

Sustainable experimental cultivation of the Greek endemic *Helichrysum amorginum* Boiss. and Orph.: Assessment of the total phenolic content at different flower harvest stages

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Abstract

Helichrysum amorginum Boiss. and Orph. constitutes a range-restricted, endemic species of Greece with known pharmaceutical potency. The current work presents the results of a targeted evaluation of the total phenolic content (TPC) of methanolic extracts of *H. amorginum* flower heads under cultivation conditions at three consecutive harvest stages in two separate cultivation locations across a period of two years (2018 and 2019). The harvested inflorescence tissue was assessed for TPC via the Folin–Ciocalteu method in three and four-year old *H. amorginum* individuals. Older plants, during the second evaluation year, generally showed higher flower TPC content throughout. The pooled analysis of the results indicated significant differences in TPC among samples of extracts prepared with plant material from different harvest stages with the early flowering stage (A) presenting higher TPC in both experimental cultivation locations (142.6–156 mg GAE g⁻¹ extract on average among the two cultivation locations) followed by the full bloom stage (B) (139–145.7 mg GAE g⁻¹ extract on average) and the late bloom or early post-anthesis stage (C) which showed the lowest TPC in all cases (130.3–131 mg GAE g⁻¹ extract on average). The current results provide for the first-time basic information on the optimum inflorescence harvest stage during the prolonged flowering period of cultivated *H. amorginum* in terms of TPC. The proposed work can be incorporated into the establishment of a sustainable cultivation protocol for achieving polyphenol-rich extracts and ultimately contributes to the utilization of *H. amorginum* in the pharmaceutical/cosmetic sectors.

Keywords: agronomic utilization; endemic species; flower polyphenols; Folin–Ciocalteu; *Helichrysum amorginum*; pharmaceutical plants; phytochemical resources

Received: 01 June 2024. Received in revised form: 30 Jun 2024. Accepted: 10 Aug 2024. Published online: 20 Aug 2024.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

The utilization of local phylogenetic resources can deliver a diversified array of plant species with noted exploitation potential within the contemporary bounds of food security, or medicinal/cosmetic sectors (Singh *et al.*, 2019; Bourgou *et al.*, 2021). Range restricted plant species are usually characterized by a frail conservation status, stemming from their specific adaptation traits relating to their habitat which can be shifting due to the current climatic change, especially across temperate regions like the Mediterranean (Casazza *et al.*, 2014; Parmesan and Hanley, 2015; Shay *et al.*, 2021). However, the evolutionary course of range restricted plant species has granted them with potent genotypes that entail, among others, highly valuable secondary metabolites relating to the plants' environmental adaptation (Defosse *et al.*, 2021). Noted examples of domestication efforts of range restricted plant species with significant pharmaceutical or nutritional potential emanating from their secondary metabolism have been demonstrated recently across the wider eastern Mediterranean basin (Zheljazkov *et al.*, 2022; Mašković *et al.*, 2023; Tsiftoglou *et al.*, 2023a), enhancing at the same time, the conservation status of such species.

The flora of Greece includes a plethora of range restricted plant species stemming from the high level of endemism among the region's biodiversity (Strid and Tan, 1997; Myers *et al.*, 2000). A noteworthy genus is *Helichrysum* Mill. (Asteraceae) which entails a plethora of herbaceous perennial species with distinctive flowers ranging across the Mediterranean area, five of which are endemic to Greece: *Helichrysum amorginum* Boiss. and Oph., which concerns the current study, but also *H. doerfleri* Rech.f., *H. heldreichii* Boiss., *H. taenari* Rothm., and *H. sibthorpii* Rouy (Strid and Tan, 1997; Argyriou *et al.*, 2020). *Helichrysum* species are characterized by their distinctive and diverse-coloured flowers that can have prolonged vivid appearance after they are cut, which has given many of them the common name of everlasting flowers or immortelles (Akaberi *et al.*, 2019). In addition, many *Helichrysum* species are being used traditionally for the medicinal and cosmetic properties of their flowers (Akaberi *et al.*, 2019). Contemporary research has shown that *Helichrysum* spp. flowers contain highly significant secondary metabolites mainly phenolic compounds with high pharmaceutical potency (Viegas *et al.*, 2014; Akaberi *et al.*, 2019). Noted examples are the antioxidant and neuroprotective properties of *H. stoechas*, or the anti-inflammatory activity of *H. arenarium* (Chinou *et al.*, 1997; Les *et al.*, 2017; Mao *et al.*, 2017).

