

# Apigenin Accumulation in *Matricaria chamomilla* and *Petroselinum crispum* Produced in a Vertical Hydroponic System

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**Abstract.** Apigenin, an anticancer secondary metabolite, is produced in selected organs of a few plant taxa, including chamomile (*Matricaria chamomilla*) flowers and parsley (*Petroselinum crispum*) leaves. In this study, two cultivars of chamomile (Bodegold and Zloty Lan) and three cultivars of parsley (Darki, Giant of Italy, and Wega) were included in an indoor vertical farm trial to determine apigenin accumulation and biomass production. Vertical farming was selected for its ability to produce a quality crop with a tightly controlled growing environment. The plants were started from seed in a growth chamber and transferred to the vertical farm when they reached two sets of true leaves. The plants were maintained solely under light-emitting diodes with daily light integrals of 19 and 17 molm<sup>-2</sup>d<sup>-1</sup> for parsley and chamomile, respectively. The photoperiod was set to 16 hours for both species to induce flowering in the chamomile. After 15 weeks, mature parsley leaves and unopened chamomile inflorescences were harvested for analysis. All plants matured quickly during the growing period; however, only 63% of the ‘Zloty Lan’ chamomile plants produced flowers. At harvest, the total dry mass of each plant was also recorded. The Giant of Italy cultivar produced significantly more usable biomass compared with that of any other cultivar of parsley or chamomile, with 49.3 g usable tissue per plant. Apigenin was extracted from lyophilized samples and quantified using high-performance liquid chromatography–ultraviolet detection. The results showed that total apigenin accumulation was significantly higher in the ‘Bodegold’ chamomile compared to any parsley cultivar, with 0.70 mg·g<sup>-1</sup> dried tissue. Additionally, ‘Bodegold’ generated significantly more usable biomass, suggesting that this cultivar shows potential for producing apigenin in a controlled environment.

Plants have long been used for their medicinal purposes, and natural products isolated from many genera still contribute to modern drug development (Jamshidi-Kia et al. 2018). Through domestication, cultivation, and breeding, medicinal plants have an increased growth rate, higher concentration of desirable compounds, and greater biomass of the target tissues (Faehnrich et al. 2021). The medicinal benefits of plants are the result of secondary metabolite production and accumulation of alkaloids, phenolic compounds, and terpenoids (Kabera et al. 2014). For example, the alkaloid colchicine is an antitumor agent

derived from autumn crocus (*Colchicum autumnale*), the furocoumarin khellin is a bronchodilator isolated from toothpick weed (*Ammi visnaga*), and the glycoside acetyldigoxin is a cardiotonic derived from woolly foxglove (*Digitalis lantana*) (Fabricant and Farnsworth 2001).

Phenolic compounds are one of the most prevalent secondary metabolites and have several functions in plants such as contributing to flavor and color and providing defense against biotic and abiotic stressors (Righini et al. 2019; Soto-Vaca et al. 2012). Flavonoids are the largest group of phenolics, with at least

2000 compounds found widely in plants (Soto-Vaca et al. 2012). Apigenin, an important flavonoid, is produced in several fruits, vegetables, and herbs, including celery (*Apium graveolens*) (Yan et al. 2014), chamomile (*Matricaria chamomilla*) (Letchamo 1996), citrus (*Citrus* spp.) (Abad-García et al. 2014), oregano (*Origanum* spp.) (Mueller et al. 2008), and parsley (*Petroselinum crispum*) (Poureini et al. 2022). Apigenin is one of the most cytotoxically active flavones against many cancers, including bladder (Zhu et al. 2013), breast (Pham et al. 2021), cervical (Chen et al. 2022), colorectal (Cheng et al. 2021), and prostate (Costea et al. 2020) cancers. It has also been approved by the Food and Drug Administration for use as combination cancer therapy to reduce resistance to traditional cancer treatments (Nozhat et al. 2021). Other medicinal applications of apigenin include antibacterial (Kim et al. 2020), antifungal (Singh et al. 2014), anti-inflammatory (Wang et al. 2014), and antioxidant properties (Tian et al. 2021). Beyond prescriptive use, the dietary intake of apigenin is considered nutritionally safe, with no signs of toxicity up to 5 g·kg<sup>-1</sup> in mice (Nozhat et al. 2021).

Apigenin accumulation differs across plant tissues. For example, in physiologically mature celery, younger leaves have the lowest concentration of apigenin, with concentrations increasing in the more developed leaves (Yan et al. 2014). Additionally, apigenin accumulation differs in the flowers, leaves, petioles, and seeds of celery, with the greatest concentration occurring in the leaves (Yan et al. 2014). Although apigenin can be produced in different plant organs, it is typically isolated from the leaves of parsley (Poureini et al. 2022) and oregano (*Origanum* spp.) (Mueller et al. 2008). However, in chamomile, apigenin accumulates primarily in the flowers, where the highest concentration occurs in the newly opened buds, with apigenin levels steadily declining as the flower head matures (Letchamo 1996).

