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Allelopathic Activity of Three Wild Mediterranean Asteraceae: *Silybum marianum*, *Cynara cardunculus* var. *sylvestris*, *Galactites tomentosus*

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Abstract: The manipulation of allelopathic mechanisms, such as the isolation of plant allelochemicals for bioherbicide production, is currently providing a new tool for weed management methods of reducing or potentially eliminating the use of synthetic herbicides. In Mediterranean agroecosystems, wild Asteraceae are the prevalent taxa, likely due to their allelopathic activity. Hence, the objective of this study was to evaluate the allelopathic effects of the aqueous extracts obtained from milk thistle [*Silybum marianum* (L.) Gaertn], wild cardoon (*Cynara cardunculus* L. var. *sylvestris*) and purple milk thistle (*Galactites tomentosus* Moench) on the seed germination, mean germination time, and seedling growth of three target weeds: *Portulaca oleracea* L., *Taraxacum officinale* (Weber) ex Wiggers and *Anagallis arvensis* L. The total polyphenol (TP), flavonoid (TF), flavonol (TFL), and phenolic acid (TPA) content in the aqueous extracts was also evaluated. Overall, the allelopathic effects were species-dependent and root length was the most affected parameter. All extracts completely inhibited root development in *P. oleracea*. Averaged over target weeds, *C. cardunculus* extract had the greatest allelopathic activity, followed by *G. tomentosus* and by *S. marianum*. In particular, *C. cardunculus* reduced seed germination by over 50% and increased the mean germination time by 154%, likely due to the highest TP (13.2 g kg^{-1} DM) and TPA (11.4 g kg^{-1} DM) content, compared to the other Asteraceae species. These results provide evidence of the phytotoxic activity of the three wild Asteraceae members and suggest their possible future exploitation as potential bioherbicides for sustainable weed management.

Keywords: allelopathy; weed management; polyphenols; weed germination; seedling growth

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Citation: La Iacona, M.; Lombardo, S.; Mauromicale, G.; Scavo, A.; Pandino, G. Allelopathic Activity of Three Wild Mediterranean Asteraceae: *Silybum marianum*, *Cynara cardunculus* var. *sylvestris*, *Galactites tomentosus*. *Agronomy* **2024**, *14*, 575. https://doi.org/10.3390/agronomy14030575

Academic Editor: Ivo Toševski

Received: 28 January 2024
Revised: 20 February 2024
Accepted: 11 March 2024
Published: 13 March 2024

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1. Introduction

It is well known that excessive and uncontrolled weeds are the main cause of yield reductions in field conditions [1]. Despite the current trend towards increasing agroecosystem biodiversity, weed control is still of outstanding importance, especially in low-input cropping systems. Unfortunately, the improper use of synthetic herbicides has led to the emergence of weed resistance and the development of a selected weed flora. For these reasons, there is a need to implement alternative and eco-friendly weed control tactics. Allelopathy is emerging as a promising phenomenon in pursuing this goal. According to Rice [2], allelopathy involves any direct or indirect beneficial or harmful effect from one plant to another (including microorganisms) through the release of chemical compounds (also referred to as allelochemicals) into the environment. Allelopathy is a multidimensional phenomenon involving different types of biological interactions (plant–plant, plant–microorganism, plant–insect, etc.) and consistently observed in both natural and anthropogenic ecosystems [3–5]. In recent years, the study of allelopathy has focused on

the selection of plant extracts and allelochemicals for their potential as bioherbicides [6]. Among the most active allelochemicals, many phenolic compounds, have been identified, purified, and applied for weed control [7]. Many allelochemicals are water-soluble and are often applied as aqueous extracts, a feature that makes them easier to apply and more cost-effective to extract [8]. Plant extracts and allelochemicals, however, are often tested under controlled conditions because of the difficulty of obtaining field-based evidence from them [9].

Several Asteraceae members are well recognized as potent allelopathic plants [10]. Chon et al. [11] reported that the aqueous leaf extracts of sixteen Asteraceae plant species showed allelopathic effects on seed germination and growth of alfalfa (*Medicago sativa* L.). The highest inhibitory capacity was observed in *Cirsium japonicum* DC., *Lactuca sativa* L., and *Xanthium occidentale* Bertol. extracts. Within the Asteraceae family, the genera with important allelopathic properties are *Artemisia*, *Cirsium*, and *Xanthium* [12].

Milk thistle [*Silybum marianum* (L.) Gaertn.], a native species of the Mediterranean basin, shows a pronounced allelopathic activity and high invasiveness in the Mediterranean environment [13]. Lehoczky et al. [12] reported that the aqueous extracts of *S. marianum* inhibited the ability of *Triticum aestivum* L. to self-regulate its phosphorus transporters in root cells, thus causing phosphorus phytotoxicity. Further allelopathic effects caused by *S. marianum* aqueous extracts were observed on seed germination and mean germination time of red beans, mung beans, chickpeas, and soybeans [13].