The current study concerns *H. amorginum*, a range restricted rosette-type perennial species that occurs in Cyclades, mainly on Amorgos Island in Greece as a chasmophyte on limestone rocks close to the sea (Argyriou *et al.*, 2020). *H. amorginum* naturally flowers from spring through mid-summer producing flushes of inflorescences that bear glandular hairs on the leaves. The flowers bear white-pinkish bracts that turn all-white with yellow male-female reproductive parts in the centre as floral development progresses (Phitos *et al.*, 1995). The limited research on the essential oil of *H. amorginum* floral organs has thus far demonstrated a number of secondary metabolites present like spathulenol and β -pinene with antimicrobial potential (Chinou *et al.*, 2004).

Based on the above-mentioned utilization potential of native species like *H. amorginum*, sustainable exploitation research lines have been brought forward in the literature which usually start with the development of a viable propagation protocol facilitated either via *in vitro* methods, like the case of *H. italicum* (Perrini *et al.*, 2009), or via seeds with the latter adding to the conservation status of range restricted target taxa (Anestis *et al.*, 2023; Varsamis *et al.*, 2023). In the case of *H. amorginum* herein practices for the efficient germination of the seeds have been proposed as part of a systematized research effort that has been initiated for the conservation and sustainable exploitation of the Greek germplasm (Argyriou *et al.*, 2020). Consecutively, *in vitro* methods were successfully used for propagation of Greek *H. amorginum* facilitating the establishment

of a multi-year experimental cultivation trial on the island of Amorgos, which is still ongoing, aspiring to enable the upscaled commercial exploitation of the pharmaceutical properties of *H. amorginum* in Greece.

Considering all the above, the current work evaluated the total phenolic content (TPC) of methanolic extracts of the floral organs of experimentally cultivated *H. amorginum* at three different harvest stages in two separate field environments across a period of two years. The overall aim of the work was to determine the optimum inflorescence harvest stage during the prolonged flowering period of *H. amorginum* in terms of TPC. Thus, the current work complements previous research and builds on the development of an economically viable cultivation protocol for the sustainable utilization of *H. amorginum* in the pharmaceutical/cosmetic sectors providing at the same time novel potential cultivation options for the local economy of the Aegean islands.

Materials and Methods

Greek Helichrysum amorginum germplasm

The plant material that was used herein consisted of *H. amorginum* individuals that were grown in two experimental cultivation locations on the island of Amorgos that were set in 2016 as part of an integrated research scheme that was initiated for the sustainable utilization of Greek *H. amorginum*. The original plant material for the experimental cultivation consisted of asexually propagated *H. amorginum* individuals that were produced from wild-occurring material collected from the species natural habitat in Amorgos. The botanical collections of *H. amorginum*, the validation and documentation of the collected material, and its ex-situ asexual propagation was conducted by the Institute of Plant Breeding and Genetic Resources (IPBGR, ELGO-Dimitra, Thessaloniki, Greece) under a special permit (Permit 82336/879 updated on 18 May 2019, and 26895/1527 of 21 April 2021) issued by the Greek Ministry of Environment and Energy. The produced material was then transferred back to the island of Amorgos early in 2016, when its experimental cultivation started.

Experimental cultivation conditions and harvest stages

The experimental cultivation of the *H. amorginum* took place on two distinct locations on the island of Amorgos, Aegean Sea, Greece from 2016 onwards. Selected topographic and soil properties of the two experimental fields are given in Table 1.

