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# Effects of water limitation on the production of key secondary metabolites with medicinal properties in *Plantago lanceolata* and *Tanacetum parthenium*

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Short title: Impact of water availability on plants with medicinal properties

Keywords: drought, medicinal plants, parthenolide, verbascoside,

## 1 Abstract

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2 Plant secondary metabolites play vital roles in growth, defence, and human pharmacological applications, with medicinal plants historically used to treat diseases. However, climate- change- induced drought may threaten medicinal plant growth and metabolite production. This study examines drought effects on two medicinal plants common in the UK: *Plantago lanceolata* and *Tanacetum parthenium*. Under glasshouse conditions, we compared growth, chlorophyll content, and photosystem II efficiency (Fv/Fm) in well-watered vs. low-watred plants. High-performance liquid chromatography (HPLC) measured the concentraton of parthenolide in *T. parthenium* and verbascoside in *P. lanceolata* leaves, with both compounds being the two most abundant with medicinal relevance. Results showed significantly reduced growth for both species under drought, though photosynthetic performance remained unaffected. Parthenolide content in *T. parthenium* decreased with low water, while verbascoside in *P. lanceolata* showed no significant change. This study highlights drought's impact on medicinal plant growth and metabolite production, emphasizing the need for further research on environmental factors affecting secondary metabolites under climate change.

## 16 1 Introduction

17 Medicinal plants have long been an important component of health care in human populations, with written records of the preparation of drugs from plants found on clay slabs dating back to over 5000 years ago (Hassan 2015) and archaeological remains dating back over 70,000 years (Wadley et al. 2011). Of the approximately 308,000 vascular plant species which have been described and accepted globally to date (Christenhusz and Byng 2016), over 50,000 are known to be used (historically or currently or both) for medicinal purposes (Chen et al. 2016). Plants produce a diverse array of secondary metabolites that play different roles in plant growth and development, including regulating flowering and defence against

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5 25 pathogens and herbivores (Teoh 2016; Erb and Kliebenstein 2020). These secondary metabolites  
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7 26 have a variety of applications for humans, for example, as food additives, agrochemicals,  
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9 27 biopesticides (Jamwal et al. 2018), and pharmaceuticals (Tungmunnithum et al. 2018). The value  
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11 28 of plants as sources of medicine has been supported historically through the production of  
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13 29 important plant-derived drugs such as artemisinin found in *Artemisia annua* L and used in the  
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15 30 treatment of malaria (Qamar et al. 2024), paclitaxel extracted from *Taxus brevifolia* and widely  
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17 31 used in chemotherapy (Weaver 2014), or galanthamine obtained from the family Amaryllidaceae  
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19 32 and used in the treatment of Alzheimer's disease (Sramk et al. 2000). The contribution of plants to  
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21 33 the treatment and prevention of disease is still enormous today, with 11% of the 252 drugs the  
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23 34 World Health Organization (WHO) considers 'essential' and 'basic' being exclusively derived from  
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25 35 plants (Veeresham 2012). It is generally agreed

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27 36 that plants still hold great potential for the discovery and development of new drugs (Miller  
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29 37 2011; Sezer et al. 2024), and despite sustained research activity in the field, further work is  
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31 38 required to improve our understanding of the impact of climate change on medicinal plants  
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33 39 (Applequist et al. 2020).

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35 40 Climate plays a primary role in defining the distribution of species along the latitudinal and  
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37 41 elevational gradients of our planet (Stevens 1989; Stevens 1992; Rahbek 2005; Lenoir et al.  
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39 42 2020). Changes in climatic conditions affect plant species, which need to move or adapt to the  
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41 43 novel growth conditions to avoid perishing (Araújo and Pearson 2005; Parmesan 2006).  
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43 44 Climate change in ecosystems, therefore, may lead to changes in plant species distribution and  
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45 45 composition, including species invasions and extinctions, but may also affect species  
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47 46 physiology, phenology, and interaction with other organisms and their environment (Bellard et  
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49 47 al. 2012; Fontúrbel et al. 2021; Kumari et al. 2024). Overall, climate change is considered a  
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51 48 major threat to biodiversity and ecosystem functioning. In the context of medicinal plants,  
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53 49 climate change may not only affect species distribution (and survival) but may also affect