Wild cardoon [*Cynara cardunculus* L. var. *sylvestris* (Lamk) Fiori] is a perennial, geophyte species and native to the Mediterranean Basin, where it shares a distribution pattern with olive production areas. *C. cardunculus* is considered the progenitor of globe artichoke and cultivated cardoon. Many findings have demonstrated the allelopathic effects of *C. cardunculus* [14–17]. For instance, Scavo et al. [14] found that *C. cardunculus* aqueous extracts reduced the germination rates and increased the mean germination time of six weed species, including *Amaranthus retroflexus* L., *Portulaca oleracea* L., *Diplotaxis erucoides* (L.) DC., *Brassica campestris* L., and *Solanum nigrum* L.

Purple milk thistle (*Galactites tomentosus* Moench) is a Mediterranean biennial hemicryptophyte commonly found in arid and uncultivated soils ranging from sea level up to an altitude of 1300 m. A study published by Bäumer and Ruppel [18] indicates that *G. tomentosus* leaves contain allergenic compounds also found in *C. cardunculus* and *S. marianum*, belonging to the class of sesquiterpene lactones (e.g., cynaropicrin) and phenolics (e.g., chlorogenic acid and caffeic acid), molecules previously identified as allelochemicals. To the best of our knowledge, its allelopathic activity has never been studied. However, given the presence of well-known allelopathic compounds and its ability to dominate other species creating shifting in weed communities (by empirical experience), we decided to investigate the allelopathic activity of *G. tomentosus* for weed control.

In Mediterranean agroecosystems under a semi-arid climate, Asteraceae is the most representative botanical family in soil seedbank and aboveground weed communities [19], where *S. marianum*, *C. cardunculus* and *G. tomentosus* are prevalent species. We hypothesized that, in addition to competitive capacity, their spread could be attributed to allelopathic interactions. Hence, the present work aims to investigate the potential allelopathic effects of the three wild Asteraceae members (*S. marianum*, *C. cardunculus sylvestris* and *G. tomentosus*) against three typical weed species in Mediterranean agroecosystems. To reach this aim, three objectives have been defined: (1) chemical characterization of the extracts; (2) assessment of the inhibitory potential of extracts on germination parameters of target weeds; (3) the effect of extracts on seedling growth. This is the first report on the allelopathic activity of *G. tomentosus*. Moreover, allelopathy of *S. marianum* on weeds has never even been evaluated, and one of the target plants used herein was utilized for the first time as a target weed for *C. cardunculus*.

2. Materials and Methods

2.1. Donor Plants Sampling and Morpho-Biometric Characterization

The fresh material of *C. cardunculus*, *S. marianum* and *G. tomentosus* was collected in spring when the plants were at the flowering stage. The three donor plants were randomly collected from natural fields located in three Sicilian (southern Italy) locations characterized by a typical Mediterranean climate: [37°36′1.4″ N, 12°58′6.8″ E] for *G. tomentosus*, [37°34′2.7″ N, 14°54′8.27″ E] for *S. marianum*, and [37°14′16.47″ N, 14°30′42.2″ E] for *C. cardunculus*. Table 1 shows the main morpho-biometric characteristics of the three donor plants. *S. marianum* and *G. tomentosus* flourish from late spring to summer, while *C. cardunculus* flourish from June to late summer. *G. tomentosus* shows smaller and lighter seeds compared to *C. cardunculus*. However, its seed production head^{-1} is significantly lower than *C. cardunculus* but comparable to the values reported by Azoz et al. [20] for *S. marianum*. Regarding biomass partitioning, in *S. marianum*, the leaves and stem contribute equally and more than heads; in *C. cardunculus*, the heads contribute more than leaves and stems, whereas in *G. tomentosus*, the major portion of the total biomass was represented by leaves.

Table 1. Morpho-biometric characteristics of the three donor plants under study.

Donor Plant	Anthesis Period	Aboveground DW (kg Plant^{-1})	Biomass Partitioning (% on Total DW)			Heads (N. Plant^{-1})	1000 Seeds Weight (g)	Seeds (N. Head^{-1})
			Leaves	Stem	Head			
Silybum marianum	May–August	0.7	42	43	15	3 [c]	21.5 [c]	63 [c]
Cynara cardunculus	June–August	1.8 [a]	33 [a]	30 [a]	37 [a]	28 [b]	21 [b]	103 [b]
Galactites tomentosus	April–July	0.8	59	4	37	46	6.72	76

[a] Ierna et al. [21]; [b] Foti et al. [22]; [c] Azoz et al. [20].

