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Original Research Article

**EFFECT OF WATER LIMITATION ON *GYNURA PROCUMBENS* GROWTH AND PHYTOCHEMICAL CONTENT**

**ABSTRACT**

The cultivation of *Gynura procumbens* holds immense value due to its pharmaceutical and therapeutic properties. A critical factor in its cultivation involves understanding how water availability impacts its biomass production and phytochemical profile. Water stress generally induces physiological changes in plants, such as reduced growth and increased production of secondary metabolites as a survival strategy. Under water-limited conditions, plants prioritize essential functions, including maintaining cellular integrity, often at the expense of biomass accumulation, which subsequently influences the phytochemical composition of *G. procumbens*. This study aimed to investigate the effects of water limitation before harvest on the biomass and phytochemical content of *G. procumbens*, providing insights into its adaptive response to water stress. Water limitation treatments were applied for varying durations before harvest: T1 (no water limitation), T2 (4 days), T3 (7 days), and T4 (15 days). The experiment followed a Randomized Complete Block Design (RCBD) with four replications. After a 60-day cultivation period, plant growth, biomass, total phenolic content (TPC), chlorogenic acid levels, and antioxidant activity were assessed. The findings revealed that water limitation from 4 to 15 days before harvesting led to reduced growth and biomass, with more extended periods causing greater adverse effects. Notably, the T4 treatment (15 days of water limitation) resulted in the highest TPC, while T2 (4 days of water limitation) optimized chlorogenic acid production. Both T2 and T4 treatments demonstrated enhanced antioxidant activity, linked to chlorogenic acid and TPC levels, respectively. This study highlights that increasing the duration of water limitation before harvest significantly affects both the biomass and phytochemical composition of *G. procumbens*.

*Keywords: Water limitation, chlorogenic acid, Gynura procumbens, growth, phytochemical*

**1. INTRODUCTION**

*Gynura procumbens*, commonly referred to as "longevity spinach," is highly valued for its rich phytochemical content and therapeutic benefits. A crucial aspect of its cultivation involves exploring the impact of water availability on its biomass production and phytochemical profile. Investigating the effects of varying durations of water limitation before harvest provides insights into how this environmental stress influences the plant's productivity and bioactive compound levels. Water stress typically induces physiological changes in plants, such as reduced growth and biomass accumulation, as an adaptive survival strategy (Lee et al., 2020). Under water-limited conditions, plants prioritize essential processes like maintaining cellular integrity and optimizing water uptake, often compromising biomass production in the process (Selmar and Kleinwächter, 2013).

Water limitation before harvesting plays a significant role in shaping the phytochemical profile of *Gynura procumbens*, a plant highly regarded for its medicinal and nutritional properties (Savitri and Khusnia, 2019). Rich in phytochemicals such as flavonoids, phenolic acids, and alkaloids, *G. procumbens* exhibits potent antioxidant, anti-inflammatory, and other bioactive properties (Dash, 2016, Arifullah et al., 2014, Rosidah et al., 2008). Research indicates that environmental stressors, including water limitation, can influence the synthesis and accumulation of phytochemical compounds (Lee et al., 2019). While some phytochemicals increase under stress as part of the plant's defence mechanisms, others may decrease due to metabolic shifts or resource reallocation (Fhaizal et al., 2015).

Understanding how water limitation affects the phytochemical composition of *G. procumbens* is crucial for optimizing cultivation practices to enhance its therapeutic potential. By uncovering the complex interactions between water availability, plant growth, and phytochemical synthesis, we can devise strategies to improve both the yield and quality of *G. procumbens* under water-limited conditions. This research supports the sustainable cultivation and utilization of *G. procumbens* as a valuable resource for promoting human health and well-being. To address these objectives, this experiment was conducted to evaluate the effects of water limitation prior to harvest on the biomass and phytochemical content of *G. procumbens*, providing critical insights into its response to water stress and its implications for medicinal and nutritional properties.

## 2. MATERIAL AND METHODS

### 2.1 Study Area

The experiment was conducted within a government compound at MARDI Serdang, Selangor, Malaysia (2° 59' 31.7292" N, 101° 41' 56.706" E), over the period from January 2023 to April 2023. The study utilized a side-netted rain shelter measuring 30 m in length, 10 m in width, and 4.5 m in height. The structure was constructed with galvanized steel frames and enclosed with insect-repellent netting (0.1 mm x 0.1 mm) on the sides. To minimize the risk of insect entry, access to the shelter was controlled through a double-door system.

