

## **EFFECT OF STEM CUTTING POSITIONS ON FORAGE GROWTH, YIELD, NUTRITIONAL AND PHYTOCHEMICAL CONTENT, AND PALATABILITY OF *Gynura procumbens* (Lour.) Merr**

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### **ABSTRACT**

The current research aimed to evaluate the potential of *Gynura procumbens* (*G. procumbens*) as forage by evaluating its growth, yield, nutritional characteristics, and palatability which were cultivated from different-position stem cutting. The experiments were laid out under completely randomized design. The treatments were three positions of cutting stem: top (P1), middle (P2), and bottom (P3), with 50 replications. The observed parameters were the growth (morphological cutting stem, plant height, the number of leaves, and branches), yield (forage biomass), then the composite sample from all treatments was analyzed for nutrient composition, in vitro digestibility, phytochemical content, and palatability. Data were analyzed statistically using R i386 3.6.1 through analysis of variance (ANOVA). The Tukey contrast test was conducted to determine the level of significant differences among different treatments. The results showed that there were differences in cutting stem diameter and weight from different positions ( $P \leq 0.05$ ). The cutting stem on top position increased the number of branches, plant height, and forage biomass ( $P \leq 0.01$ ;  $P \leq 0.05$ ), yet the number of leaves had no different effects. *G. procumbens* plant growth had different patterns on the number of leaves and branches and plant height. The crude protein, ash, and digestibility in leaves were higher than in the stalk. The degree of palatability was higher for cattle and goats. It was concluded from this study that the best position to induce *G. procumbens* plant growth was the top stem-cutting and its yield could be utilized as forage from leaves and stems based on the in vitro digestibility, forage biomass, nutritional characteristics, and palatability.

**Keywords:** Crop, Cutting Stem, Forage, *Gynura procumbens*, Stalk Position

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### **INTRODUCTION**

Animal farming has developed rapidly to fulfill the community's needs for food in Indonesia (Agus and Widi, 2018). However, animal farming development has to face several problems, including the fluctuating forage supply (Kusumastuti *et al.*, 2015); the decreasing farmers' number, age, and education (Kumalasari *et al.*, 2021); also, animal outbreak diseases, especially foot and mouth disease in 2021 (Pamungkas *et al.*, 2023). The problems should be resolved through a simple and applicable way for older farmers. Forage from tropical plants can be one alternative for ruminant feed sources (Paul *et al.*, 2020) as it can be grown easily, close to animal farms, and accessible (Peters *et al.*, 2021).

*Gynura procumbens* (Lour.) Merr. (*G. procumbens*) is an endemic plant to tropical Asia

countries such as China, Thailand, Malaysia, Vietnam, and Indonesia (Tan *et al.*, 2016). *G. procumbens* is locally known as 'sambung nyawa' or 'daun dewa' and is commonly used as herb medicine, especially for therapeutic pharmacological potential medicine to treat various diseases (Ashraf, 2019). Throughout the years, most *G. procumbens* were harvested in its natural range to consume raw or added in the cooking process as food (Idris and Markom, 2019).

*G. procumbens* leaves are already used as a forage supplement on sheep in ensilage feed and could be used as anthelmintic (Yusfani *et al.* 2024) and applied on chicken to increase egg production, feed efficiency, and suppress harmful excreta microflora (Lokhande *et al.*, 2014). The positive effect on animal performance related to *G. procumbens* addition might related to the secondary metabolites and antioxidant content (Ahmed *et al.*, 2021).

The *G. procumbens* cultivation expansion as forage should optimize the stem utilization as planting material. *G. procumbens* cultivation is developed through vegetative materials because it is difficult to obtain the seed (Yusoff *et al.*, 2019). Commonly, *G. procumbens* propagation using stem cuttings methods, despite the adventitious root can be used as planting material (Muthoharoh *et al.*, 2019).