2.2. Preparation of Aqueous Extracts

Reagents and solvents were purchased from VWR (Leighton Buzzard, UK) and were of analytical grade. Gallic acid and rutin were obtained from Extrasynthese (Lyon, France). Ferric chloride hexahydrate, sodium acetate trihydrate, ethanol and Folin–Ciocalteu reagent were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Milli-Q system (Millipore Corp., Bedford, MA, USA) ultrapure water was used throughout this research.

2.3. Preparation of Aqueous Extracts

In the laboratory, the fresh leaves collected from each donor plant were dried in an oven at 35 °C up to constant weight. The dry material of *S. marianum* was reduced to powder, while that of *C. cardunculus* and *G. tomentosus* was cut in chunks. For the preparation of aqueous extracts, a 1:10 v/w extraction ratio was established (30 g of dry material in 300 mL of bidistilled water). Maceration in bidistilled water took place in the dark and at room temperature (20 ± 1 °C) for 72 h; after that, an ultrasound treatment was performed for 15 min. Then, the 100% (v/w) master solutions were filtered with a Whatman #2 sterilized paper layer. Lastly, the three extracts of the donor species were centrifuged at 4000 rpm for 3 min at 20 °C. During phytotoxicity tests, the extracts were stored in a refrigerator at 3 °C for further use and analysis of allelochemicals.

For each extract, the pH was determined with a Cyberscan pH meter 2100, and the extract yield was calculated using the following formula:

$$\text{Percentage extract yield } (\%) = \frac{\text{mL solvent}}{\text{mL solution after maceration}} \times 100 \quad (1)$$

Table 2 shows pH values and yield of each extract under study. *C. cardunculus* had the highest extract yield and the lowest pH, whereas *S. marianum* showed an opposite trend.

Table 2. pH values and yield of the donor plant extracts under study.

Donor Plant	pH	Extract Yield (%)
Silybum marianum	6.7	53.3
Cynara cardunculus	5.9	61.5
Galactites tomentosus	6.1	56.7

2.4. Seed Germination and Seedling Growth Bioassays

The target plants of this study were *Portulaca oleracea* L., *Anagallis arvensis* L. and *Taraxacum officinale* (Weber) ex Wiggers. The choice of these species derived from their widespread presence in Mediterranean agroecosystems. Moreover, these species have never been tested with *G. tomentosus* and *S. marianum* extracts, while *T. officinale* is used herein for the first time as target weed for *C. cardunculus* extract. The mature seeds of *P. oleracea* were purchased from Magic Garden Seeds GmbH (Regensburg, Germany). *T. officinale*'s mature seeds were purchased from Sementi Dotto (Mortegliano, Italy), and *A. arvensis* seeds were gathered from natural populations in the Catania Plane of Sicily (37°28′ N, 14°57′ E, at an average altitude of 50–150 m a.s.l.). Before germination bioassays, the seeds were selected using a stereomicroscope to obtain a higher degree of homogeneity in size and color.

Germination tests were performed by placing 20 seeds per Petri dish (each of 9 cm diameter). For each target plant × donor plant combination, 5 mL of the different aqueous extracts was used to moisten a double-layer of Whatman #2 sterilized paper. Bidistilled water was used as a control. Petri dishes were sealed with a parafilm layer to prevent evaporation of the solution and then placed inside germination chambers, which were set considering the optimal temperature and photoperiod of the three target species: dark at a constant temperature of 35 °C for *P. oleracea*, and alternating light (dark/light cycle: 14/10 h) at a constant temperature of 17 °C for *T. officinale* and *A. arvensis*. *Portulaca oleracea* dishes were also wrapped with aluminum film. Seeds were considered germinated when a 2 mm root protrusion was observed and seeds did not germinate for three consecutive days.

Germinated seeds were moved to new Petri dishes with further 5 mL of the same extract to perform the growth tests. Two weeks after germination, the epicotyl length (EL, mm) and the root length (RL, mm) were measured on each seedling.