Table 1. Characteristics of the two experimental cultivation locations of *Helichrysum amorginum* in the island of Amorgos, Greece

Topographic / soil property	Aegiali	Katapola
Coordinates (HGRS87) (Lat, Lon)	36° 54' 55,6 N 26° 00' 22,4 E	36° 49' 36,8 N 25° 52' 39,9 E
Altitude (m)	247	20
Distance from sea (m)	1500	400
Soil type	SCL	SCL
Electrical conductivity ($\mu\text{S}/\text{cm}$)	160.6	188.6
CaCO ₃ (g/100g)	28488.1	3152.38
Organic matter (%)	4.83	3.27
Salinity (mg/Kg)	251	120.7

One-year old asexually propagated *H. amorginum* plants were established in the two experimental fields early in 2016 following basic cultivation of the topsoil at 20 cm depth. Plants were drip-irrigated via nearby

artesian wells on a weekly basis throughout the year at a rate that ranged from every 2-7 days depending on the weather conditions.

The climatic conditions on the island of Amorgos can be described as typical Mediterranean conditions where December - January are typically the coldest months and June – August are typically the hottest months of the area on a yearly basis. Similarly, June - September are the driest months of the year in terms precipitation, whereas December - March are usually the wettest months of the year. The annual mean temperature fluctuation among the years of the current study did not diverge significantly from the 10-year average and ranged from 13.2-15.5 in December and January to 25.5-27.3 on July and August. The precipitation fluctuation showed noted variation with more than double rainfall volumes being recorded for the months of January, February, April, and November 2019 compared to the same months in 2018 and the region's 10-year average

Table 2. Records of average/maximum/minimum/10 years average temperatures & precipitation for a 3-years period at experimental cultivation plots (www.meteo.gr, accessed on 12 April 2023)

Average temperatures (°C)												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2018	13.8	15.2	16.8	18.7	22.4	25.0	27.3	26.2	24.5	20.5	17.9	13.8
2019	13.2	13.7	15.2	17.0	20.4	24.8	25.6	25.5	23.1	21.8	20.5	15.5
AVG 10 years	13.3	14.2	14.8	17.3	20.7	24.1	25.5	25.9	24.2	20.7	18.1	14.9
Maximum temperatures (°C)												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2018	15.6	16.9	18.8	21.9	25.4	28.1	30.0	28.3	26.6	22.1	19.3	15.6
2019	15.1	15.4	17.5	19.0	23.6	27.3	28.5	28.1	25.2	23.7	22.2	17.3
AVG 10 years	15.1	16.0	16.7	19.9	23.8	26.9	28.2	28.5	26.6	22.7	19.8	16.6
Minimum temperatures (°C)												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2018	11.6	12.9	14.7	15.8	20.2	22.5	25.2	24.7	22.6	18.9	16.5	11.7
2019	10.3	11.5	12.4	14.5	17.1	22.8	23.1	23.4	21.2	20.0	18.2	13.3
AVG 10 years	11.0	11.9	12.4	14.6	18.0	21.7	23.3	23.9	22.2	18.8	16.1	12.9
Precipitation (mm)												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2018	46.6	80.8	6.0	0.0	0.0	2.8	0.0	0.0	6.2	7.4	58.3	113.2
2019	295.8	256.8	78.8	82.6	4.2	0.0	0.0	0.0	0.0	6.2	84.8	106.0
AVG 10 years	110.5	69.4	54.7	16.2	6.6	5.5	0.0	0.0	4.4	12.1	35.3	71.6

Basic principles of organic horticulture (Ferguson, 2004) were followed in the experimental cultivation herein applying biological methods for pests and disease management when needed (biological fungal-based fungicides and *Bacillus thuringiensis* (Bt) based pesticides were used) coupled with manual weeding between the planting rows.