In spite of the adventitious root, *G. procumbens* propagation using stem cuttings methods can be used as planting material (Muthoharoh *et al.*, 2019). Previous research reported that the stem from the middle part of the plant stalk is more suitable for propagation (Yusoff *et al.*, 2019). On the other hand, there was no information about the optimum stem-cutting position for *G. procumbens*. The stem-cutting position may affect the survival rate of newly raised seedlings (Pamungkas *et al.*, 2021), plant growth (Hartati *et al.*, 2021), the number of branches (Gebremedhn, 2016) and biomass production (Solikin, 2018b). Hence, this research determined the optimum position of *G. procumbens* stem cuttings for forage development and production. The research also evaluated the potential of *G. procumbens* growth, production, nutrient composition, and *in vitro* digestibility as forage for ruminants.

## MATERIALS AND METHODS

**General condition of the experimental area:** This research was conducted in the Field Laboratory of Agrostology, Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Indonesia. The research area was located at 6°33'10.624" S latitude and 106°43'21.719" E longitude at 202 m.asl. Based on Bogor Climatological Station during the experimental period, the annual rainfall research area ranged from 3496 mm and the mean temperature and humidity were 25.38°C and 86.28%, respectively.

**Sample preparation:** For this research, stem cuttings were collected from the mature plant of *G. procumbens* (2 years old) with a stem age was 6 months after pruning. A total of 150 cuttings with 30 cm length were categorized based on the position, i.e., top (P1), middle (P2), and bottom (P3). The stem cuttings were immersed in fresh water for a week in order to stimulate the callus development before cultivation. Media for planting used was soil in the polybag with a capacity of 2 kg and the addition of manure fertilizer application and NPK fertilizer at the dosage of 8 tons/ha and 200 kg/ha, respectively. Stem cuttings were planted in the polybag with the organic fertilizer application and NPK fertilizer at the dosage of 8 tons/ha and 200 kg/ha, respectively for cultivation. *G. procumbens* was planted using cutting stems in 8 rows on a well-prepared soil. All the different cutting stem positions were planted in pits with a depth of

10 cm on the polybag. The polybag spacing between rows in the nursery was 100 cm x 50 cm and between plants was 50 cm x 50 cm, respectively.

**Data and sample collection:** The morphological data such as plant height, number of leaves, and number of branches were recorded from all plants of each plot once a week. The plants were harvested 12 weeks after cultivation. The leaf-to-stem ratio was determined by plucking all the leaves and then trimming the stem, leaving a stubble height of 30 cm. The fresh biomass was measured immediately after each harvest.

**Nutritional characteristic.** Representative samples taken from each plot at each site were dried under the sun for three days then followed in an air drought oven at 60 °C for 48 hours. The dry matter (DM) was determined according to SNI 01.2891-1992 number 5.1. The ash was determined by AOAC (2005) method 4.1.10, crude protein by AOAC (2005) 4.2.11, crude fiber by SNI 01.2891-1992 number 11, and ether extract by AOAC (2005) 4.5.06. The forage digestibility was analyzed by a simple gas production method (Theodorou *et al.*, 1994). The phytochemical screening of flavonoid, alkaloid, tannin, saponin, quinone, steroid, and triterpenoid were analyzed based on Takaidza *et al* (2018). Phytochemical constituents of the *G. procumbens* leaf were determined through a powdered leaf sample (0.5 g) stirred in 5 mL of 1% aqueous hydrochloric acid in a water bath at 65°C, filtered using a No. 1 Whatman filter paper and the filtrate was divided into two portions. Aliquots (1 mL) of the first and second portions were treated with a few drops of Mayer's reagent and Drangendorff's reagent, respectively. Turbidity or precipitation with either of those reagents was taken as preliminary evidence of the presence of alkaloids in the extract.

**Forage palatability.** The palatability test in this research was determined through observations of animals like the forage, accept and willing to consume the plant as part of the diet. The palatability test was conducted on cattle, goats, sheep, and rabbits that were fed fresh leaves in the amount of 1% of animal body weight in the afternoon as the first meal. The palatability was classed by the degree of palatability classes method based on Hussain and Durrani (2009), i.e., highly palatable (HP), moderately palatable (MP), less palatable (LP), rarely palatable (RP) and non-palatable (NP). The five categories were modified based on the eaten forage percentage, i.e.: HP: almost all served forage was eaten (>80%), MP: forage was eaten with average preference (50-80%), LP: forage was eaten with less preference (20-50%), RP: forage was eaten under compulsion when no other forage was available (≤20%) and NP: not eaten by animals at any stage; possibly toxic or harmful.