### 2.2 Planting Materials

*Gynura procumbens* plants were propagated using shoot cuttings. Each cutting, measuring 30 cm in length with 7–9 nodes, served as the planting material. The side shoots were removed, leaving only the leaves on the main shoots. The stem cuttings were planted in rockwool, which was used as the rooting medium. Rooting began five days after planting. After 14 weeks, the rooted cuttings had grown into mature plants and were ready for transplantation into polybags filled with coco peat as the growth medium.

### 2.3 Treatments and Experimental Design

The experiment was designed to evaluate the impact of varying durations of water limitation on the biomass and phytochemical composition of *Gynura procumbens*. Four treatments were applied, corresponding to different periods of water limitation before harvesting: T1: No water limitation (0 days), T2: Water limitation for 4 days, T3: Water limitation for 7 days, T4: Water limitation for 15 days. A Randomized Complete Block Design (RCBD) was used, with four replications for each treatment. Each treatment consisted of 10 plants, resulting in a total of 160 plants included in the study. A fertigation system was employed for irrigation and nutrient delivery. For treatments involving water limitation, the cessation of water flow and nutrient solution application was implemented according to the respective durations specified for each treatment period prior to harvesting.

### 2.4 Plant Nutrient Supplementation

A water-soluble fertilizer, formulated by the Malaysian Agricultural Research and Development Institute (MARDI) specifically for leafy herbs, was used for nutrient supplementation. The nutrient solution was prepared in 100 L batches labeled as stock A and stock B, with the pH adjusted to a range of 5.5–6.5 before application. These stock solutions were mixed in a 200 L tank at a 1:1 ratio to achieve an electrical conductivity (EC) between 1800  $\mu\text{S}/\text{cm}$  and 2400  $\mu\text{S}/\text{cm}$ . The nutrient solution was applied to the plants twice daily via a drip irrigation system. During the first three weeks, each plant received 150 ml of the solution daily, which was increased to 500 ml per plant from the fourth week until the initiation of water limitation treatments. Routine horticultural practices, including pest, disease, and weed management, were carried out as needed using biopesticides to ensure optimal plant health throughout the cultivation period.

### 2.5 Extraction of Samples

The *Gynura procumbens* plants were ground into fine powder. For the extraction process, 1 g of *G. procumbens* powder was mixed with 10 mL of 70% methanol solvent in a 1:10 (w:v) ratio. The mixture was subjected to sonication at a temperature of 40–50°C to enhance the extraction process. After sonication, the mixture was centrifuged at 10,000 rpm for 15 minutes to separate the supernatant from the sediment. The extraction process was repeated twice under the same conditions to ensure maximum yield of the crude extract.

### 2.6 Determination of Total Phenolic Content

79 The total phenolic content of *Gynura procumbens* extract was determined using the Folin-Ciocalteu colorimetric method.  
80 A 50 µL aliquot of the test sample was mixed with 100 µL of Folin-Ciocalteu phenol reagent. After allowing the mixture to  
81 react for 3 minutes, 100 µL of a 10% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) solution was added. The reaction mixture was then  
82 incubated in the dark for 60 minutes to ensure complete development of the blue-colored complex. The absorbance of the  
83 resulting complex was measured at 725 nm using a spectrophotometer, and the analysis was conducted in triplicate for  
84 accuracy. Chlorogenic acid was used as the reference standard, and the total phenolic content was expressed as  
85 chlorogenic acid equivalents (CGAE) in milligrams per gram of sample, calculated using a standard curve.

## 86 **2.7 Determination of Chlorogenic Acid Content Using HPLC**

87 The identification and quantification of chlorogenic acid in *Gynura procumbens* extracts were performed using a High-  
88 Performance Liquid Chromatography (HPLC) system. Separation of the compounds was achieved chromatographically  
89 with a Kinetex 5µ C18 column (250 mm x 4.6 mm x 3.5 µm) from Waters, USA. The column oven temperature was  
90 maintained at 25°C. A binary linear gradient was employed as the mobile phase, consisting of Mobile Phase A: Water with  
91 0.1% formic acid and Mobile Phase B: Acetonitrile with 0.1% formic acid. All solvents used were of HPLC grade. The flow  
92 rate of the mobile phase was set at 1 mL/min, and an injection volume of 20 µL was used for the analysis. Detection of  
93 chlorogenic acid compounds was carried out using a Diode Array Detector (DAD) set at an absorption wavelength of 280  
94 nm.