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50 plant growth and the synthesis and production of plant secondary metabolites with medicinal  
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plant growth and the synthesis and production of plant secondary metabolites with medicinal properties (Sun et al. 2023; Alum 2024). Climate change can affect plant morphology, including changes in the root-to-shoot ratio (Eziz et al. 2017), specific root length (Nicotra et al. 2010), leaf area, foliage distribution (Gratani 2014), and the overall plant biomass (Misra and Srivastava 2000), which can also affect the quantity of desirable metabolites harvested from plants (Applequist et al. 2020). Abiotic stresses associated with climate change, such as changes in temperature and water availability, can affect the secondary metabolite content of medicinal plants (Gairola et al. 2010; Applequist et al. 2020; Sun et al. 2023; Alum 2024), which in turn may affect the medicinal properties and safety in therapeutic use—e.g., posing a health risk if stress results in an unexpected increase in the concentration of secondary metabolites that can become toxic at higher concentrations (Applequist et al. 2020). Prior

work has found that drought tends to increase the content of secondary metabolites in plants, particularly of those involved in drought protection response (Yang et al. 2018), and also tends to increase overall metabolite content specifically in medicinal plants (Selmar and Kleinwächter 2013). However, the effect of environmental stress on plant secondary metabolites appears variable and has been found to depend on the type of stress and the identity of the chemicals considered (Yang et al. 2018). For example, baicalin content, a flavonoid with therapeutic uses extracted from *Scutellaria baicalensis* Georgi was found to increase under mild to moderate drought stress conditions but to decrease under severe stress conditions (Cheng et al. 2018). Similarly, glycyrrhizin content, a triterpenoid saponin with therapeutic properties extracted from *Glycyrrhiza glabra* L., decreases under conditions of excessive drought compared to mild and moderate drought (Hosseini et al. 2018). Moreover, in *Mentha piperita* L. and *Catharanthus roseus* (L.) G. Don the concentration of some secondary metabolites such as total phenols, flavonoids and saponins decrease whilst the concentration of others such as tannins, terpenoids, and alkaloids increase when exposed to

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5 75 the same level of drought stress (Alhathloul et al. 2019). These studies highlight the varying  
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7 76 responses of secondary metabolites in medicinal plants when exposed to varying levels of  
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9 77 drought stress. Further research is, therefore, required in order to extend our knowledge on the  
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11 78 effects of climate change, in particular water deprivation, to other medicinal plant species and  
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13 79 their secondary metabolites.

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16 80 Here, we aimed to investigate the effects of water availability on the growth and performance of  
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18 81 two herbs with known medicinal properties, i.e., *Tanacetum parthenium* L. (Feverfew) and  
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20 82 *Plantago lanceolata* L. (Ribwort Plantain). We further quantified changes in two key secondary  
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22 83 metabolites with known medicinal properties, parthenolide in *T. parthenium* and verbascoside in  
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24 84 *P. lanceolata*, in response to the different water regimes. Verbascoside and  
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26 85 parthenolide were chosen as they are the most abundant secondary metabolites with known  
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28 86 therapeutical properties in our study species (Bahadori et al. 2020; Kashkooe et al. 2024).

## 29 30 31 87 2 Materials and Methods

### 32 33 88 2.1 Study Species

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35 89 Two species with medicinal uses that can be found widespread across Northern Europe were  
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37 90 used in this study: *Plantago lanceolata* (Ribwort Plantain) and *Tanacetum parthenium* (Feverfew).  
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39 91 Both species have well-established therapeutic uses (Pareek et al. 2011; Abate et al. 2022).

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43 93 *T. parthenium* is a member of the Asteraceae (Compositae) family. Its common name— Feverfew—  
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45 94 reflects its long-term use as a medicinal plant, as it stems from ‘febrifuge’, meaning a medicine to  
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47 95 ‘reduce fever’ (Kashkooe et al. 2024). Parthenolide is the primary active phytochemical found in *T.*  
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49 96 *parthenium* and is one of the many sesquiterpene lactones this species is known to produce  
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51 97 (Kashkooe et al. 2024). Among other applications, this metabolite has been studied with regards  
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53 98 to its use for the treatment of acute myelogenous

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5 99 leukemia (Guzman et al. 2005), reducing atherosclerotic lesions (López-Franco et al. 2006), and  
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7 100 its anti-inflammatory properties (Kwok et al. 2001). *T. parthenium* is mainly used in the  
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9 101 treatment and prevention of migraines (Kashkooe et al. 2024).