2.5. Total Content of Polyphenols, Flavonoids, Flavonols and Phenolic Acids

The total content of polyphenols, flavonoids and flavonols was assessed as described by Lucini et al. [23]. To determine the total polyphenol content (TPC) of the leaves, an aliquot (0.1 mL) of the extract was diluted with 3.9 mL of distilled water, then mixed with 0.1 mL of Folin–Ciocalteu reagent and 0.4 mL of 10% sodium carbonate (*v*/*v*). Then, after 1 h at room temperature, in the dark, absorbance at 760 nm was recorded. A calibration curve was prepared using aliquots of gallic acid ethanol solutions and the results were expressed as g kg^{-1} equivalents of dry matter (DM) of gallic acid. Regarding the total flavonoid content (TFC), an aliquot of the leaf extract (1 mL) was mixed with 1 mL of $AlCl_3$ in ethanol (20 g L^{-1}) and absolute ethanol (2.5 mL). Subsequently, the samples were placed at 20 °C for 40 min and the absorbance recorded at 415 nm. A calibration curve was prepared using aliquots of rutin ethanol solutions and the results were expressed as g kg^{-1} DM equivalent of rutin. Regarding the total flavonol content (TFLC), an aliquot of the leaf extract (1 mL) was mixed with 1 mL of $AlCl_3$ in ethanol (20 g L^{-1}) and 3 mL of sodium acetate (50 g L^{-1}). Subsequently, the samples were placed at 20 °C for 2.5 h and recorded absorbance at 440 nm. Results were expressed as equivalent g kg^{-1} of DM of rutin. The total phenolic acid content (TPA) was determined by subtracting the total content of flavonols and flavonoids from the total content of polyphenols, according to the following formula:

$$TPA = [TPC - (TFC + TFLC)] \quad (2)$$

2.6. Data Collection

To evaluate the allelopathic effects of the three donor plants under investigation, the following parameters were calculated:

- Final germination percentage (G, %), calculated as the ratio between the number of germinated seeds and the total number of seeds in each Petri dish;
- Mean germination time (MGT, days), computed according to the equation proposed by Ranal et al. [24]:

$$\mathrm{MGT} = \sum_{i=1}^{k} n_i \times t_i / \sum_{i=1}^{k} n_i \quad (3)$$

where n_i and t_i are, respectively, the number of seeds germinated in time ith and the duration from the beginning of the experiment to the observation ith; k = last day of germination.

- epicotyl length (EL, mm) and root length (RL, mm).

2.7. Statistical Analysis

The experiments were conducted according to a completely randomized block design with 4 replicates, as suggested by Jefferson and Pennacchio [25], and Turk and Tawaha [26]. All data were statistically analyzed by a two-way analysis of variance (ANOVA), considering the 'target plant' and the 'donor plant' as fixed factors, and means were separated with a least significant difference (LSD) test at $p \leq 0.05$. Prior to ANOVA, G and MGT data were log-transformed to achieve normal data distribution. Homoscedasticity was verified by inspecting the residuals. The data about TPC, TFC, TFLC and TPA were analyzed by one-way ANOVAs.

3. Results

The two-way ANOVA carried out on G, MGT, EL and RL showed significant 'target plant × donor plant' interactions (Table 3). In particular, the 'target plant' is the factor that contributed most of the total variance for G, whereas in the other cases, the 'donor plant' had the highest incidence.

Table 3. Absolute values of the *F*-Fisher's values for the two main factors and their interaction resulting from the analysis of variance (ANOVA).

	Target Plant (TP)	Donor Plant (DP)	(TP) × (DP)
df	2	3	6
G	448.6 ***	108.1 ***	31.1 ***
MGT	83.6 ***	314.3 ***	110.1 ***
EL	13.3 ***	74.0 ***	13.0 ***
RL †	0.1 NS	45.9 ***	3.7 *

*** and * indicate significance at $p \leq 0.001$ and $p \leq 0.05$, respectively; NS indicates non-significant; df: degrees of freedom; G: seed germination percentage; MGT: mean germination time; EL: epicotyl length; RL: root length. † For RL, *Portulaca oleracea* was excluded from the ANOVA, meaning df = 1 for TP.

3.1. Total Polyphenol, Flavonoid, Flavonol and Phenolic Acid Content in the Aqueous Extracts

The content of total polyphenols, flavonoids, flavonols and phenolic acids was significantly influenced by the donor plant. *C. cardunculus* showed the highest TPC, followed by *G. tomentosus* and *S. marianum* (Figure 1). Specifically, *C. cardunculus* reported a higher TPC (approximately 2.0 and 1.2 times) compared to *S. marianum* and *G. tomentosus*, respectively. On the contrary, TFC in *G. tomentosus* was significantly higher than in *S. marianum* and *C. cardunculus* (+26% and +42%, respectively). *G. tomentosus* also showed the highest TFLC, greater by approximately 1.64 and 2.72 times compared to *C. cardunculus* and *S. marianum*, respectively (Figure 1). TPA had the same trend as TPC, since the greatest content was detected in *C. cardunculus* (11.43 g kg^{-1} DM, representing 87% of TPC).