## 95 **2.8 The scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical**

96 To assess the scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, various concentrations of extracts in  
97 methanol were prepared. Each extract was mixed with a methanol solution containing DPPH radicals, resulting in a final  
98 concentration of 0.06 mM. The reaction mixture was then shaken and left in the dark for 30 minutes. All procedures were  
99 performed in triplicate. The scavenging activity was determined by measuring the absorbance of the mixture at 517 nm  
100 using a microplate reader. The results were expressed as IC<sub>50</sub> values, which represent the inhibitory concentration  
101 required to scavenge 50% of the DPPH radicals.

## 102 **2.9 The ferric reducing antioxidant power (FRAP) activity**

103 The FRAP assay determines antioxidant capacity based on the reduction of ferric-tripyridyltriazine to the blue-colored  
104 ferrous form. To perform the assay, a FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM  
105 TPTZ solution, and 20 mM ferric chloride at a 10:1:1 ratio. The reagent was heated to 37°C before use. A total of 7 µl of  
106 sample and 20 µl of distilled water were added to 200 µl of the FRAP reagent, and the mixture was incubated at 37°C for  
107 4 minutes. The absorbance of the reaction mixture was then measured at 593 nm. Ferrous sulfate heptahydrate was used  
108 to generate a standard curve. The results were expressed as the antioxidant concentration (in µmol/mg) capable of  
109 reducing ferric ions in the sample.

## 110 **3.0 Parameters measurements**

111 Plant growth was assessed by measuring multiple parameters. These included plant height, girth, canopy diameter,  
112 number of stalks, SPAD value, and vegetative biomass (fresh and dry weight of leaves, stems, and roots). Plants were  
113 harvested after 60 days of planting to determine biomass yield. Weights were measured immediately after harvest to  
114 minimize water loss and desiccation.

## 115 **3.1 Statistical analysis**

116 Statistical analysis was conducted using analysis of variance (ANOVA) to evaluate the significance of the effects of all  
117 variables (Steel and Torrie, 1960). The analysis was performed using SAS software (version 9.2). Means and standard  
118 deviations (SD) were calculated. ANOVA was used to determine significant differences between treatments. The level of  
119 statistical significance was set at 0.05 for all tests.

## 122 **3. RESULTS AND DISCUSSION**

### 124 **3.1 Effects on plant growth**

125 Significant differences in plant growth and biomass were observed among treatments at  $p < 0.05$  following the application  
126 of water limitation (Table 1). As the duration of water limitation increased from T1 (0 days) to T4 (15 days), a clear trend  
127 emerged across the measured parameters. At T1, plants exhibited the greatest growth, with the tallest height (83 cm), the  
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widest canopy diameter (69 cm), and the largest stem diameter (0.88 cm), indicating optimal growth under normal irrigation conditions. However, as water stress intensified, there was a consistent decline in plant height, canopy diameter, and stem diameter across treatments, reflecting inhibited growth due to water limitation. Notably, at T4, plant height decreased to 72.9 cm, and canopy diameter was reduced to 53 cm compared to T1.

Moreover, biomass allocation within the plants shifted notably with prolonged water limitation before harvesting. The highest growth rates and biomass accumulation were recorded at T1, with plant fresh weight reaching 562 g, leaf fresh weight 293 g, stem fresh weight 226 g, root fresh weight 24.4 g, number of leaves per plant 295, and total leaf area 7128.22 cm<sup>2</sup>, reflecting robust growth under optimal irrigation. However, as water limitation persisted, there was a marked decline in biomass across all measured parameters, highlighting reduced photosynthetic capacity and diminished branching, likely as adaptive responses to conserve water. Root weight also gradually decreased, indicating a reduced ability to absorb nutrients and water from the growth medium (Yang et al., 2021). These results collectively demonstrate that extended water limitation before harvesting adversely impacts plant growth and biomass allocation, reducing overall vigor and productivity (Fhaizal et al., 2015).