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11 102 *P. lanceolata* belongs to the family Plantaginaceae and has been historically used as a food  
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13 103 source and traditional medicine (Bahadori et al. 2020). Its medicinal purposes include the  
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15 104 treatment of wounds and inflammation (Abate et al. 2022)) and as an antimicrobial agent  
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17 105 (Ferrazzano et al. 2015). The active phytochemicals present in *P. lanceolata* include  
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19 106 verbascoside—a phenylethanoid glycoside (Alipieva et al. 2014)—and iridoid glycosides  
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21 107 aucubin and catalpol (Abate et al. 2022). Studies have investigated several applications of  
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23 108 verbascoside, including in the treatment of oxidative-stress-related neurodegenerative  
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25 109 diseases  
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27 110 (Esposito et al. 2010), inflammatory diseases (Pesce et al. 2015), and *Staphylococcus aureus*  
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29 111 infections (Yang et al. 2021). Aucubin and catalpol have also been found to improve the  
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31 112 prognosis of Parkinson’s disease and Alzheimer’s disease (Yang et al. 2022) offer  
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33 113 neuroprotection in primary diabetic encephalopathy (Xue et al. 2008), and alleviate diabetic  
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35 114 complications (Bai et al. 2019).

## 36 114 2.2 Germination and experimental set up

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38 115 *P. lanceolata* and *T. parthenium* plants were grown from seeds (King’s seeds, Colchester, UK)  
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40 116 under glasshouse conditions with natural lighting and temperature ranging from 16 °C – 29  
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42 117 °C, in the Talybont Greenhouses plant growth facilities, as part of our Plant Growth  
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44 118 Technology Hub (School of Biosciences, Cardiff University). Seeds were sown on the 21st  
45  
46 119 April 2022 into germination trays filled with a mix of John Innes n° 2, Brett™ horticultural  
47  
48 120 sand, and perlite (5:5:1). Three weeks after sowing, on the 13th May 2022, 60 seedlings per  
49  
50 121 species were transplanted into individual 8 cm round pots filled with the same mix of  
51  
52 122 compost, sand, and perlite and kept in the same glasshouse. Soil pH (measured with a pH

123 tester HI98103, Hanna Instruments) was  $7.6 (\pm 0.1)$ , which is within the pH range adequate  
124 for both species (Fitter and Peat 1994).

125 Following transplanting, these 60 seedlings per species were allowed to establish and on the  
126 6th of June 2022 were then randomly allocated to two different levels of water availability.  
127 Well-watered (WW) plants ( $n = 30$  for *P. lanceolata* and  $n = 30$  for *T. parthenium*) were watered  
128 to field capacity every other day. The soil of plants in the low-watered (LW) treatment ( $n = 30$   
129 for *P. lanceolata* and  $n = 30$  for *T. parthenium*) was allowed to dry between watering, with  
130 plants showing signs of wilting before rewatering to field capacity (at 5-6 days of water  
131 deprivation). LW plants showed signs of wilting that fitted the description of stage 2 of  
132 observable wilt, with visible wilted leaves and dropping stems, as described by Jackson and  
133 Fonteno (2017). To monitor soil water content and determine the extent of water restriction, we  
134 used the gravimetric method by weighing all LW pots and a subsample of HW pots before and after  
135 watering at field capacity. The difference in pot mass before and after watering reflects the change  
136 in soil water content, with LW pots showing an average decrease of 19.7% in mass prior to re-  
137 watering at field capacity and HW pots showing an average decrease of 8.2% in mass prior to re-  
138 watering at field capacity. This indicates that LW pots experienced a water restriction of  
139 approximately 58% compared to HW plants. Plants were harvested on the 28th of June 2022, 22  
140 days after water treatments started (46 days after transplantation), during which the LW plants  
141 underwent wilting four times.

142

### 143 2.3 Chlorophyll content and photosynthetic performance

144 Chlorophyll content was estimated prior to harvest on the 28th June 2022, by measuring the  
145 first to third youngest fully expanded leaves from the shoot apex of each plant ( $n = 60$  (LW=30,  
146 WW =30) for *P. lanceolata* and  $n = 30$  (LW=30, WW =30) for *T. parthenium*)



147 using a handheld chlorophyll meter (SPAD-502, Minolta Camera Co., Osaka, Japan), which  
148 calculates an index based on absorbance at 650 and 940 nm. SPAD values are positively  
149 correlated with the chlorophyll content of leaves (Richardson et al. 2002).