Although apigenin can be chemically synthesized, the process requires four-step, multiday synthesis with a low (55%) yield (Wang et al. 2015). Because of its low natural abundance, apigenin is costly, thus making it a desirable target for enhanced production as a biopharmaceutical (Wang et al. 2018). Other research increased apigenin production in *Astragalus trigonus* through agrobacterium-mediated transformation with the *Chalcone isomerase A (chiA)* gene from petunia (*Petunia hybrida*). Production increased from 0.95 mg·g<sup>-1</sup> in the control cells to 19.81 mg·g<sup>-1</sup> in the transformed cells (Elarabi et al. 2021). Although genetic transformation is a viable method for increasing apigenin production, many consumers and markets are resistant to accepting products derived from transgenic food crops (Teferra 2021).

An alternative method of increasing productivity of apigenin is production in controlled environments, namely, indoor vertical farms. Through their unconventional use of space, vertical farms can yield greater biomass per acre compared with that of greenhouses or field production and can optimize

growth through their tightly controlled growth conditions. Although advancements in lighting, irrigation, and automation allow for more efficient production of crops compared to conventional agriculture, energy usage is still a common concern for indoor systems (Folta 2019). Additionally, the high cost of starting a new operation caused more than half of controlled environment farms to be unprofitable in 2017 (O'Sullivan et al. 2019). Therefore, selecting a high-value biopharmaceutical crop may be necessary to make vertical farming more cost-effective (Chen and Yeh 2018). Chamomile and parsley, which naturally produce apigenin, are both suitable crops for an indoor vertical farm because they have a short production time and compact structure. We hypothesized that chamomile and parsley grown in an indoor vertical farm will biosynthesize apigenin in harvestable quantities. The aim of this research was to determine biomass production and apigenin accumulation in selected cultivars of chamomile and parsley as potential biopharmaceutical crops for indoor vertical farm production.

## Materials and Methods

**Crop production.** Two cultivars of chamomile (*Matricaria chamomilla* L.), Bodegold and Zloty Lan (Jelitto Perennial Seeds, Louisville, KY, USA), and three cultivars of parsley (*Petroselinum crispum* Mill. Nyman ex A.W. Hill.), Darki, Giant of Italy, and Wega (Johnny's Selected Seeds, Winslow, ME, USA), were selected for a trial in a hydroponic indoor vertical farm located at the University of Georgia (College of Agricultural and Environmental Sciences, Department of Horticulture, CEA Crop Physiology and Production Laboratory) in Athens, GA, USA, in Jun 2023. Sixteen seeds of each cultivar were directly sown onto 3.5- × 5.5-cm plugs of soilless substrate (Preforma; Jiffy Growing Solutions, Lorain, OH, USA) and germinated in a growth chamber. The light-emitting diode (LED) light intensity was set to 250  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for a 16-h photoperiod with setpoint values of 25 °C, 70% humidity, and 800  $\text{mg}\cdot\text{L}^{-1}$   $\text{CO}_2$ . The medium was kept consistently moist through subirrigation. The seedlings were ready to transplant 3 weeks after sowing for chamomile and 5 weeks after sowing for parsley. When two sets of true leaves appeared, the seedlings were transferred to a deep-water culture system and placed in net pots spaced evenly on foam rafts in square 60- × 60- × 10-cm containers.

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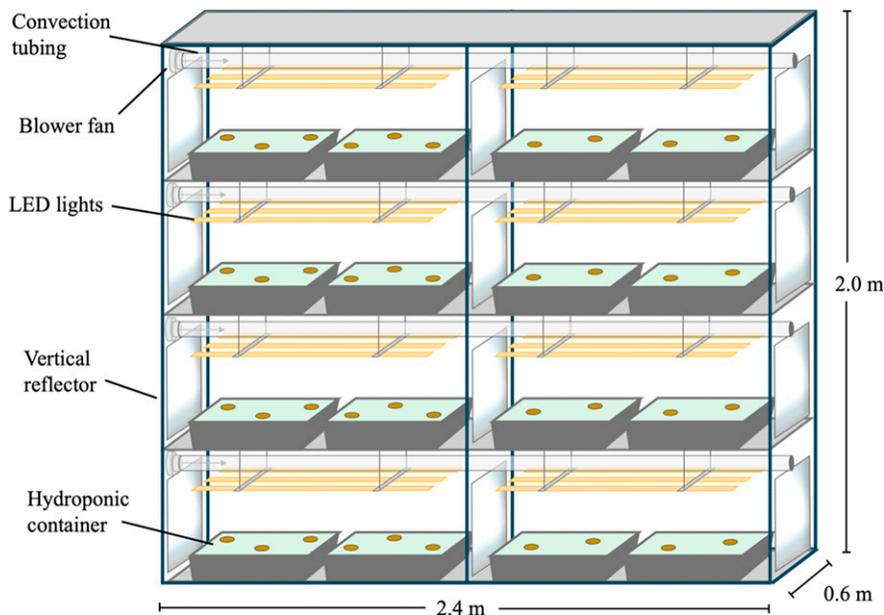