Flower heads from two separate *H. amorginum* plants that did not receive any external fertilization or other experimental treatment were collected and mixed into a composite sample at the three consecutive harvest stages from each experimental field for two consecutive years (2018 and 2019). The harvest stages were conducted consecutively and approximately 15 days apart from each other based on the phenological progression of flowering and were divided into: (I) an early flowering stage (stage A) where approx. 50% of the flowers opened with newly formed bracts that were of white-pinkish colour, (II) the onset of full bloom (stage B) with 50-80% of the flowers opened with mainly white bracts, and (III) late bloom and onset of post-anthesis

stage (stage C) with the reproductive parts of the flowers being fully developed and anther dehiscence and pollination were commenced (Figure 1).



Figure 1. Harvested Greek *Helichrysum amorginum* inflorescences at the three harvest stages designated in the current study
From right to left: Harvest stage A (early flowering), harvest stage B (onset of full bloom), and harvest stage C (late bloom).

Assessment of the total phenolic content of the floral organs

Extraction process

Capitula of the flowering stems were initially grinded with a household mill into fine homogeneous powders and extracted with ultrasound assisted the extraction (UAE). An Elma S 100H (Elmasonic, Richmond, VA, USA) instrument was used with methanol (100%) as extraction solvent for 15 min at room temperature and ratio plant per solvent 1/10 (w/v). The extraction procedure was repeated twice for each sample. Methanol was evaporated to dryness under reduced pressure using a rotary evaporator (Buchi Rotavapor R-200) at 40 °C.

Determination of the total phenolic content

The Total Phenolic Content (TPC) of the produced extracts was determined by employing the Folin–Ciocalteu method, according to a reported methodology, with minor modifications (Singleton *et al.*, 1999). Folin–Ciocalteu solution was prepared with 10% dilution in distilled water and 7.5% sodium carbonate. In 96-well plates, 25 μL of each extract was dissolved in DMSO, 125 μL Folin–Ciocalteu solution and 100 μL Na_2CO_3 solution were mixed. The plates were incubated for 30 min in the dark. A microplate reader (Infinite 200 PRO, TECAN, Mannedorf, Switzerland) was used for the measurement of sample absorbance at 765 nm. The results were expressed as milligrams of gallic acid equivalent (GAE) per gram of extract, based on the reference gallic acid calibration curve (at a linearity range of 1-10 $\mu\text{g mL}^{-1}$ with the equation $y = 0.083x + 0.046$, $R^2 = 0.998$).

Experimental design and statistical analysis

The experimental design entailed the harvest stage factor with three levels (harvest stages A, B, and C), the cultivation location factor with two levels (experimental fields in Aegiali and Katapola) and the cultivation year (or plant age) factor with two levels (year of cultivation 2018 with three-year old plants and 2019 with

four-year old plants). As such, the sampling scheme that is described above included six treatment samples for each year from both experimental fields resulting in 12 composite samples in total that were analysed for TPC content in triplicate ($n=3$). The resulting TPC data were analysed via analysis of variance (ANOVA) to determine factor effects. Following the ANOVA results, further pooled analysis was conducted to determine the effects of the cultivation year and location factors irrespective of harvest stage (pooled harvest stage data) on flower TPC, and similarly to determine the effects of harvest stage under either the different cultivation years (pooled cultivation location data) or the different cultivation locations (pooled cultivation year data) using Tukey's HSD post-hoc test ($p<0,05$) for mean comparison. All analyses were conducted using the SigmaPlot 12 software (Systat Software Inc., San Jose, CA, USA), and graphs were drawn using Microsoft Excel 365 (Microsoft corp., Washington, USA).

Results

Effects of the cultivation year, location, and harvest stage on the total phenolic content of methanolic extracts of the floral organs in cultivated Helichrysum amorginum

The statistical analysis of the effects of the cultivation year, cultivation location and flower harvest stage on flower TPC showed that all three factors significantly affected the TPC of the floral organs of Greek *H. amorginum* herein (Table 3). The cultivation parameters of year and location and the interaction term showed a significant effect on flower TPC (Table 3). The harvest stage presented a significant interaction with cultivation year and location and, thus incurred differentiated effects on flower TPC in different years and cultivation locations (Table 3).