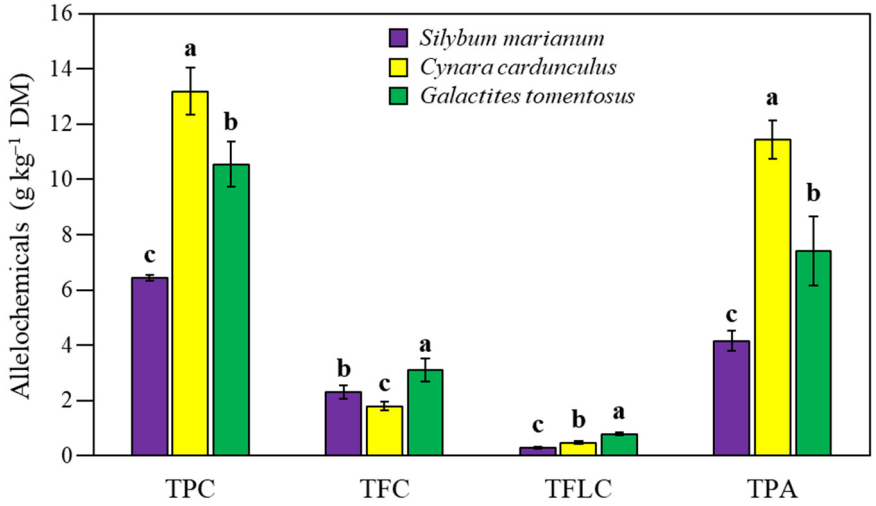
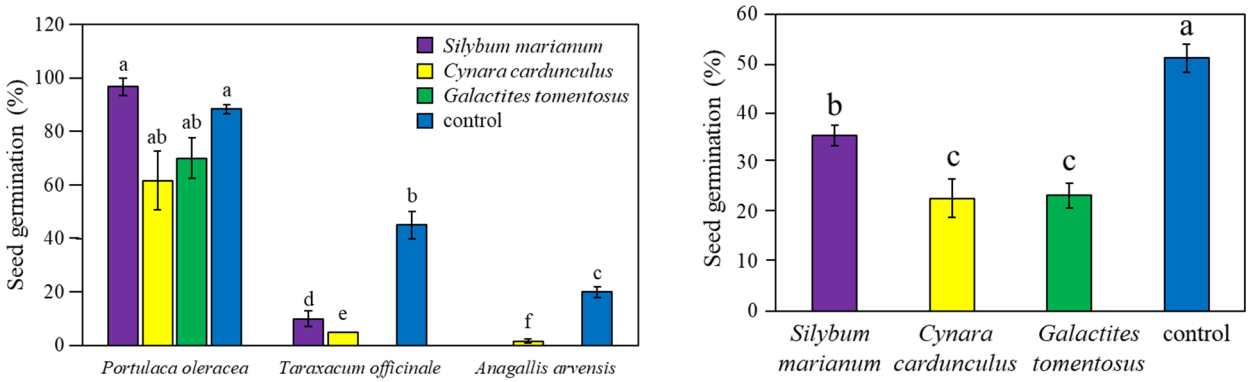


Figure 1. Total polyphenol content (TPC), total flavonoid content (TFC), total flavonol content (TFLC) and total phenolic acids (TPA) (± standard deviation) in leaf aqueous extracts obtained from three Asteraceae species. Different letters indicate significance at $p \leq 0.05$ (LSD test).

3.2. Allelopathic Effects on Seed Germination Percentage

Figure 2a shows the two-way interaction on G. Except for *S. marianum* extract on *P. oleracea*, all extracts under study significantly reduced G compared to the control for the three target plants, with species-specific results. In particular, the *C. cardunculus* extract decreased G by 30% in *P. oleracea*, 89% in *T. officinale*, and 92% in *A. arvensis*. *S. marianum* extract, except for a 9% increase in *P. oleracea*, reduced G by 78% and 100%, respectively, in *T. officinale* and *A. arvensis*. The *G. tomentosus* extract reduced G by 21% in *P. oleracea*, and completely inhibited seed germination in *T. officinale* and *A. arvensis*. Averaged over target plants (Figure 2b), the extract with the highest inhibitory capacity on G was the *C. cardunculus* one (−55% compared to the control), followed by *G. tomentosus* (−53%), and *S. marianum* (−30%).



(**a**) (**b**)

Figure 2. (**a**) Effect of the 'target plant × donor plant' interaction on the final seed germination percentage; (**b**) effect of the donor plant on G. Vertical bars indicate ± standard error. Different letters indicate significance at $p \leq 0.05$ (LSD test).