Fig. 1. Schematic of the vertical hydroponic system divided into two 1.2- × 0.6- × 2.0 m sections to separate the parsley and chamomile trials. Blower fans forced air through convection tubing above the plant canopy to prevent humid pockets. The lighting was supplied by light-emitting diodes (LEDs) with vertical reflectors to distribute the light. The hydroponic containers held one plant from each cultivar (three for the parsley and two for the chamomile).

The vertical farm was designed with two 1.2- × 0.6- × 2.0-m sections separating the chamomile and parsley trials. Both sections were subdivided into four vertically stacked shelves with two deep-water culture containers per shelf (Fig. 1). For parsley, each hydroponic container held a single replicate of the three trialed cultivars, which were uniformly spaced and randomized in their placement. Similarly, each chamomile hydroponic container held two uniformly spaced plants (one of each cultivar). The plants were grown under an array of three LEDs (RAY Physiospec Spectrum; Fluence, Austin, TX, USA) with spectral output of 360 to 780 nm and 30.5-cm spacing between the media surface and the light source. Based on the recommended light intensities, the chamomile and parsley were grown with daily light integrals of 17 and 19  $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ , respectively (Litvin-Zabal 2019; Otto et al. 2017). Because chamomile is a long-day flowering species, both herbs were grown under a photoperiod of 16 h to induce flowering (Otto et al. 2017). Vertical reflectors were installed on both sides of the shelves to help distribute light across the canopy. The daytime and nighttime

temperatures were set to 24 and 20 °C, respectively, with 800  $\text{mg}\cdot\text{L}^{-1}$  of supplemental  $\text{CO}_2$  during the day, and a humidity range of 50% to 75%. Air was circulated around the plant canopy through convection tubing at a rate of 1.2  $\text{m}\cdot\text{s}^{-1}$  with two blower fans per shelf (SEAFLO, South Bend, IN, USA).

The net pots were held by a foam raft that allowed the plant roots to be continuously submerged in a fertilizer solution. The fertilizer solution was oxygenated with an air pump and air stone (Fig. 2), and it was adjusted biweekly with a stock solution of 16N-1.8P-14.3K (Jack's Hydro Feed; JR Peters, Inc. Allentown, PA, USA). Because the seedlings were not fertilized in the growth chamber, the electrical conductivity was gradually increased from 0.75 to 1.5  $\text{dS}\cdot\text{m}^{-1}$  over a period of 4 weeks in the vertical farm to prevent shock. The pH was adjusted with KOH or  $\text{H}_3\text{PO}_4$  to maintain a range of 6.0 to 6.5, and  $\text{CaCO}_3$  was added to each container to buffer the solution.

**Harvest.** Based on known apigenin accumulation, the leaves of parsley and unopened inflorescences of chamomile were considered the usable tissues. When the chamomile plants began flowering, unopened (i.e., before dehiscence

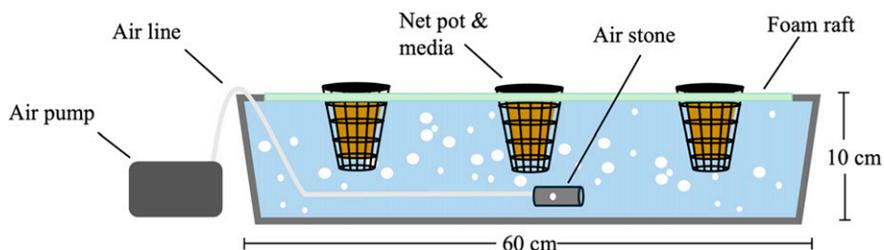


Fig. 2. Schematic of the deep-water culture hydroponic containers where seedlings and media were held in net pots and spaced on a floating foam raft. The roots were submerged in an aerated nutrient solution.

of the disc florets) buds were harvested from individual plants twice per week. The flower buds were collected in screw-top tubes (Falcon, Corning Inc., Corning, NY, USA) and immediately placed in liquid nitrogen. Then, the samples were transferred to a lyophilizer (Labconco; Marshall Scientific, Hampton, NH, USA) and dried at  $-84^{\circ}\text{C}$  and 0.133 mbar for 24 h. When the samples were fully desiccated, they were stored in the dark until the chemical analysis was performed. At 14 weeks after transplant, samples of the fully expanded parsley leaves were harvested from each plant and lyophilized as described. The vegetative tissue of the chamomile plants and the remaining leaves of the parsley plants were separated and dried at  $80^{\circ}\text{C}$  for 48 h in a forced air oven (Shel Lab; Stellar Scientific, Baltimore, MD, USA). After the drying period, the dry weight of the isolates was recorded.