Table 3. Analysis of variance (ANOVA) table showing the significance of the effects of individual factors (cultivation year, cultivation location, and flower harvest stage) and their interactions on flower TPC (mg GAE g⁻¹ extract) of cultivated *Helichrysum amorginum*

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-statistic (sig.)
Replicates	2	2.93	1.46	0,199 NS
Year	1	2433.4	2433.4	331.2 ***
Location	1	380.6	380.6	51.8 ***
Harvest stage	2	2130.2	1065.1	145.0 ***
Year × Location	1	29.4	29.4	4.0 *
Year × Harvest stage	2	894.6	447.3	60.9 ***
Location × Harvest stage	2	287.6	143.8	19.6 ***
Year × Location × Harvest stage	2	227.3	113.6	15.5 ***
Residual	22	161.6	7.35	

NS: non-significant effect, *: statistically significant effect at $p<0.05$, ***: statistically effect at $p<0.001$

According to the pooled analysis conducted following the ANOVA results, higher flower TPC content was generally observed in the second evaluation year (2019) which was the third year of the cultivation of *H. amorginum* when the plants were four-year old (Figures 2 and 3). When the data among the two cultivation fields were pooled, the early flowering harvest stage (A) showed the highest flower TPC content in 2018 (145.1 mg GAE g⁻¹ extract on average), whereas in 2019 the highest flower TPC content was measured in harvest stage B which was on the onset of full bloom (157.6 mg GAE g⁻¹ extract on average) (Figure 2). On the other hand, flower TPC content was increased in 2019 in both experimental fields, with the experimental field of Aegiali presenting higher flower TPC content compared with Katapola in both evaluation years (151.7 and 146.3 mg GAE g⁻¹ extract in Aegiali and Katapola respectively in 2019) irrespective of flower harvest stage (Figure 3).

Similarly, when the cultivation year data were pooled, the experimental field of Aegiali showed higher flower TPC content in harvest stages A and B with a prevalence of the early flowering stage A, whereas the late bloom stage C showed the lowest flower TPC content irrespective of cultivation location (Figure 4). The pooled flower TPC content of both evaluation years was found on average at 156, 145.7, and 130.3 mg GAE g⁻¹ extract in harvest stages A, B, and C respectively in Aegiali and 142.6, 139, and 131 mg GAE g⁻¹ extract in harvest stages A, B, and C respectively in Katapola (Figure 4).

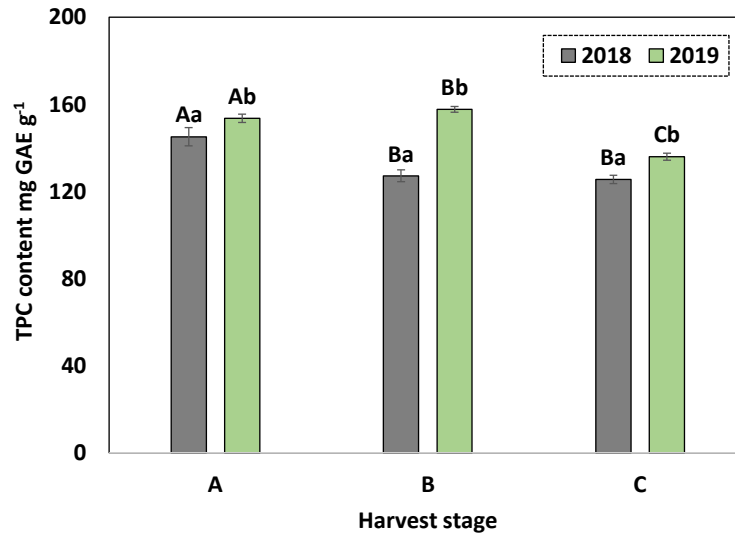


Figure 2. Effect of the cultivation year (2018 with three-year old plants and 2019 with four-year old plants) for the three flower harvest stages (A, B, and C) on total phenolic content (TPC, mg GAE g⁻¹) of *H. amorginum* flowers for both experimental fields combined. Standard error bars are shown on the graphs Capital letters denote differences among harvest stages for the same year. Lowercase letters denote differences between cultivation years for the same harvest stage (Tukey HSD, $p < 0.05$).