The data also provided valuable insights into the physiological responses of plants to water limitation, as reflected by the measured SPAD values. SPAD values, which serve as indicators of chlorophyll content and photosynthetic activity, were highest at T1 (41), signifying optimal chlorophyll levels and photosynthetic efficiency. However, as water limitation increased, SPAD values steadily declined, reaching their lowest at T4 (33). This reduction suggests a decrease in chlorophyll content and photosynthetic capacity, likely due to water stress-induced physiological responses such as stomatal closure and reduced leaf expansion (Wang et al., 2018). The decline in SPAD values aligned with the observed reduction in plant growth and biomass accumulation under prolonged water limitation. These findings highlight the sensitivity of plant physiological processes to water availability and underscore the critical role of effective water management in maintaining plant health and productivity, especially in water-scarce environments. To achieve maximum plant biomass, a water limitation of up to four days is recommended. No significant differences were observed between T2 and T1 (the control) in terms of plant height, stem diameter, leaf fresh weight, stem fresh weight, and the number of leaves per plant, suggesting that short-term water limitation does not adversely affect these parameters. Water limitation decreased water availability in the media that disrupted physiological and morphological activities and eventually cause cessation plant growth (Gopinath and Pavadai, 2015). Water limitation create oxygen deficiency or anoxia, the process of plant growth and development in mainly leaf organ will decrease (Chirkova and Yemelyanov, 2018).

**Table 1: Plant growth and biomass allocation after 60 days of cultivation periods**

Treatment	Plant height (cm)	Stem diameter (cm)	Canopy diameter (cm)	Plant fresh weight (g)	Leaves fresh weight (g)	Stem fresh weight (g)	Root fresh weight (g)	Number of leaves per plant	Total leaf area (cm <sup>2</sup> )	SPAD value
T1	83 <sup>a</sup>	0.88 <sup>a</sup>	69.0 <sup>a</sup>	562 <sup>a</sup>	293 <sup>a</sup>	226 <sup>a</sup>	24.4 <sup>a</sup>	295 <sup>a</sup>	7128.22 <sup>a</sup>	41 <sup>a</sup>
T2	78.5 <sup>a</sup>	0.82 <sup>a</sup>	61.7 <sup>b</sup>	545 <sup>b</sup>	285 <sup>a</sup>	203 <sup>a</sup>	8.4 <sup>b</sup>	285.25 <sup>a</sup>	6621.1 <sup>b</sup>	36 <sup>b</sup>
T3	76.0 <sup>b</sup>	0.78 <sup>b</sup>	55.7 <sup>c</sup>	518 <sup>c</sup>	272 <sup>b</sup>	201 <sup>a</sup>	7.6 <sup>b</sup>	250 <sup>b</sup>	6524.3 <sup>c</sup>	37 <sup>b</sup>
T4	72.9 <sup>c</sup>	0.71 <sup>c</sup>	53.0 <sup>c</sup>	500 <sup>d</sup>	208 <sup>c</sup>	195 <sup>a</sup>	6.3 <sup>c</sup>	218 <sup>b</sup>	6130.1 <sup>d</sup>	33 <sup>c</sup>

\* Mean values in the same column followed by the same letter are not significantly different at  $p < 0.05$

### 3.2 Effect on phytochemical content

Significant differences were observed in the impact of water limitation on phytochemical compounds and antioxidant levels in plants across varying durations (Table 2). The treatments, ranging from T1 to T4, provided insights into how different levels of water stress affect plant biochemistry. Among the parameters measured, total phenolic content (TPC) emerged as a critical indicator of secondary metabolites with antioxidant properties. Under optimal watering conditions at T1, the TPC was moderate (0.572 mg CGAE/g), representing baseline secondary metabolite levels. However, as water limitation intensified, particularly at T4 (15 days), TPC increased substantially to 1.093 mg CGAE/g, indicating a significant upregulation of secondary metabolite production as a physiological response to water stress. Conversely, chlorogenic acid content exhibited an inverse relationship with the duration of water limitation. At T2, its content was relatively high (0.923 mg/g), but it declined markedly with prolonged water stress, reaching its lowest level at T4 (0.200 mg/g). This decline suggests a potential reallocation of metabolic resources, with the plant prioritizing other stress mitigation mechanisms over chlorogenic acid production under severe water stress conditions. These findings highlight the complex biochemical adjustments plants make in response to water limitation, emphasizing the dual role of phenolic compounds

176 and antioxidants in stress adaptation and the nuanced trade-offs involved in resource allocation under environmental  
177 stress (Ren et al., 2014).