The combined dried mass of the leaves and stems was calculated as the total biomass production for parsley. The combined dry mass of the inflorescences, leaves, and stems was calculated as the total biomass for chamomile. RStudio (version 2023.12.0+369) was used for the data analysis.

**Sample preparation.** The stock solution of apigenin was prepared at a concentration of  $1\text{ mg}\cdot\text{mL}^{-1}$  by dissolving the apigenin standard (95% purity) in high-performance liquid chromatography (HPLC) grade methanol/DMSO (90/10, v/v) (Sigma-Aldrich, St. Louis, MO, USA). The working standard solutions (e.g., calibration standards) were prepared daily by diluting the stock solution with methanol before use. The freeze-dried tissue samples were pulverized using a mortar and pestle. Powdered samples (100 mg for parsley and 50 mg for chamomile) were placed in 2-mL plastic tubes and mixed with 1 mL of 50% methanol. The samples were vortexed for 5 min and centrifuged at  $13,500\text{ g}$  for 10 min. The supernatants were collected, passed through  $0.2\text{-}\mu\text{m}$  membrane filters, and injected into the HPLC system.

**High-performance liquid chromatography analysis.** A chemical analysis was performed using HPLC (Shimadzu Nexera; Shimadzu Corp., Tokyo, Japan) equipped with a photodiode array detector. Apigenin was separated on a  $4.6 \times 150\text{-mm}$  analytical column with a particle size of  $5\text{ }\mu\text{m}$  equipped with a guard column (ZORBAX Eclipse XDB-C18; Agilent Technologies, Santa Clara, CA, USA). The column temperature was set to  $40^{\circ}\text{C}$ . The mobile phase was composed of water/acetonitrile (65/35, v/v, %) containing 0.1% formic acid (Oakwood Products Inc., Estill, SC, USA). The flow rate was  $1\text{ mL}\cdot\text{min}^{-1}$  with an injection volume of  $10\text{ }\mu\text{L}$ . After chromatographic separation, apigenin was detected at a ultraviolet wavelength of  $336\text{ nm}$  and identified by comparing the retention times and ultraviolet spectra with the apigenin standard. Apigenin was quantified using calibration curves. LabSolutions software (version 5.124) was used for the HPLC data interpretation and analysis.

Table 1. Dried usable biomass, unusable biomass, and apigenin accumulation in cultivars of chamomile (*Matricaria recutita*) and parsley (*Petroselinum crispum*) produced in an indoor deep-water culture system. Inflorescences were the usable biomass for chamomile, with all vegetative tissue counted as unusable biomass. For parsley, the leaves were considered usable and the stems were considered unusable biomass. Values are reported as the mean  $\pm$  standard deviation.

Cultivar	Usable biomass (g)	Unusable biomass (g)	Apigenin concn ( $\text{mg}\cdot\text{g}^{-1}$ dried sample)	Total apigenin ( $\text{mg}/\text{plant}$ )
<i>M. recutita</i> Bodegold	$18.53 \pm 20.79$	$183.87 \pm 69.52$	$0.7036 \pm 0.1726$	$15.32 \pm 20.32$
<i>M. recutita</i> Zloty Lan	$4.39 \pm 4.83$	$75.22 \pm 39.25$	$0.7334 \pm 0.1544$	$5.49 \pm 4.11$
<i>P. crispum</i> Darki	$21.39 \pm 5.11$	$8.04 \pm 1.74$	$0.0250 \pm 0.0463$	$0.58 \pm 1.13$
<i>P. crispum</i> Giant of Italy	$49.30 \pm 15.43$	$34.96 \pm 15.88$	$0.0032 \pm 0.0013$	$0.15 \pm 0.07$
<i>P. crispum</i> Wega	$32.82 \pm 11.38$	$14.12 \pm 6.17$	$0.0050 \pm 0.0050$	$0.16 \pm 0.15$

## Results

**Biomass production.** Because apigenin accumulates in the highest concentrations in the leaves of parsley (Poureini et al. 2022) and the flowers of chamomile (Letchamo 1996), these were considered the usable tissues for biosynthesis of apigenin. The Bodegold chamomile cultivar generated  $18.5 \pm 20.8\text{ g}$  of flowers per plant, accounting for 9% of the total biomass (Table 1 and Fig. 3). The large standard deviation for ‘Bodegold’ was observed because the flowering of three plants was much more prolific than that of the other five plants. The Zloty Lan cultivar produced  $4.3 \pm 4.8\text{ g}$  of flowers per plant, accounting for 5% of the total biomass. Again, the large deviation was attributable to two ‘Zloty Lan’ plants that did not produce flowers. A *t* test indicated that there was no statistically significant difference in the biomass of flowers of the two cultivars.