3.3. Allelopathic Effects on Mean Germination Time

From the ANOVA (Table 3), it emerged that the two-way interaction was highly significant for MGT ($p \leq 0.001$), but contrary to G, the variance was mostly affected by the 'donor plant' (62%). Except for the *S. marianum* extract on *P. oleracea*, all extracts increased the MGT compared to the control for the three target plants (Figure 3). The *C. cardunculus* extract caused the greatest increase in MGT on *P. oleracea* (+128% compared

to the control), in *T. officinale* (+112%), and in *A. arvensis* (+219%). The *S. marianum* extract, on the one hand, reduced MGT by 20% in *P. oleracea* while, on the other hand, increased it by 79% in *T. officinale*, while no effect on MGT was recorded in *A. arvensis* as it completely inhibited G. On average over all target plants, the *C. cardunculus* extract induced the highest MGT (15.4 days), a +154% increase compared to the control, denoting a significant allelopathic effect in terms of both reducing seed germination and prolonging the mean germination time.

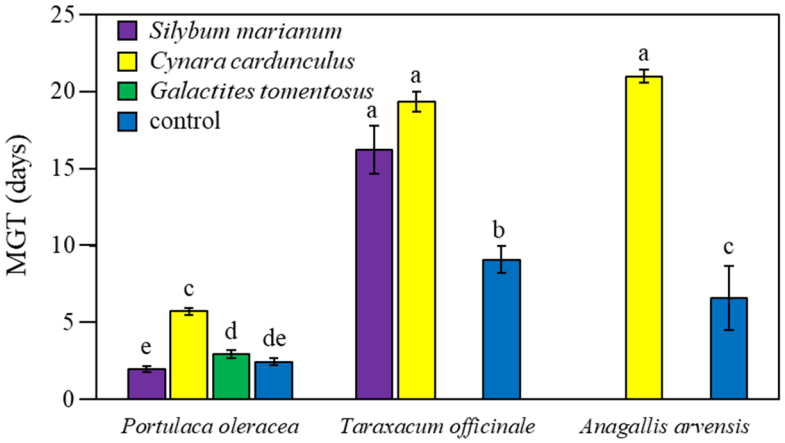


Figure 3. Effect of the 'target plant × donor plant' interaction on the mean germination time (MGT). Vertical bars indicate ± standard error. Different letters indicate significance at $p \leq 0.05$ (LSD test).

3.4. Allelopathic Effects on Epicotyl Length

For epicotyl length (EL), the ANOVA highlighted the same trend as the MGT (Table 3). The two-way interaction (Figure 4a) shows that all extracts significantly reduced EL compared to the control. The greatest inhibitory activity was observed in *A. arvensis*, where *S. marianum* and *C. cardunculus* extracts completely inhibited epicotyl growth, and the *G. tomentosus* extract reduced it by 97% compared to the control. In *P. oleracea*, the *C. cardunculus* extract showed the highest inhibitory activity (−93%), while in *T. officinale*, the *G. tomentosus* extract completely inhibited EL. Pooling over target plants (Figure 4b), the *C. cardunculus* extract had the highest allelopathic activity (−96% compared to the control), followed in decreasing order by the *S. marianum* extract (−93%) and the *G. tomentosus* extract (−85%). Overall, the results indicate a pronounced inhibition of epicotyl growth in all three target species.

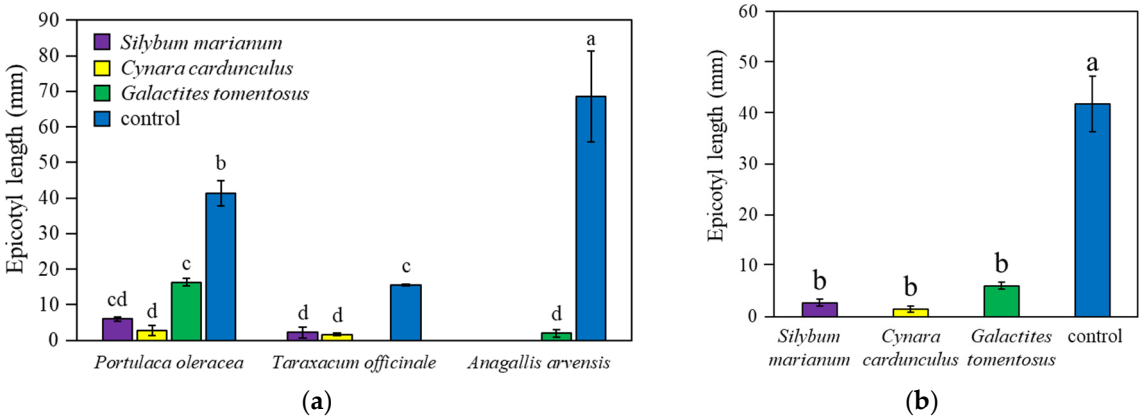


Figure 4. (**a**) Effect of the 'target plant × donor plant' interaction on the epicotyl length (EL); (**b**) effect of the donor plant on EL. Vertical bars indicate ± standard error. Different letters indicate significance at $p \leq 0.05$ (LSD test).

