). Anyway, milk thistle biomass is used as silage, as reported by Grabowicz et al. (2001) for Poland or its biomass residues may be blended with other crops residues and traditional forages. Tagliapietra et al. (2014) reported that the in vitro fermentability of low quality forage from milk thistle can be improved by combining it with agro-industrial by-products (apple pomace and citrus pulp). Moreover, the use of seeds (Korczak and Grabowicz 2003) or silymarin extracts (Tedesco et al. 2004) as feed supplement have been investigated.

Some biometric parameters and the recorded DM yields were comparable to previous results (Ledda et al. 2013), confirming the remarkable potential of this species for the production of biomass. This potential and its chemical composition, suggest us to consider the abundant yields from this species as a potential fodder source to be blended with other crop



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**Table 5** Correlations ($R^2$ and equation) established between neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and antioxidant capacity (ABTS, DPPH), total phenolics (ToTP), non tannic phenolics (NTP), tannic phenolics (TP), flavonoids (ToTF) in milk thistle at vegetative and flowering stages

| | ABTS | | | | DPPH | | | |
|---|---|---|---|---|---|---|---|---|
| | Vegetative | | Flowering | | Vegetative | | Flowering | |
| NDF | 0.0892ns | Y = −0.1576x + 11.979 | 0.7222*** | Y = [illegible]6 + 9.3662 | 0.3556ns | Y = −0.319x + 19.806 | 0.6462*** | Y = −0.151x + 11.752 |
| ADF | 0.1215ns | Y = −0.262x + 12.639 | 0.5787*** | Y = −[illegible]7 + 8.5085 | 0.4811ns | Y = −0.5285x + 21.087 | 0.4866** | Y = −0.1684x + 10.423 |
| ADL | 0.1109ns | Y = −0.5141x + 12.734 | 0.0139ns | Y = 0.0822x + 3.2478 | 0.4828ns | Y = −1.0877x + 22031 | 0.0352ns | Y = 0.1829x + 3.1572 |
| | **ToTP** | | | | **NTP** | | | |
| | Vegetative | | Flowering | | Vegetative | | Flowering | |
| NDF | 0.2199ns | Y = −0.3449x + 28.117 | 0.6323*** | Y = −0.2865x + 25.144 | 0.1956ns | Y = −0.3328x + 24.817 | 0.7001*** | Y = −0.182x + 16.789 |
| ADF | 0.1891ns | Y = −0.4556x + 26.228 | 0.6256*** | Y = −0.3[illegible] + 24.281 | 0.2335ns | Y = −0.5216x + 25.293 | 0.6239*** | Y = −0.2[illegible] + 15.82 |
| ADL | 0.2011ns | Y = −0.9652x + 27.451 | 0.0094ns | Y = −0.1[illegible]x + 12.66 | 0.2531ns | Y = −1.1074x + 26,734 | 0.0021ns | Y = −0.0515x + 8.369 |
| | **TP** | | | | **ToTF** | | | |
| | Vegetative | | Flowering | | Vegetative | | Flowering | |
| NDF | 0.0007ns | Y = −0.0122x + 3.36 | 0.3566** | Y = −0.1044x + 8.3546 | 0.2771ns | Y = −0.4016x + 26.291 | 0.7343*** | Y = −0.2672x + 19.748 |
| ADF | 0.008ns | Y = 0.0596x + 1.1134 | 0.4181* | Y = −0.1454x + 8.4603 | 0.4178ns | Y = −0.7024x + 28.97 | 0.5402*** | Y = −0.2946x + 17.274 |
| ADL | 0.0108ns | Y = 0.1423x + 0.7168 | 0.0205ns | Y = −0.1308x + 4.2912 | 0.4079ns | Y = −1.[illegible]x + 29.927 | 0.0736ns | Y = 0.4419x + 3.4711 |

*ns* not significant

* Significance level at $P \leq 0.05$

** Significance level at $P \leq 0.001$

*** Significance level at $P \leq 0.0001$



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residues and forages according to the local context. In addition, a galactogue effect of milk thistle leaves and its safely consumption has been acknowledged (Mohanty et al. 2014).

Furthermore, based on the outcomes of our research, the antioxidant capacity found in milk thistle plant organs can be useful also in animal feeding. In fact, an increasing attention is being paid to the use of natural antioxidants in animal diets (Tedesco et al. 2004; Gladine et al. 2007; Casamassima et al. 2012). According to Ahmad et al. (2013), various tissues of *S. marianum* exhibit higher antioxidant activities than vitamin C and E. Natural antioxidants prevent the oxidation chain reactions and protect the body from induced oxidative stress of toxic free radicals. In fact, the toxic free radicals attack on nucleic acids (DNA and RNA) can lead to mutational events. These toxic radicals can also attack enzymes, proteins and lipids causing degenerative diseases. Natural antioxidants from different tissues of medicinal plants function as free-radical scavengers and radical chain reaction breakers, complexers of pro-oxidant metal ions and quenchers of singlet-oxygen formation. In addition, natural antioxidants can exhibit anti-inflammatory, antimicrobial, antiviral, antiallergic and vasodilatory activities and are also used as anticancer, antimutagenic and antiaging agents (Ahmad et al. 2013).