Regarding parsley, the Darki, Giant of Italy, and Wega cultivars produced  $21.4 \pm 5.1\text{ g}$ ,  $49.3 \pm 15.4\text{ g}$ , and  $32.8 \pm 11.4\text{ g}$  of leaf tissue per plant, representing 73%, 59%, and 70% of the total plant biomass, respectively. Although the Darki parsley cultivar had the highest percentage of usable biomass relative to total biomass production, the Giant of Italy cultivar was the most productive overall. An analysis of variance (ANOVA) for parsley showed that the dried biomass of the leaves was significantly different among the cultivars [ $F(2) = 12$ ;  $P = 0.0003$ ]. Tukey’s honestly

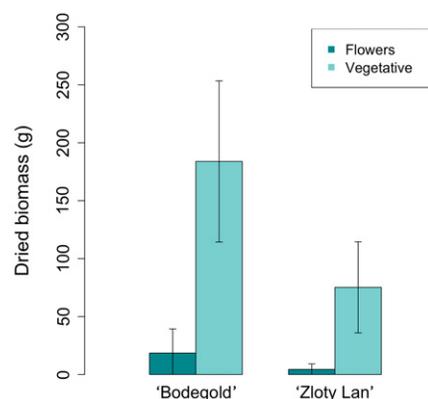


Fig. 3. The Bodegold and Zloty Lan chamomile (*Matricaria chamomilla*) cultivars produced  $18.5 \pm 20.8\text{ g}$  and  $4.3 \pm 4.8\text{ g}$  of flowers per plant, respectively. No statistically significant difference in flower production was found between these two cultivars. Because apigenin accumulates in the flowers of chamomile, the vegetative tissue was considered unusable.

significant difference test indicated that the Giant of Italy cultivar generated significantly more usable biomass than that of cultivars Darki or Wega (Table 1 and Fig. 4). Compared with chamomile, all parsley cultivars produced more usable biomass, which was expected because the leaves of parsley account for most of the plant biomass. Because apigenin accumulates in the chamomile flowers, none of the vegetative tissue is used for extraction.

**HPLC method evaluation.** Figure 5 shows the chromatograms of apigenin in a standard solution and a sample. Apigenin was clearly separated from matrices, and its retention time was 5.2 min. No matrix effect was observed around the retention time of apigenin, confirming the good selectivity of the method. Linearity (quantification capacity) was achieved by plotting calibration curves of apigenin within the ranges of 6.25 to  $200\text{ }\mu\text{g}\cdot\text{mL}^{-1}$  for chamomile and 0.078 to  $2.5\text{ }\mu\text{g}\cdot\text{mL}^{-1}$  for parsley. For apigenin in chamomile, the calibration data showed that the linear range was 6.25 to  $200\text{ }\mu\text{g}\cdot\text{mL}^{-1}$  and could be described by the linear regression equation  $y = 5171x - 50$  ( $r^2 = 0.999$ ). For apigenin in parsley, the linear range extended from 0.078 to  $2.5\text{ }\mu\text{g}\cdot\text{mL}^{-1}$  with a regression equation of  $y = 4610x - 832$  ( $r^2 = 0.997$ ).

**Apigenin accumulation.** The Bodegold and Zloty Lan chamomile cultivars produced  $0.704 \pm 0.173$  and  $0.733 \pm 0.154\text{ mg}$  apigenin/g dried tissue, respectively (Table 1 and Fig. 6). A *t* test showed no significant difference in the

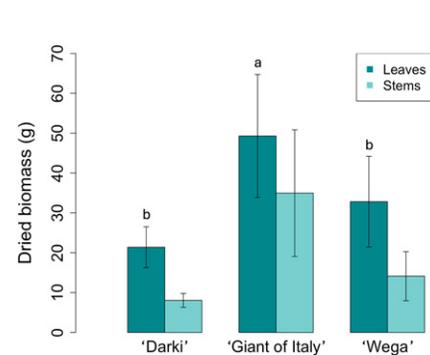


Fig. 4. Darki, Giant of Italy, and Wega parsley (*Petroselinum crispum*) cultivars produced  $21.4 \pm 5.1\text{ g}$ ,  $49.3 \pm 15.4\text{ g}$ , and  $32.8 \pm 11.4\text{ g}$  of leaf tissue per plant, respectively. The Giant of Italy cultivar produced significantly more leaf tissue than that of the other cultivars [ $F(2) = 12$ ;  $P = 0.0003$ ]. Because apigenin accumulates in the leaves of parsley, the stem tissue was considered unusable.