3.5. Allelopathic Effects on Root Length

The ANOVA highlighted a highly significant effect of the 'donor plant' on RL, while the 'target plant' was not significantly affected (Table 3). All extracts inhibited radicle growth in *P. oleracea*, and consequently, it was excluded from Figure 5. From the second-order interaction (Figure 5a), it emerged that the *C. cardunculus* extract completely inhibited RL in both *T. officinale* and *A. arvensis*; the *S. marianum* extract reduced RL by 63% in *T. officinale* and completely inhibited it in *A. arvensis*; the p *G. tomentosus* extract completely inhibited RL in *T. officinale* and reduced it by 75% in *A. arvensis*. Averaged across the two target species (Figure 5b), the *C. cardunculus* extract showed the highest allelopathic effect on RL, followed by *G. tomentosus* (−87%) and *S. marianum* (−82%).

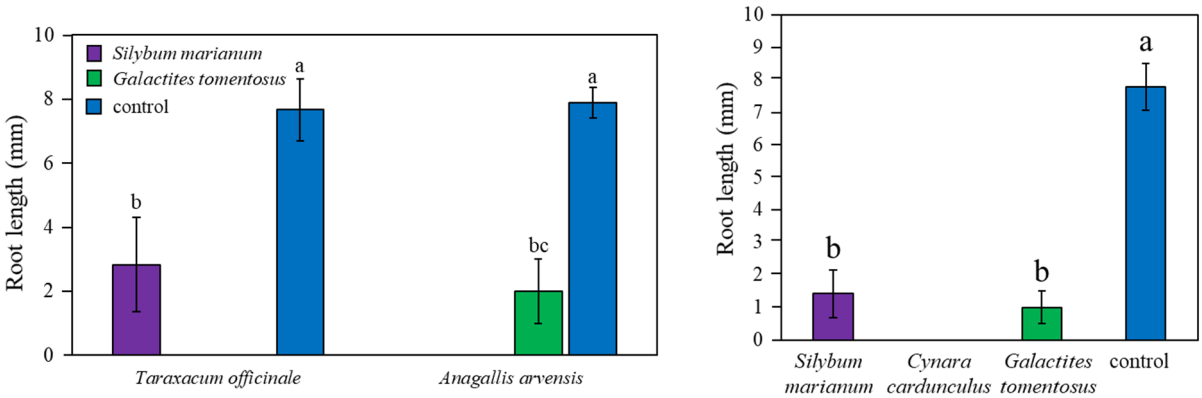


Figure 5. (**a**) Effect of the 'target plant × donor plant' interaction on the root length (RL); (**b**) effect of the donor plant on RL. Vertical bars indicate ± standard error. Different letters indicate significance at $p \leq 0.05$ (LSD test).

4. Discussion

The present study was focused on the potential allelopathic activity of three different wild Asteraceae members on three chosen Mediterranean weed species. The three donor plants showed consistent allelopathic effects, although with species-dependent results. The differentiated responses could be attributed to the qualitative and quantitative profile of secondary metabolites in donor plant extracts [14–16,27,28]. In this study, the aqueous extract of *C. cardunculus* stood out, having the greatest allelopathic capacity in reducing G, MGT, RL and EL. The same extract also showed the highest content of total polyphenols and phenolic acids compared with the other wild Asteraceae under study. This extract was followed by the *G. tomentosus* extract, which showed the highest content of total flavonoids and an intermediate value of total polyphenols. The portions of TPC made up of phenolic acids in *S. marianum*, *C. cardunculus*, and *G. tomentosus* were 65%, 87%, and 70%, respectively. This leads us to hypothesize that the allelopathic activity of the wild Asteraceae under study could be mainly attributed to phenolic acids (especially *C. cardunculus)*, in which they are particularly rich, as reported in previous studies [29,30]. Kaab et al. [31], for example, demonstrated that *C. cardunculus* methanolic extract showed a significant reduction in germination and seedling growth of *Trifolium incarnatum* L., *S. marianum*, and *Phalaris minor* Retz., and that its allelopathic activity was mainly due to five phenolic acids: syringic acid, *p*-coumaric acid, myricitrin, quercetin, and naringenin. The key role of phenolic acids in different allelopathic interactions is corroborated by other studies. Rice [2] indicated that many hydrolysable tannins (with high allelopathic activity) are formed by complex mixture of phenolic acids. Einhellig [32] noted that water-soluble phenolic acids are leached from the leaves of various herbaceous and shrubby species, contributing to their phytotoxic effects on associated herbaceous and grassy vegetation. Rial et al. [33], studying the joint

action of binary mixtures of *C. cardunculus* allelochemicals, reported a higher allelopathic activity when major compounds act in synergism.