150 The maximum quantum yield of PSII,  $F_v/F_m$ , was also measured on the 28th June, 2022, by  
151 measuring the two youngest fully expanded leaves from the shoot apex of each plant ( $n = 60$   
152 (LW=30, WW =30) for *P. lanceolata* and  $n = 60$  (LW=30, WW =30) for *T. parthenium*) using a  
153 MINI-PAM II fluorometer (Walz, Germany), which measures chlorophyll fluorescence. DLC-8  
154 dark leaf clips were attached to these leaves to ensure a 20-minut dark- adaptation period,  
155 which is required for  $F_v/F_m$  measurements, before taking readings.  $F_v/F_m$  is a non-invasive  
156 measurement of plant photosynthetic performance, with alues lower than  
157 0.7 indicating stress and/or photoinhibition (Maxwell and Johnson 2000).

#### 158 2.4 Plant growth and metabolite analysis

159 On the 28th June 2022 plants were harvested and their biomass was separated into aboveground  
160 and belowground parts. Roots wee separated from the soil by washing through a 2 mm<sup>2</sup> sieve  
161 from which roots were rtrieved. Above- and belowground dry mass were obtained after drying in  
162 an oven at 50°C for 72 hours (to constant mass) followed by weighing using an analytical balance  
163 (ME204, Mettler-Toledo, Switzerland, accuracy 0.0001 g).

164 Extraction of parthenolide and verbascoside from the aboveground dry mass was adapted  
165 from (Hakiman and Maziah 2009). The aboveground dry mass of each plant ( $n = 60$  (LW=30,  
166 WW =30) fr *P. lanceolata* and  $n = 60$  (LW=30, WW =30) for *T. parthenium*) was ground to  
167 a fine powder, and a sample of 1g was mixed with 10 mL of methanol (99.9%) for 30 min,  
168 agitating it every 10 min. Extracts were then filtered using Whatman No 2 filter paper, and the  
169 methanol was evaporated under a flow of nitrogen until 1 mL of extract remained. Extracts  
170 were then centrifuged at 13,000 rpm for 5 min, and the supernatant resuspended in methanol

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5 171 (99.9 %) in a ratio of 9:1 (methanol: supernatant) prior to analysis using high-performance  
6  
7 172 liquid chromatography (HPLC). HPLC analyses were carried out using a Waters®  
8  
9 173 Autopurification™ HPLC system using standard protocol and conditions, as described in  
10  
11 174 Webster et al. (2020), with an injection volume of 20 µL. A standard solution of parthenolide  
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13 175 (10 mg/ml concentration) was prepared by dissolving 3.6 mg of parthenolide standard (CAS  
14  
15 176 20554-84-1 – Calbiochem, ≥97% HPLC, Sigma-Aldrich) in 360 µL of HPLC-grade methanol  
16  
17 177 (99.9%, Thermo Fisher Scientific) (as described in Mahood et al., 2022). A standard solution  
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19 178 of verbascoside was prepared in the same manner using verbascoside standard (CAS 61276-  
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21 179 17-3, 99.21%, APExBIO). The mobile phases consisted of water with 0.1% formic acid  
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2 180 (solvent A) and acetonitrile with 0.1% formic acid (solvent B). A solvent gradient was used to  
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3 181 achieve separation, beginning with 95% of solvent A and 5% of solvent B and ending with  
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2 182 5% of solvent A and 95% of solvent B after 15 minutes. The peak retention time for  
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2 183 parthenolide was between 6.68 and 6.74 min. For verbascoside, the peak retention time was  
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2 184 between 4.28 and 4.29 min. Parthenolide and verbascoside peak heights were found using  
7  
2 185 MassLynx V4.1 and V4.2 software (Waters®) from chromatograms filtered by wavelengths  
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2 186 of 214 nm and 330 nm, respectively, and peak areas were calculated.