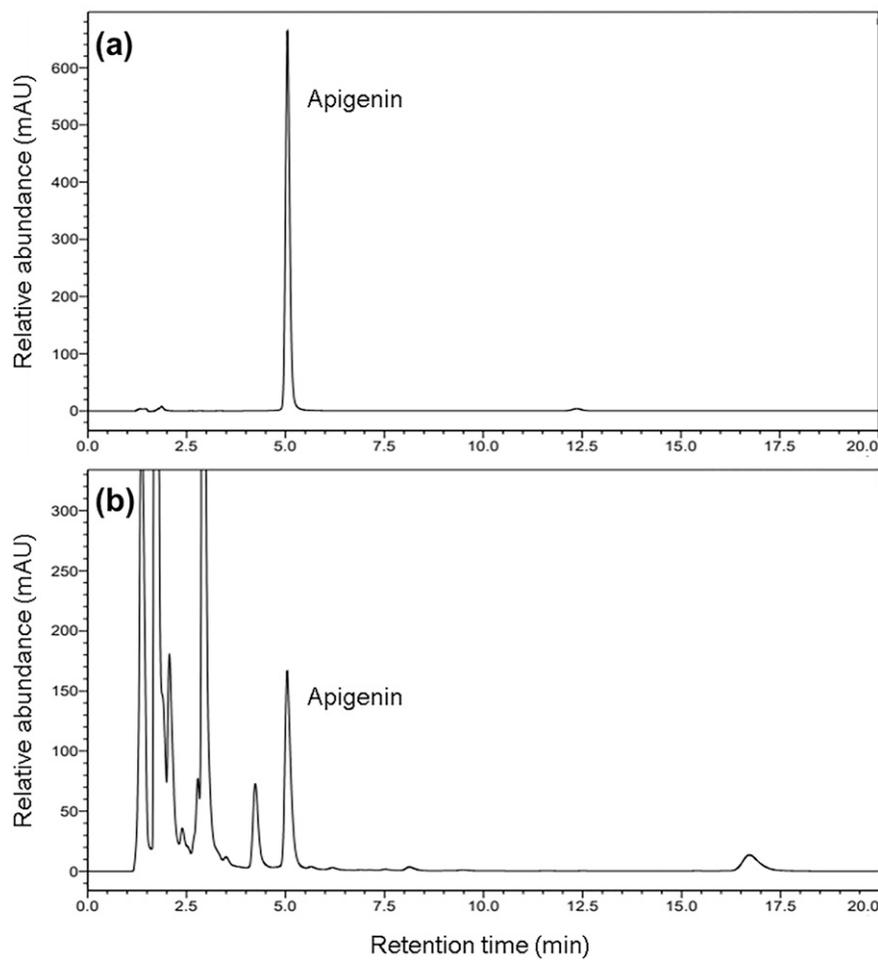


Fig. 5. High-performance liquid chromatography (HPLC) chromatograms of apigenin from the standard (A) and a sample from chamomile (B).

apigenin concentrations of the two cultivars. The parsley cultivars accumulated lower concentrations of apigenin compared with that of chamomile, with  $0.025 \pm 0.046$ ,  $0.003 \pm 0.001$ , and  $0.005 \pm 0.005$  mg apigenin/g dried tissue in the Darki, Giant of Italy, and Wega cultivars, respectively (Table 1 and Fig. 7). However, one plant from the Darki parsley cultivar was an outlier, with 0.139 mg apigenin/g dried tissue, which slightly skewed the average for this

cultivar. An ANOVA also showed no significant difference in the apigenin concentrations among the parsley cultivars, which was unexpected. Previous research of celery and chrysanthemum (*Chrysanthemum ×morifolium*) indicated that apigenin production is cultivar-dependent (Wang et al. 2018; Yan et al. 2014). Although the present research did not find a cultivar dependence for the trialed chamomile or parsley, apigenin levels may differ in other cultivars.

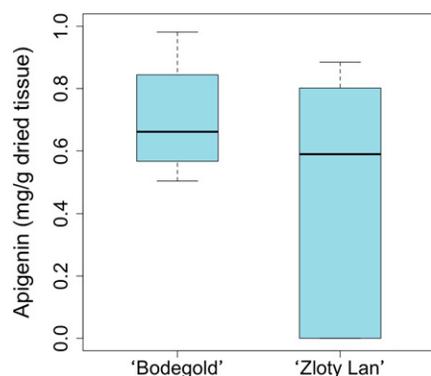


Fig. 6. In the inflorescences, the Bodegold and Zloty Lan chamomile (*Matricaria chamomilla*) cultivars produced  $0.704 \pm 0.173$  and  $0.733 \pm 0.154$  mg apigenin/g dried tissue, respectively, with no significant difference in the apigenin concentration between these two cultivars.