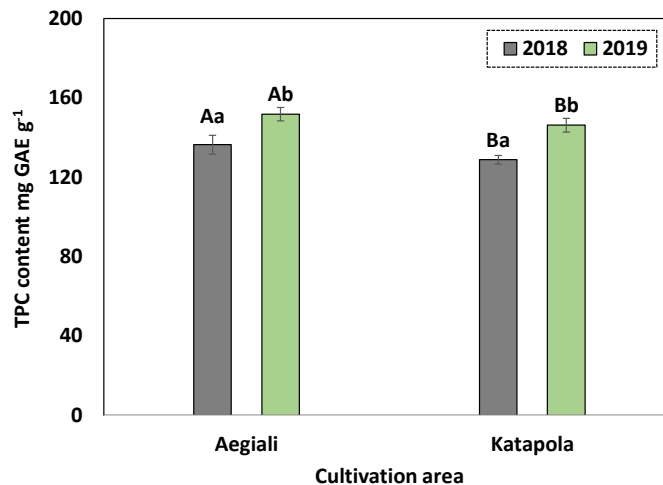


Figure 3. Effect of the cultivation year (2018 with three-year old plants and 2019 with four-year old plants) for the two experimental fields (Aegiali and Katapola) on total phenolic content (TPC, mg GAE g⁻¹) of *H. amorginum* flowers for all three harvest stages combined. Standard error bars are shown on the graphs Capital letters denote differences among experimental field for the same year. Lowercase letters denote differences between cultivation years for the same experimental field (Tukey HSD, $p < 0.05$).

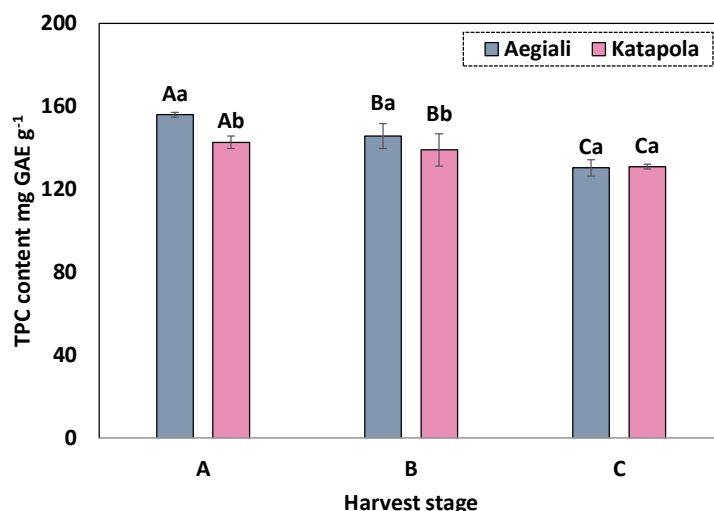


Figure 4. Effect of the cultivation field/area (Aegiali and Katapola) for the three flower harvest stages (A, B, and C) on total phenolic content (TPC, mg GAE g⁻¹) of *H. amorginum* flowers for the two cultivation years combined

Standard error bars are shown on the graphs. Capital letters denote differences among harvest stages for the same area. Lowercase letters denote differences between cultivation fields/areas for the same harvest stage (Tukey HSD, $p < 0,05$).