## 31 187 2.5 Data analysis

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3 188 All statistical testing was carried out using R version 4.1.1. (R Core Team 2022). The effects of  
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3 189 watering treatment on aboveground, belowground, and total dry mass, root:shoot ratio,  
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3 190 chlorophyll content, Fv/Fm, and parthenolide and verbascoside content were determined by  
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3 191 means of a ne-way analysis of variance (ANOVA), using the 'lm' function in R with water  
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3 192 availability (WW, LW) as fixed factor, separately for each plant species. P-values were obtained  
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3 193 by calling the Anova function in the 'car' package, using sum of squares type 'III'. Models were  
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3 194 assessed visually for assumption of normality and homoscedasticity, and variables were  
9  
4 195 transformed to meet model assumptions. Chlorophyll content and root:shoot

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5 196 ratio of *T. parthenium* were transformed to the power of two. Aboveground dry mass and  
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7 197 parthenolide content of *T. parthenium* and the belowground dry mass, root:shoot ratio, and  
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9 198 verbascoside content of *P. lanceolata* were log<sub>10</sub>-transformed. The chlorophyll content of *P.*  
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11 199 *lanceolata* was square root-transformed, and the reciprocal of the square root  
12  
13 200 transformation was applied to the aboveground dry mass of *P. lanceolata*.

## 16 201 3 Results

### 19 202 3.1 *Tanacetum parthenium*

21 203 The aboveground, belowground and total dry mass of *T. parthenium* plants were significantly  
22 204 reduced in response to water limitation (Figures 1a, b, and c, respectively). LW plants  
23 205 accumulated, on average, 38.7% less total dry mass than control plants (Fig. 1c); however,  
24 206 biomass allocation was not significantly affected by water availability as the root:shoot ratio  
25 207 was similar in WW and LW plants (Figure 1d). Similarly, the chlorophyll content and the  
26 208 maximum quantum yield of PSII (Fv/Fm) were also not significantly affected by the water  
27 209 availability (Figures 2a and 2c, respectively). Fv/Fm was on average 0.835, indicating that the  
28 210 plants were not affected by water limitation.

31 211 At the time of harvest, LW plants had significantly lower parthenolide content than WW  
32 212 plants (Figure 3a).

### 33 213 3.2 *Plantago lanceolata*

34 214 Similarly to *T. parthenium*, *P. lanceolata* plants accumulated less aboveground, belowground and  
35 215 total dry mass under restricted water conditions (Figures 4a, b, and c, respectively), and the  
36 216 root:shoot ratio was not statistically affected by water availability (Figure 4d). However, the  
37 217 chlorophyll content was significantly greater in LW than in WW plants (Figure 2b), and

218 again, no differences were found in the maximum quantum yield of PSII (Fv/Fm) in response  
219 to the changing water availability (Figure 2d).

220 In contrast to the response of parthenolide in *T. parthenium* plants, at the time of harvest, no  
221 differences were found in the verbascoside content (mg/g dry leaf matter) in *P. lanceolata*  
222 plants in response to different water availability (Figure 3b).

223

#### 224 4. Discussion

225 More intense and more abrupt droughts are becoming increasingly common globally as a  
226 result of climate change (Mukherjee et al. 2018). These changes are affecting not only the  
227 survival and distribution of medicinal plants but also the production of their secondary metabolites  
228 (Applequist et al. 2020). In this study, we investigated the growth responses of two commonly  
229 used plants with medicinal properties, *T. parthenium* and *P. lanceolata*, and measured the  
230 accumulation of two compounds with well-established medicinal properties. By reducing water  
231 availability/frequency in our experiment, we expected to see an increase in parthenolide and  
232 verbascoside content in *T. parthenium* and in *P. lanceolata*. Even though our water limitation  
233 treatment reduced plant growth in both species, the production of the secondary metabolites  
234 investigated was only negatively affected in *T. parthenium*, whilst verbascoside content was not  
235 affected in *P. lanceolata*.

236 It is well known that environmental stress affects the quantity and composition of metabolites in  
237 medicinal plants (Zehra et al. 2019), and an increase in the overall content of secondary  
238 metabolites has been found in response to drought stress in many medicinal plant species  
239 (Selmar and Kleinwächter 2013; Shil and Dewanjee 2022; Ghasemi et al. 2023). However, it  
240 appears that not all secondary metabolites are affected equally in response to drought stress. In  
241 some cases, particularly for active phytochemicals such as alkaloids, tannins, and