**Statistical analysis:** The research was laid out in a Completely Randomized Design (CRD) with 3 parts of

stem cutting as treatments and 50 replications. Data were analyzed with R i386 3.6.1 using the analysis of variance test with unbalanced data (ANOVA unbalanced), and the Tukey contrasts test was applied to determine the level of significant difference at  $P \leq 0.05$  (Dudek, 2023).

## RESULTS AND DISCUSSION

**Growth parameters of *G. procumbens* stem cuttings from different positions:** The results of *G. procumbens* stem cutting length, weight, and diameter from different positions are presented in Table 1. The result indicated that positions had a significant effect on stem diameter and weight ( $P \leq 0.05$ ). The highest diameter and weight were observed at the bottom part and decreased proportionally in terms of both parameters. The length of the cutting stem was managed uniformly by researchers, which resulted in no notable variations of mean the cuttings length, although in the total range of 28.20 to 32.60 cm.

The uniform cutting stem length from each stalk position resulted in different diameters and weights. The bottom part had the biggest size in diameter and weight due to the stem already in the mature phase where the cell wall materials growing in mass (Robinson, 2021). The bottom stem cutting has more mature cells that cell plate contains a deposition of polysaccharides such as callose, cellulose, and cross-linking glycans along with glycoproteins (Jawaid *et al.*, 2022). In this research, the bottom positions of *G. procumbens* cutting stem had high viability rates because the plant cell maturation could develop roots and grow as a new plant (Solikin, 2018a). Therefore, it is recommended that all stem-cutting positions can be used to propagate *G. procumbens*.

The growth characteristics of *G. procumbens* were affected by stem cutting position, especially in the number of branches and plant height ( $P \leq 0.01$ ) (Table 2). Most of the cutting positions produced new plants successfully, from the P1, P2, and P3. The result indicated that 96% to 100% of new seedlings of *G. procumbens* could be raised through stem cutting. The number of branches was significantly ( $P \leq 0.01$ ) affected by stem cutting position. The highest number of branches was observed at the bottom and the number of branches was proportionally increased at the higher position. On the other hand, the plant height was significantly ( $P \leq 0.01$ ) affected by stem cutting position in different patterns. The highest plant height was observed at the top and bottom cutting stem with different growth patterns in each week (Figure 1). Stem position has a significant effect on the number of leaves per plant and an interesting pattern in that leaves grow rapidly in the early stage from the top stem followed by the bottom stem after three weeks of cultivation.

The high number of branches from the top stem cutting could be due to the number of active cells that

transform into meristematic (Zigene and Kassahun, 2016). However, the number of leaves after 8 weeks was no different from each cutting stem position related to the cell maturity in each cutting stem position (Satterlee *et al.*, 2020). In the initial stage, the bottom cutting stem grew the root then after the establishment, it could stimulate and support a rapid stem and leaf growth (Greb and Lohmann, 2016). This finding was similar to Sun *et al.* (2019) where the leaf number from different stems cutting was similar because the stem serves as a proxy for the nutrient resources cross-section.

**Biomass production of *G. procumbens* from different stem-cutting positions:** Biomass production of *G. procumbens* was divided into two parts, i.e., leaves and edible stalk (Table 3). The results showed that *G. procumbens* leaves and edible stalks were affected by different stem-cutting positions. The stem-cutting position from the bottom parts had the highest biomass production on both leaves and edible stalk of  $24.78 \pm 2.15$  and  $6.95 \pm 1.15$  g/plant, respectively, while the middle position had the smallest biomass of  $21.97 \pm 2.15$  and  $5.25 \pm 1.15$  g/plant, respectively. These biomass productions had different patterns from the number of branches and plant height.