178 The data also assessed antioxidant activity using two assays: DPPH radical scavenging activity and ferric-reducing  
179 antioxidant power (FRAP), both of which provided valuable insights into the overall antioxidant capacity of *G. procumbens*  
180 plant extracts. The DPPH assay evaluates the ability of antioxidants to neutralize free radicals by donating an electron or  
181 hydrogen atom, thus stabilizing the DPPH molecule (Soares et al., 1997). In the study, the DPPH radical scavenging  
182 activity, expressed as IC<sub>50</sub>, showed a significant decrease with increasing water limitation. The lowest IC<sub>50</sub> value,  
183 indicating the highest antioxidant capacity, was recorded at T2 (34.265 mg/mL), followed by T4 (44.986 mg/mL).  
184 However, the differences in IC<sub>50</sub> values between these two treatments were not statistically significant. The FRAP assay,  
185 which measures the antioxidant power of plant extracts based on their ability to reduce ferric ions, revealed an increasing  
186 trend in antioxidant capacity with prolonged water limitation. The highest FRAP value was observed at T4 (1.332 μmol  
187 Fe/g), followed by T2 (1.143 μmol Fe/g), although these differences were also not statistically significant. These findings  
188 demonstrate that water limitation influences antioxidant capacity differently depending on the assay used. While DPPH  
189 scavenging activity declined, FRAP values increased, reflecting the complex interplay between different antioxidant  
190 mechanisms under water stress conditions (Soubeyrand et al., 2014). This highlights the importance of employing multiple  
191 assays for a comprehensive evaluation of antioxidant capacity. Previous study showed that water deficit increased  
192 flavonoid content in *Sonchus arvensis* L. compared to control (Yang et al., 2007).

193 This discrepancy between the DPPH and FRAP assays underscores the complexity of antioxidant responses to water  
194 limitation, suggesting that different antioxidant mechanisms may be activated under varying levels of stress (Nima et al.,  
195 2024). Overall, the data highlight the dynamic interplay between water availability, phytochemical composition, and  
196 antioxidant activity in plants. The results demonstrate the plant's ability to modulate its biochemical profile in response to  
197 water stress, potentially as an adaptive strategy to mitigate oxidative damage and maintain cellular homeostasis (Wahab  
198 et al., 2022). These findings provide valuable insights into plant stress physiology, emphasizing the importance of  
199 understanding the biochemical responses that underpin resilience to environmental stressors. They also have practical  
200 implications for crop management strategies, particularly in optimizing water use to enhance crop resilience and  
201 productivity in water-limited conditions. In water limitation condition, phenylpropanoid metabolism is very active with high  
202 activation phenylalanine ammonia lyase (PAL) which increased phenol content (Ghasemi et al., 2019).

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**Table 2: Phytochemical content and antioxidant activity of *G. procumbens* extract**

Treatment	Total Phenolic Content (TPC) (mg CGAE/g)	Chlorogenic acid compounds content (mg/g)	DPPH radical scavenging activity, IC <sub>50</sub> (mg/mL)*	ferric-reducing antioxidant power (FRAP) (μmol Fe/g)
T1	0.572 <sup>b</sup>	0.623 <sup>b</sup>	111.464 <sup>c</sup>	0.889 <sup>ab</sup>
T2	0.282 <sup>c</sup>	0.923 <sup>a</sup>	34.265 <sup>a</sup>	1.143 <sup>ab</sup>
T3	0.751 <sup>b</sup>	0.352 <sup>c</sup>	87.091 <sup>b</sup>	0.874 <sup>ab</sup>
T4	1.093 <sup>a</sup>	0.200 <sup>d</sup>	44.986 <sup>a</sup>	1.332 <sup>a</sup>

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\*A lower IC<sub>50</sub> value indicates higher antioxidant activity.  
Min labeled with the same letter is not significant at P<0.05 according to Tukey's test.

#### 4. CONCLUSIONS

210 Studies have shown that prolonged water limitation significantly impacts both the biomass and phytochemical composition  
211 of *G. procumbens*. Extended periods of water stress were detrimental to plant growth and biomass but led to notable  
212 alterations in chlorogenic acid content and increased antioxidant levels. Among the treatments, T4 (15 days of water  
213 limitation) resulted in the highest total phenolic content (TPC), while T2 (four days of water limitation before harvesting)  
214 was the optimal condition for maximizing chlorogenic acid content. Both T2 and T4 exhibited high antioxidant activity,  
215 corresponding to their respective chlorogenic acid content and TPC levels. These findings underscore the critical role of  
216 water management strategies in optimizing the balance between biomass production, phytochemical composition, and  
217 antioxidant potential of *G. procumbens*. This knowledge is vital for maximizing the plant's medicinal and nutritional  
218 benefits while ensuring sustainable cultivation practices.

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225 Details of the AI usage are given below:

226 1. Llama 3.2 were used for grammatically error check.

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