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5 242 terpenoids, drought stress increases their content, whilst content is reduced for phenols,  
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7 243 flavonoids, and saponins (Alhaithloul et al. 2019; Shil and Dewanjee 2022). Varying responses can  
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9 244 occur even within the same group of metabolites, for example, within terpenoids it has been found  
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11 245 that drought-treated plants of the same species showed an increase in overall monoterpene  
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13 246 synthesis and decline in total sesquiterpene synthesis (Chandrasekaran et al. 2022).  
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15 247 Sesquiterpene lactones—a subgroup of terpenoids to which parthenolide belongs—have been  
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17 248 found to decrease in other species, such as artimisin found in *Artemisia annua*, in response to  
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19 249 increasing drought stress (Yadav et al. 2014). Previous work on *T. parthenium* has found reduced  
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21 250 levels of parthenolide in plants which have experienced drought stress but an increase in total  
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23 251 phenolic content (Fonseca et al. 2006; Fonseca et al. 2008). Not much is known regarding the  
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25 252 changes in verbascoside—a  
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27 253 phenylethanoid glycoside—in *P. lanceolata* in response to water stress, and the lack of response  
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29 254 here may be in part explained by the resilience of this species to the drought intensity imposed in  
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31 255 our experiments (not severe, as indicated by the physiological parameters investigated), with  
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33 256 some studies suggesting that it is moderately drought-tolerant (Morales et al. 2021; Miszalski et  
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35 257 al. 2023). However, it has also been found that prolonged drought causes contrasting changes in  
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37 258 the levels of different secondary metabolites of *P. lanceolata*, with some being enhanced and  
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39 259 others reduced (Orians et al. 2019).  
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260 Fonseca et al. (2008) point out that trade-offs may occur between the contents of different  
261 metabolites in response to changing environments, with increases in certain metabolites  
262 occurring at the expense of decreasing the content of other metabolites. This highlights the  
263 complexity surrounding the production of secondary metabolites in plants (Li et al. 2020),  
264 which is influenced not only by the plant genetics (Li et al. 2020; Qaderi et al. 2023) but also  
265 by environmental conditions such as water availability, temperature, light, and soil nutrients  
266 (Gobbo-Neto and Lopes 2007) and any changes herein. Furthermore, differences in the

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5 267 intensity and duration of the imposed stress may lead to different responses in the development  
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7 268 and production of secondary metabolites in medicinal plant species (Qaderi et al. 2023).  
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11 270 As expected, the aboveground, belowground, and total dry mass of *P. lanceolata* and *T.*  
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13 271 *parthenium* were significantly reduced in plants that experienced periods of water deficit.  
14  
15 272 However, the root-to-shoot ratios were not affected by the changing water regimes in either of the  
16  
17 273 study species. Increased root:shoot ratio under water stress has been widely reported across  
18  
19 274 many different plant species, and it is considered a drought avoidance strategy whereby growing  
20  
21 275 plants allocate their resources more heavily to the root systems to increase water uptake capacity  
22  
23 276 at the expense of developing aboveground organs (e.g., Makbul et al. 2011;  
24  
25 277 Eziz et al. 2017). However, equally, decreased root:shoot ratios have been reported across  
26  
27 278 different plant species (Kou et al. 2022). It has been suggested that in some cases plants  
28  
29 279 develop a survival strategy when experiencing periods of drought, allowing part of the roots to  
30  
31 280 die rather than investing more on them and reinvesting in new root development only when  
32  
33 281 favourable conditions return (Whitmore and Whalley 2009; Kou et al. 2022). Such variation in  
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35 282 root allocation strategies may occur in response to the intensity and duration of drought  
36  
37 283 stress, along with the identity of th plant species (Xu et al. 2015).

38  
39 284 To fully understand the mechanisms responsible for the changes in secondary metabolites  
40  
41 285 because of drought, a more detailed molecular and genetic study should have been  
42  
43 286 performed, in addition to measuring the primary metabolism (i.e. photosynthetic capacity) of  
44  
45 287 the plants. Moreover, it would be interesting to consider how potential acclimation to water  
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47 288 stress (see Charng et al. 2022 and references there) may have affected secondary compound  
48  
49 289 concentrations in our study species. Unfortunately, this one was not possible in our study.  
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51 290 Nevertheless, we measured chlorophyll content and Fv/Fm as proxies of the physiological  
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53 291 status of the plants. Chlorophyll content was significantly higher in low-watered plants of *P.*