Biomass production of *G. procumbens* including leaves and edible stalk were affected by different stem-cutting positions. The highest biomass production resulted in the bottom cutting stem ( $24.78 \pm 2.15$  g/plant), followed by the top ( $23.67 \pm 2.15$  g/plant). The stem position of the plant related to diameter size resulted in root growth that increased water and nutrient uptake (Sulaiman *et al.*, 2005). The bottom cutting stem might had a higher energy reserve and a better-established root system, which could potentially lead to quicker establishment and better initial growth (Eed *et al.*, 2015). The top cutting stem usually contained a higher concentration of growth hormones, which could promote faster root development and encourage strong upward growth in the new plant (Kéllou *et al.*, 2022). Consequently, it resulted in higher biomass production than the middle cutting stem.

**Nutritional characteristic and phytochemical content of *G. procumbens*:** current study shows that leaf nutrient content was higher in ash content, crude protein, magnesium concentration, and forage digestibility than the edible stalk ( $P \leq 0.01$ ,  $P \leq 0.05$ ) (Table 4). Yet, the edible stalk was significantly higher in the percentage of dry matter, ether extract, crude fibre, the calcium and phosphor concentration, and in vitro digestibility ( $P \leq 0.01$ ,  $P \leq 0.05$ ). Table 5 shows the phytochemical compound in *G. procumbens*. Both leaves and edible stalks contained flavonoids, saponins, and steroids. However, the tannin component was found only in the leaves.

The result showed that *G. procumbens* had great

potency as a feed resource for livestock, especially the leaves. Based on crude protein content, *G. procumbens* could be categorized as prime forage (Tucak *et al.*, 2021). It would be an advantage to use *G. procumbens* as a forage source due to its high crude protein and low crude fiber content. Moreover, the crude fiber in the leaves of *G. procumbens* had a lower composition than other forage. For example, *Pennisetum purpureum* leaves had crude fiber in the range of 28.17% - 36.50% (Malahubban *et al.*, 2021) while the crude fiber range of alfalfa leaves was 11.72% - 12.07% (Popovic *et al.*, 2000). In vitro digestibility in leaves and edible stalks was in the range of 42.2% – 52.6%. The higher crude fiber percentage affected the lower in vitro digestibility in edible stalks. The low digestibility could be happened due to the phytochemical compound that can be found in both parts.

The phytochemical component was able to be an anti-nutritional content that could influence the digestibility percentage (Table 5). Flavonoid, saponin, and steroid were detected in both leaves and stalk, while tannin was detected on leaves only. These results align with the previous research that found *G. procumbens* was a rich source of an antioxidant such as flavonoid (Sathiyaseelan *et al.*, 2021). Antioxidant are secondary metabolites produced by *G. procumbens* as a plant protection mechanism in response to limiting nutrient conditions (Ahmed *et al.*, 2021). In animal feed, tannin and saponin have been reported to have the potential to

decrease dry matter digestibility (DMD) and organic matter digestibility (OMD) in ruminants, although they can have a positive effect on reducing methane production (Yogianto *et al* 2014).

***G. procumbens* palatability:** One of the important parameters of forage was plant palatability which refers to how well animals consume. *G. procumbens* palatability was measured as indirect forage quality due to the plant characteristics that determine a forage consumed by animals (Table 6). The result revealed that *G. procumbens* were highly palatable for cattle and goat, but moderately palatable for sheep and rabbits. Palatability by parts used was based on the type of *G. procumbens* plant parts, i.e., leave and shoot. The results showed that cattle consumed both leaves and chewy stalks that can be eaten easily, while sheep preferred the leaves and rabbits consumed the hard stalk.

Palatability is related to physical characteristics such as fiber content, toughness, stemminess, leafiness, level of maturity, and succulence (Horrocks and Vallentine, 1999). The differences in palatability degree and plant parts due to the animals have specified preferences for the selection of various plant species or parts of the plant (Abdullah *et al.*, 2017). *G. procumbens* leaves are palatable for most experimental animals, while the shoot is palatable for cattle and rabbits is related with animal morphology, especially the dental structure (Harcourt-Brown, 2017).