Discussion

The current study evaluates for the first time the polyphenol potential of the floral organs of *Helichrysum amorginum*, building upon the basis of its sustainable exploitation and filling targeted research gaps. The preservation of economically potent neglected and underutilized species like the range restricted *H. amorginum* under the current climatic and economic status quo facilitates the establishment of novel exploitation options of the available germplasm resources in both a local and a global context (Grigoriadou *et al.*, 2020; Sharrock, 2020; Krigas *et al.*, 2022). The exploitation of local or endemic plants for their phytochemical profile has been the purpose of widespread research efforts over the recent years in biodiversity rich areas (Evergetis and Haroutounian, 2014; Volenzo and Odiyo, 2020; Cogoni *et al.*, 2021). Traditionally, local plants were studied for their pharmaceutical properties (Balunas and Kinghorn, 2005), with many of the applied studies being triggered from long-term ethnobotanical use of target taxa (Farnsworth, 2007; Porras *et al.*, 2020).

The results herein revealed the patterns of total phenolic compounds concentration at different developmental stages of the flower tissue of *H. amorginum* under cultivation conditions at two different altitudinal sites of the same island. The available literature on the biochemical properties of *H. amorginum* is very scarce including one older study that demonstrated the chemical composition and antimicrobial activity of the essential oil of the flowering tissue of *H. amorginum* revealing 29 chemical constituents representing 85.0% of the total oil and included spathulenol (36.6%), β -pinene (12.5%), α -pinene (4.3%) and myrtenal (5.5%) (Chinou *et al.*, 2004). Similarly, the biochemical profile and antibacterial potential of other *Helichrysum* species occurring in Greece like *Helichrysum stoechas* ssp. *barrelieri* and *H. taenari* have been demonstrated in another older study (Chinou *et al.*, 1997). In more general terms the polyphenolic compounds in the flowering tissue of other species of Asteraceae family have been studied for their pharmaceutical properties in traditionally used herbaceous species like *Hypericum perforatum* (St John's wort) where the plant's biochemical potency has

long been demonstrated facilitating its systematized use and successful utilization (Barnes *et al.*, 2001; Makarova *et al.*, 2021; Kwiecień *et al.*, 2021).

In this study the plant material originated from the experimental field with the higher altitude (Aegiali) performed increased values of total polyphenolic content. Similarly, in another insular region of Mediterranean two different varieties of *Vitis vinifera*, 'Ruby Cabernet' and 'Listán negro', cultivated at two different altitudes on the Tenerife 's Island were measured with statistically significant differences regarding the TPC. More specifically, the grapes of both varieties originated from lower vineyards showed a decrease in total phenolics. The TPC of 'Ruby Cabernet' variety was 1.422 g GAE/kg from grapes of the higher altitude and 1.109 g GAE/kg from the lower vineyard while the values for 'Listán negro' variety was 0.933 g GAE/kg and 0.827 g GAE/kg respectively (Miguel-Tabares *et al.*, 2002). Although the altitude of cultivation seems to affect the secondary metabolism of the plants there is no a clear pattern for all the medicinal plants. In a study on eight (8) cultivated species of medicinal plants in Cyprus at two environmental conditions characterized of different altitudes the results suggested that only *Artemisia abrotanum* L and *Mentha spicata* L. showed statistically higher TPC from the mountainous fields rather than the plain fields. On the contrary, extracts from the species *Pelargonium roseum* L., *Laurus nobilis* L. and *Salvia officinalis* L cultivated in mountainous plots were counted with statistically decreased TPC compared with the corresponding plain cultivations. The altitude had no effect on the other studied species: *Rosmarinus officinalis* L, *Lavandula angustifolia* L and *Aloysia triphylla* L. (Chrysargyris *et al.*, 2000).