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5 292 *lanceolata* than in those that were well-watered, but no differences were found between the  
6  
7 293 treatments in *T. parthenium*. The effect of drought on chlorophyll content has been found to  
8  
9 294 vary depending on plant genotype and environmental conditions (including frequency and  
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11 295 duration of drought) (Junaid et al. 2023). It is generally accepted that drought stress causes a  
12  
13 296 reduction in plant chlorophyll content, likely due to damage to the thylakoid membranes within  
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15 297 chloroplasts as a result of water deficit (Monteoliva et al. 2021). However, some research has  
16  
17 298 also found that the level of chlorophyll in leaves may increase in water-stressed plants  
18  
19 299 (Ramírez et al. 2014)—similar to our findings for *P. lanceolata*. It has been suggested that  
20  
21 300 different causes, including a loss of turgor or a reduction in leaf growth caused by drought,  
22  
23 301 could lead to higher chlorophyll concentration (Rolando et al. 2015), which would explain the  
24  
25 302 high SPAD readings measured in *P. lanceolata* in response to lack of water. The  
26  
27 303 lack of a significant reduction in chlorophyll content under reduced water availability could  
28  
29 304 partly explain the maintenance of the maximum quantum efficiency of photosystem II (Fv/Fm)  
30  
31 305 – with similar values in low- and well-watered plants –, as any potential damage to the  
32  
33 306 photosynthetic machinery as a result of stress would typically impair the energy capture and  
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35 307 transfer processes within the photosystem, leading to a decline in Fv/Fm values (Murchie and  
36  
37 308 Lawson 2013).

### 309 *Conclusions*

310 Compared to agricultural crops, there is still limited knowledge of the effects of drought and  
311 drought tolerance in medicinal plants (Bistgani et al. 2024). Overall, this study has shown that  
312 the growth of *T. parthenium* and *P. lanceolata* is negatively affected by drought. Moreover, we  
313 found variable effects of changing watering conditions on plant performance and metabolite  
314 production in these two species, with parthenolide content decreasing, whilst verbascoside was  
315 not affected in response to water deficit. Our findings highlight the potential

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4 316 effects that climate change and drought are having on medicinal plant populations and the  
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6 317 production of pharmaceutical compounds.  
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11 319 Conflict of Interest  
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14  
15 320 *The authors declare that the research was conducted in the absence of any commercial or*  
16  
17 321 *financial relationships that could be construed as a potential conflict of interest.*  
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32 330 analysis of metabolites.  
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34 331  
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36 332 Author Contributions  
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38 333 EG & JSV: Data curation, Formal analysis, Investigation, Methodology, Writing – original  
39 334 draft. JSV & BdG: Supervision, Resources, Writing – review & editing. SV: Resources,  
40 335 Writing – review & editing. All authors read and approved the final manuscript before  
41 336 submitting it for publication.  
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338 Data availability

339 The data that support the findings of this study are available at

340 <https://doi.org/10.17035/cardiff.27691971.v1>

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9 589 Figure 1. Dry mass measurements of *T. parthenium*: a) aboveground dry mass, b)  
10 belowground dry mass, c) total dry mass, and d) root-to-shoot ratio. Bars represent mean  
11 values (n = 30), and error bars indicate standard error. One-way ANOVA output is also  
12 shown; *P*-values <0.05 are highlighted in bold.  
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19 594 Figure 2. Chlorophyll content (SPAD units) for *T. parthenium* (a), and for *P. lanceolata* (b)  
20 and Fv/Fm, maximum quantum yield of PSII for *T. parthenium* (c), and for *P. lanceolata* (d).  
21 Bars represent mean values (n=30). Error bars indicate standard error. One-way ANOVA  
22 output is also shown; *P*-values <0.05 are highlighted in bold.  
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27 599 Figure 3. a) Concentration of parthenolide (mg/ g of dried leaves) in *T. parthenium* and b)  
28 concentration of verbascoside (mg/g of dried leaves) in *P. lanceolata* under different water  
29 availability levels. Bars indicate mean values (n=30). Error bars indicate standard error. One-  
30 way ANOVA output is also shown; *P*-values <0.05 are highlighted in bold.  
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33 604 Figure 4. Dry mass measurements of *P. lanceolata*: a) aboveground dry mass, b)  
34 belowground dry mass, c) total dry mass, and d) root-to-shoot ratio. Bars represent mean  
35 values (n = 30), and error bars indicate standard error. One-way ANOVA output is also  
36 shown; *P*-values <0.05 are highlighted in bold.  
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