The importance of a Mediterranean-type diet, due to the high number of antioxidants, is acknowledged (El-Sabban 2014). Within a study regarding Mediterranean non-cultivated vegetables as dietary sources of compounds with antioxidant activity, Morales et al. (2014) found in *S. marianum* a content of polyphenols and flavonoids of 3.72 g GAE $kg^{-1}$ and 1.13 g CE $kg^{-1}$, respectively, lower than our results at vegetative stage. In such a study leaves of milk thistle were harvested before flowering, but the extract preparation was made with methanol and this could explain the different results. Very often, comparisons with other published data about polyphenols, flavonoids, antioxidant capacity in similar species, are quite difficult due to variations in methods, procedures and standards used for the analyses. If compared to flavonoid contents reported by Soumaya et al. (2013) for stems of wild cynara (*C. carduncculus* L. var. *sylvestris* (Lamk) Fiori), of cynara (*C. carduncculus* var. *altilis* DC) and globe artichoke (*C. carduncculus* var. *scolymus* L.), our results showed lower flavonoid contents in milk thistle stems.

Ahmad et al. (2013) evaluated the antioxidant activity by DPPH method in different parts of *S. marianum* and found that the tested plant materials had significant free radical scavenging activity, suggesting that such plant materials can be used as a source of antioxidants for different diseases. The same authors evaluated the antioxidant activity in different parts of the plant (leaves, stems, seeds, roots) and found highest antioxidant capacity in young leaves of a white seed variety. Unfortunately, they used methanolic extract, whereas in our study the extraction was performed in acetone/water (7:3 v/v) with both ABTS and DPPH assays, showing a high TEAC value in leaves compared to heads and stems in the two Sardinian populations. However, due to the different methodological approaches followed, the absolute values cannot be compared.

Silymarin is an important free radical scavenger (Soto et al. 2010) and it was detected in leaves and seeds of *S. marianum* during different growth stages (Omar et al. 2012). On the other hand, Sanchez-Mata et al. (2012) and Morales et al. (2014) studied wild vegetables, traditionally eaten in the Mediterranean area, and indicated *S. marianum*, as a source of bioactive compounds such as polyphenols, vitamin C, organic acids, tocopherols, etc. Therefore, the antioxidant capacity of the leaves could be attributed to both silymarin and other active compounds which are related with antioxidant capacity.

Tawaha et al. (2007) reported the linear relationship between antioxidant activity from extracts of 51 plant species of Jordanian origin including *S. marianum,* with the total phenolic contents. Our data agree with the observation of many studies that documented the relationship between antioxidant activity and total phenolic compounds (Zheng and Wang 2001; Cai et al. 2004; Soumaya et al. 2013; Piluzza et al. 2014).

In our opinion, the above mentioned information regarding antioxidant activity in *S. marianum* should be coupled with new nutritional data reported by García-Herrera et al. (2014) who suggest to consider this plant as a valuable resource with potential in human diet.

Regarding the correlation between antioxidant activity and NDF (Table 5), Hęś et al. (2014) reported a significant correlation between antioxidant activity and NDF content in barley (*Hordeum vulgare*) and buchwheat (*Fagopyrum esculentum*) groats, whereas Campion et al. (2013) found that cellulose





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accumulation is negatively correlated with total phenolic and lignin contents in common bean (*Phaseolus vulgaris* L.) seeds. Even if it has been acknowledged the role of dietary fiber for the bioaccessibility and bioavailability of antioxidants in human diet and health (Palafox-Carlos et al. 2011), the relationship between antioxidant activity and NDF content in animal response needs to be elucidated. In addition, the chemical composition of bioactive compounds in milk thistle needs more investigation.

## Conclusions

Our results highlight differences in antioxidant capacity and bioactive compound contents in the different organs of milk thistle and evidenced a highly significant linear correlation between antioxidant capacity and ToTP and TotF at flowering compared to vegetative stage. However, considering both the overall chemical composition and antioxidant capacity of young plants mainly composed by leaves, the harvest at this stage could be suggested for the exploitation as forage or food.

Such results encourage investigations dealing with the exploitation of milk thistle Mediterranean germplasm also as functional feed and food and natural antioxidants and as a complementary source of fodder and additional research is justified for new natural antioxidants from milk thistle with elucidation of their chemical composition.

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