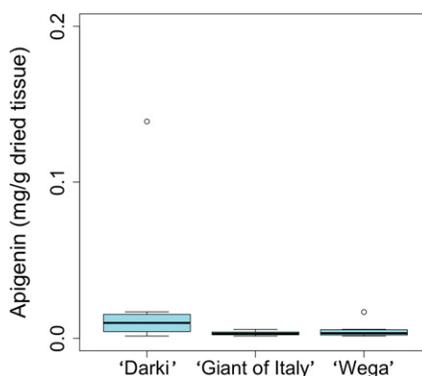


Fig. 7. The Darki, Giant of Italy, and Wega parsley (*Petroselinum crispum*) cultivars accumulated  $0.025 \pm 0.046$ ,  $0.003 \pm 0.001$ , and  $0.005 \pm 0.005$  mg apigenin/g dried leaf tissue, respectively, with no significant difference in apigenin accumulation among the cultivars.

The concentrations of apigenin in the chamomile flowers and parsley leaves were also unexpected. Previous research isolated  $7.01 \pm 0.07$  mg apigenin/g of dried chamomile flowers (Miguel et al. 2015) and  $9.48 \pm 0.11$  mg apigenin/g of dried parsley leaves (Poureini et al. 2022). The present study found considerably lower levels of apigenin accumulation in both herbs. One possible explanation is that the indoor vertical farm with a spectral output of 360 to 780 nm did not have ultraviolet-B light. Previous studies have found that ultraviolet irradiation increases production of flavonoids through activity of the chalcone synthase enzyme, which catalyzes the first step of the flavonoid biosynthetic pathway (Schmelzer et al. 1988). In plants, apigenin is a pigment that contributes to the color of white and pale-yellow flowers (Iwashina 2015) and protects against damage by ultraviolet-B radiation (Righini et al. 2019). Therefore, the lack of ultraviolet light may have reduced apigenin biosynthesis. Future research should investigate whether the addition of ultraviolet-B light to indoor production increases the accumulation of apigenin.

Because the parsley cultivars generated more usable biomass and the chamomile cultivars accumulated more apigenin in the usable tissue, the overall productivity of each cultivar was ascertained by considering apigenin production on a whole-plant basis. The Bodegold and Zloty Lan chamomile cultivars produced  $15.32 \pm 20.32$  and  $5.49 \pm 4.11$  mg apigenin per plant (Table 1 and Fig. 8). In parsley, the Darki, Giant of Italy, and Wega cultivars produced  $0.58 \pm 1.13$ ,  $0.15 \pm 0.07$ , and  $0.16 \pm 0.15$  mg apigenin per plant (Table 1 and Fig. 9). When considering the total usable biomass and concentration of apigenin in the dried tissue, there was no significant difference in overall apigenin production between the chamomile cultivars or among the parsley cultivars. However, because of the higher concentration of apigenin in chamomile flowers compared with that in parsley leaves, the chamomile cultivars generated more

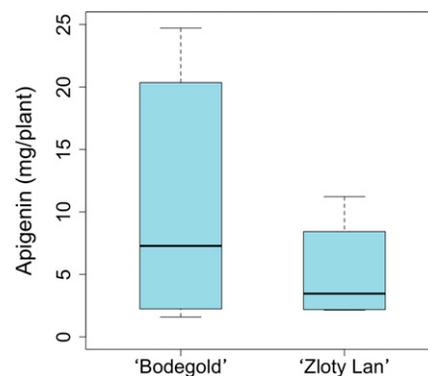


Fig. 8. Total apigenin production per plant from chamomile (*Matricaria chamomilla*) inflorescences. The Bodegold and Zloty Lan cultivars produced  $15.32 \pm 20.32$  and  $5.49 \pm 4.11$  mg apigenin per plant, respectively. No significant difference in apigenin production was found between cultivars.

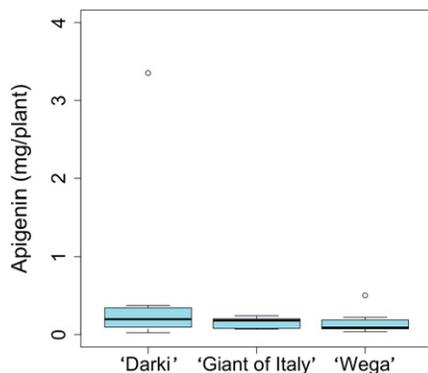


Fig. 9. Total apigenin production per plant from parsley (*Petroselinum crispum*) leaves. The Darki, Giant of Italy, and Wega cultivars produced  $0.58 \pm 1.13$ ,  $0.15 \pm 0.07$ , and  $0.16 \pm 0.15$  mg apigenin per plant, respectively. No significant difference in apigenin production was found among cultivars.

apigenin than that generated by the parsley cultivars on a whole-plant basis.