In *H. amorginum* flowers herein, the early stages of flowering presented higher polyphenol content with 156 mg GAE g⁻¹ which decreased as the flowers progressed towards reproductive maturity and fertilization. Basic literature suggests that polyphenol build up in the reproductive tissue is involved in the protection against pests or diseases coupled with the evolutionary pathways of floral attractiveness for pollination success (Glover, 2007), with recent studies including the environmental adaptation factor to be involved in species molecular diversity (Defosse *et al.*, 2021). The latter point may also relate to the differences in flower TPC content among the two cultivation fields observed herein. The total phenolic content of the reproductive tissue has been determined in a diverse array of plant species with utilizable flowers (Li *et al.*, 2014). In the case of *Helichrysum* spp., the chemical composition of the essential oil of the flowering tissue has been shown in *Helichrysum italicum*, *H. orientale*, *H. heldreichii* and *H. doerfleri* all occurring in Greece without providing data about the optimum stage of flowering period related to the TPC (Roussis *et al.*, 2000). A study on *Phyllirea latifolia*, *Cistus incanus* and *Pistacia lentiscus* regarding their diurnal and monthly TPC variations revealed that among May, July and October the total phenolics were positively affected in July and among daily harvesting hours 8.00, 14.00 and 18.00 the middays collections of the plant material demonstrated higher values polyphenols (Gori *et al.*, 2000)

A further relevant study in widespread plant species have shown that the total phenolic content of *Opuntia ficus-indica* and *Opuntia stricta* (prickly pear) flowers being the highest at late flowering or post-anthesis stages (Ammar *et al.*, 2012). From the current results in *H. amorginum* the precise mechanism of the purpose of polyphenol buildup during early floral development and its decrease at the later floral developmental stages remains ambiguous. Nevertheless, the current results provide significant information on the timing of flower harvest for maximum phenolic compounds yield.

In agronomical terms, the timing of flower harvest can be considered as a very important factor in the development of a successful cultivation protocol that is biochemically orientated. Since the flowering tissue is considered the desired product herein, the phytochemical profile, and consequently the utilization value of the final product can vary among different harvest stages. This has already been demonstrated in utilization studies of reproductive plant parts in cultivated germplasm of plants like tea, St John's wort, and globe artichoke (Pandino *et al.*, 2013; Makarova *et al.*, 2021; Paiva *et al.*, 2021).

A further indicative similar example of sustainable utilization of native plants based on their phytochemical properties can be found among shrub species with *Sambucus nigra* being successfully utilized for its antimicrobial and antiviral properties of flowers and fruits (Kaltsa *et al.*, 2020; Corrado *et al.*, 2023; Papagrigoriou *et al.*, 2023). Considering the case of the Greek flora, the biochemical potential has recently been targeted and successfully highlighted in numerous herbaceous native (or endemic) plant species of Greece, including *Campanula pelviformis*, *Thymus holosericeus*, *Achillea grandifolia*, and *Sideritis siphylea* (Anestis *et al.*, 2023; Mašković *et al.*, 2023; Tsiftoglou *et al.*, 2023b; Tsiftoglou *et al.*, 2023c).

Conclusions

Considering all the above, the framework for the sustainable agronomic exploitation of *H. amorginum* for the pharmaceutical/cosmetic sectors can be enhanced via the current results in terms of the timing of inflorescence harvest and selection of the plot cultivation in the Amorgos for achieving a polyphenol-rich extract. The experimental cultivation trials of *H. amorginum* are currently underway assessing further agronomical traits of plant growth and physiology which are expected to further refine a valid pilot cultivation protocol for the sustainable exploitation of this phyto-genetic resource.

Authors' Contributions

Conceptualization GS, PP, SAL; Data curation GS, EV; Formal analysis GS; Funding acquisition GS; Investigation IK, EK; Methodology GS, AK; Supervision SAL, PP; Validation GS, EK; Visualization PP; Writing - original draft GS, EK; Writing - review and editing GS, PP, SAL. Please note: All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

The authors gratefully acknowledge Korres Natural Products S.A for its broad support to the implementation of this work. The authors thank Social Cooperative Enterprise Amorgos Collaboration (Amorgos Botanical Park) for providing the experimental fields on the island of Amorgos.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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