The results about *P. oleracea* seed germination and seedling growth are in line with our previous findings [16]. In particular, for *P. oleracea*, a 51% reduction in epicotyl length was reported when treated with *C. cardunculus* extract; here, however, we observed a greater percentage reduction (93%). This could be due to the different environment and season of *C. cardunculus* collection, which probably determined a different polyphenolic profile. In fact, *C. cardunculus* collected in April caused more pronounced inhibitory effects on target weeds than the November and January samples due to the higher content of sesquiterpene lactones [16]. This hypothesis is also supported in other plant species. For instance, Chung et al. [34] reported differentiated allelopathic effects of wild rice collected from different areas. Moreover, it is known that the synthesis and concentration of secondary metabolites in *C. cardunculus* are affected by different abiotic and biotic factors [8]. According to Ochoa-Velasco et al. [35], secondary metabolites play a significant role in plant adaptation and are responsible for plant protection against various biotic agents such as microorganisms, insects, and weeds or abiotic stress factors including nutrient and water deficiency, etc. These conditions promote a defensive response in plants, inducing genes encoding proteins for repairing cellular damage, producing protease inhibitors and lytic enzymes, and synthesizing secondary metabolites (such as polyphenols and phenolic acids) with antimicrobial and antioxidant characteristics. We therefore hypothesize that the three Asteraceae species under study developed allelopathic potential, suggested by their phytotoxic effects and allelochemicals profiles, as a co-evolution adaptation to Mediterranean environments, which are characterized by long drought periods and high solar radiation intensity.

The *S. marianum* extract completely inhibited *A. arvensis* seed germination and caused significant reductions in *T. officinale* RL (−63%). In a previous study conducted by Sultana and Asaduzzaman [36], *S. marianum* extract was found to inhibit root length and sprout development, decrease the germination percentage, and increase the mean germination time of alfalfa and rapeseed. Phytotoxic effects of *S. marianum* were also reported by Lehoczky et al. [10] in terms of root and shoot reduction of *T. aestivum*. Similarly, significant phytotoxic effects of *S. marianum* were reported by Khan et al. [12] on the seed germination, mean germination time, and vigor of *Phaseolus vulgaris* L., *Vigna radiata* L., *Cicer arietinum* L. and *Glycine max* L. On the contrary, to our knowledge, no study has yet focused on the allelopathic effects of *G. tomentosus* on weed species. Overall, the *G. tomentosus* extract had a similar effect to that of *C. cardunculus* on seed germination, especially on *T. officinale*.

The specific modes of action of the three Asteraceae donor plants are still unknown. In a recent study, Kaab et al. [37] evaluated two physiological parameters (fluorescence and electrical conductivity) and two oxidative stresses (melondialdehyde and hydrogen peroxide) in *T. incarnatum* treated with *C. cardunculus* extract. The observed symptoms were leaf necrosis, chloroses, and electrolyte leakage that led to a disturbance in the electron transport chain of photosynthetic system, and consequently, to an increase in ROS production and a decrease in ATP levels. Given the similarities between the profiles of the *C. cardunculus*, *S. marianum* and *G. tomentosus* extracts, it is reasonable to assume that the mode of action reported for *C. cardunculus* is the same for the other two Asteraceae members.

5. Conclusions

These results reinforce that the leaf extracts of *C. cardunculus*, *S. marianum*, and *G. tomentosus* have significant allelopathic effects on three Mediterranean weed species under controlled conditions. Their allelopathic activity may be one of the reasons that they have spread extensively in Mediterranean environments. In particular, *C. cardunculus* extract showed the highest TPC and TPA, followed by *G. tomentosus* and *S. marianum*. As a consequence, allelopathic effects on seed germination parameters and seedling growth showed the same trend as the extracts' chemical characterization (*C. cardunculus* > *G. tomentosus* > *S. marianum*). However, the allelopathic activity of these Asteraceae was both target

plant- and donor plant-dependent. Therefore, further studies on other target weeds and experiments aiming to create Asteraceae allelochemical-based formulations are needed. Another future research direction will be to test the aqueous extracts of the three donor plants in open-field conditions. As a practical application, these results suggest their possible application as new bioherbicides within weed management methods, which may reduce the adoption of synthetic herbicides.

Author Contributions: Conceptualization, G.P., A.S. and G.M.; investigation, M.L.I., S.L., G.M., A.S. and G.P.; methodology, A.S. and G.P.; formal analysis, M.L.I.; writing—original draft, M.L.I.; writing—review and editing: S.L., A.S., G.M. and G.P.; supervision, G.M., A.S. and G.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data and materials for the current study are included in this published article. No other data were generated or used. The datasets used and/or analyzed during the present experiments are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

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