### Discussion

Although the chamomile cultivars produced more apigenin per plant compared to that of parsley, other important considerations are the time to maturity and labor of harvest. Both parsley and chamomile can be harvested multiple times throughout the growing period. Under field conditions, parsley can be harvested up to eight times, with dry matter content increasing with successive harvests (Alan et al. 2017). Field-grown chamomile is frequently harvested up to four times. However, the efficiency of harvest is variable because the continually blooming plants have inflorescences at different developmental stages (Ghareeb et al. 2022). Previous research of 'Bodegold' chamomile grown under field conditions isolated 70 to 137 g m<sup>-2</sup> of dried flowers in an 8-month growing season depending on the planting density (Rahmati et al. 2011). Based on spacing used in the present study, 24 'Bodegold' chamomile plants could be grown in a 1-m<sup>2</sup> footprint with 2 m of vertical growing space. Because 'Bodegold' yielded an average of 18.5 g of dried flowers per plant, the expected yield would be approximately 445 g m<sup>-2</sup> of dried flowers in an indoor vertical farm. Furthermore, the time from transplant to final harvest was 80 d, meaning it would be easily possible to generate four crop cycles in a calendar year in a controlled environment yielding approximately 1.78 kg m<sup>-2</sup> year<sup>-1</sup> of dried flowers. Therefore, indoor vertical farming of 'Bodegold' chamomile is expected to produce significantly greater yields compared with that of field production because vertical farming uses space more efficiently and indoor systems are not season-dependent.

The Bodegold chamomile cultivar yielded the greatest amount of apigenin per plant, with an average of 15.3 mg, meaning it could be possible to isolate approximately 1.5 g m<sup>-2</sup> year<sup>-1</sup> of apigenin based on the plant spacing used in this study. It is challenging to estimate the

profitability of growing chamomile as a biopharmaceutical because the economic value of apigenin is dependent on the source and purity of the commercial product. Furthermore, it would be important to consider factors such as the cost of inputs, labor, and isolating apigenin to determine the economic viability of selecting chamomile as a biopharmaceutical.

In this study, chamomile was harvested twice per week to collect inflorescences before the disc florets dehisced. It is essential to harvest chamomile at the correct developmental stage because fully opened buds have a lower concentration of apigenin (Letchamo 1996). In this study, the number of flowers increased over time as the plants matured, but the time to collect the flowers also increased. For commercial production of chamomile, the labor associated with frequent hand-harvesting would likely result in a significant profit reduction compared with that associated with the labor of harvesting parsley. One possibility would be mechanizing the harvesting of the flowers, which is a common method for field production. However, mechanical harvesters have not yet been fully developed for indoor systems, and they would likely need to be modified with lower time spacing to collect the unopened inflorescences. Future research should also investigate whether apigenin accumulation changes throughout multiple harvests to determine when the plants should be replaced in the vertical farm.

Despite the higher yields of 'Bodegold', one challenge identified in this study was the high proportion of wasted biomass. A potential solution would be to harvest and repurpose the vegetative tissue at the end of the growing period. Previous research found that chamomile is a useful filler for rubber biocomposites. Chamomile biomass added to natural rubber as 20% of the constituent material resulted in a biocomposite with higher strength than that of the base polymer and reduced the use of synthetic materials (Masłowski et al. 2021). Therefore, the vegetative by-product of chamomile, which does not accumulate high levels of apigenin, may be useful for polymer technology.

### Conclusion

Chamomile and parsley may be effective crops for the biopharmaceutical production of the anticancer compound apigenin. Both herbs grow readily in a controlled environment with compact growth well-suited for year-round indoor vertical farming. In this study, both chamomile and parsley accumulated apigenin, but the chamomile cultivars produced more apigenin than that of any parsley cultivar, yielding  $0.704 \pm 0.173$  and  $0.733 \pm 0.154$  mg apigenin/g dried flowers in the Bodegold and Zloty Lan cultivars, respectively. However, the observed yields were lower than expected. Future research should investigate the impact of adding ultraviolet-B light to the indoor system on overall apigenin accumulation. Despite the greater yield of apigenin in chamomile compared

with that in parsley, regularly harvesting the unopened inflorescences is more labor-intensive than harvesting mature parsley leaves, which may reduce the profitability of selecting chamomile as a biopharmaceutical crop. Therefore, future research should also investigate the profitability of isolating apigenin from chamomile and consider novel applications of the unharvestable biomass.

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