**Table 1. Morphological characteristics of *G. procumbens* stem cutting from different positions.**

Treatments	Stem Diameter (mm)	Stem Length (cm)	Stem Weight (g)
Top	12.10 <sup>c</sup>	31.65	39.68 <sup>c</sup>
Middle	15.20 <sup>b</sup>	31.46	62.73 <sup>b</sup>
Bottom	18.16 <sup>a</sup>	30.76	85.65 <sup>a</sup>
Minimum	8.30	28.20	23.70
Maximum	23.30	32.60	90.00
SEM	1.53	1.32	138.20
P-value	≤0.05	≤0.23	≤0.05

<sup>abc</sup> means in the same column with the different letters are significantly different at P≤0.05

**Table 2. Shoot percentage, number of branches, number of leaves per plant, and plant height of *G. procumbens*.**

Treatments	Shoot Percentage (%)	Number of Branches per Plant	Number of Leaves per Plant (sheets)	Plant Height (m)
Top	98	3.65 <sup>a</sup>	38.34	82.43 <sup>a</sup>
Middle	96	3.03 <sup>b</sup>	34.76	74.60 <sup>b</sup>
Bottom	100	2.46 <sup>c</sup>	38.42	81.39 <sup>a</sup>
Minimum	96	1.00	11.0	16.2
Maximum	100	9.00	90.0	131.2
SEM		0.26	2.49	4.78
P-value		≤0.01	0.35	≤0.01

<sup>abc</sup>Means in the same column with the different letters are significantly different at P≤0.05

**Table 3. Fresh biomass production of *G. procumbens* (g/plant).**

Treatments	Leaves Biomass	Edible Stalk Biomass	Leaves:Stalk Ratio
Top	23.67 <sup>ab</sup>	7.19 <sup>a</sup>	3:1
Middle	21.97 <sup>b</sup>	5.25 <sup>b</sup>	4:1
Bottom	24.78 <sup>a</sup>	6.95 <sup>ab</sup>	3:1
Minimum	13.4	0.42	
Maximum	30.0	2.03	
SEM	2.15	1.15	
P-value	≤0.01	≤0.05	

<sup>abc</sup> Means in the same column with the different letters are significantly different at P≤0.05

**Table 4. Nutrient composition and in vitro digestibility of *G. procumbens***

Nutrient composition	Leaves	Edible Stalk	SEM	P-value
Dry matter (%)	26.2 <sup>b</sup>	71.7 <sup>a</sup>	1.33	≤0.01
Ash (%)	10.1 <sup>a</sup>	4.80 <sup>b</sup>	0.47	≤0.01
Crude protein (%)	26.7 <sup>a</sup>	7.64 <sup>b</sup>	0.16	≤0.01
Ether extract (%)	3.8 <sup>b</sup>	5.37 <sup>a</sup>	0.29	≤0.05
Crude fiber (%)	8.3 <sup>b</sup>	25.52 <sup>a</sup>	0.18	≤0.01
Calcium (ppm)	692 <sup>b</sup>	1129 <sup>a</sup>	47.3	≤0.01
Magnesium (ppm)	1051 <sup>a</sup>	870 <sup>b</sup>	66.6	≤0.05
Phosphor (ppm)	933 <sup>b</sup>	1539 <sup>a</sup>	6.66	≤0.01
Dry matter digestibility (%)	52.6 <sup>a</sup>	48.4 <sup>b</sup>	0.45	≤0.05
Organic matter digestibility (%)	49.7 <sup>a</sup>	42.2 <sup>b</sup>	0.82	≤0.05

<sup>abc</sup> Means in the same row with the different letters are significantly different at P≤0.05

**Table 5. Qualitative phytochemical analysis of *G. procumbens***

Phytochemical	Leaves	Edible Stalk
Flavonoid	+	+
Alkaloid		
Wagner	-	-
Mayer	-	-
Dragendorff	-	-
Tannin	+	-
Saponin	+	+
Quinone	-	-
Steroid	+	+
Triterpenoid	-	-

+ : positive, - : negative

**Table 6. Palatability classification of fresh *G. procumbens* on several animals**

Animal	Degree of Palatability*	Part of <i>G. procumbens</i>	
		Leaves	Edible Stalk
Cattle	Hp	+	+
Goat	Hp	+	-
Sheep	Mp	+	-
Rabbit	Mp	-	+

\*Hp: Highly palatable, Mp: Moderately palatable

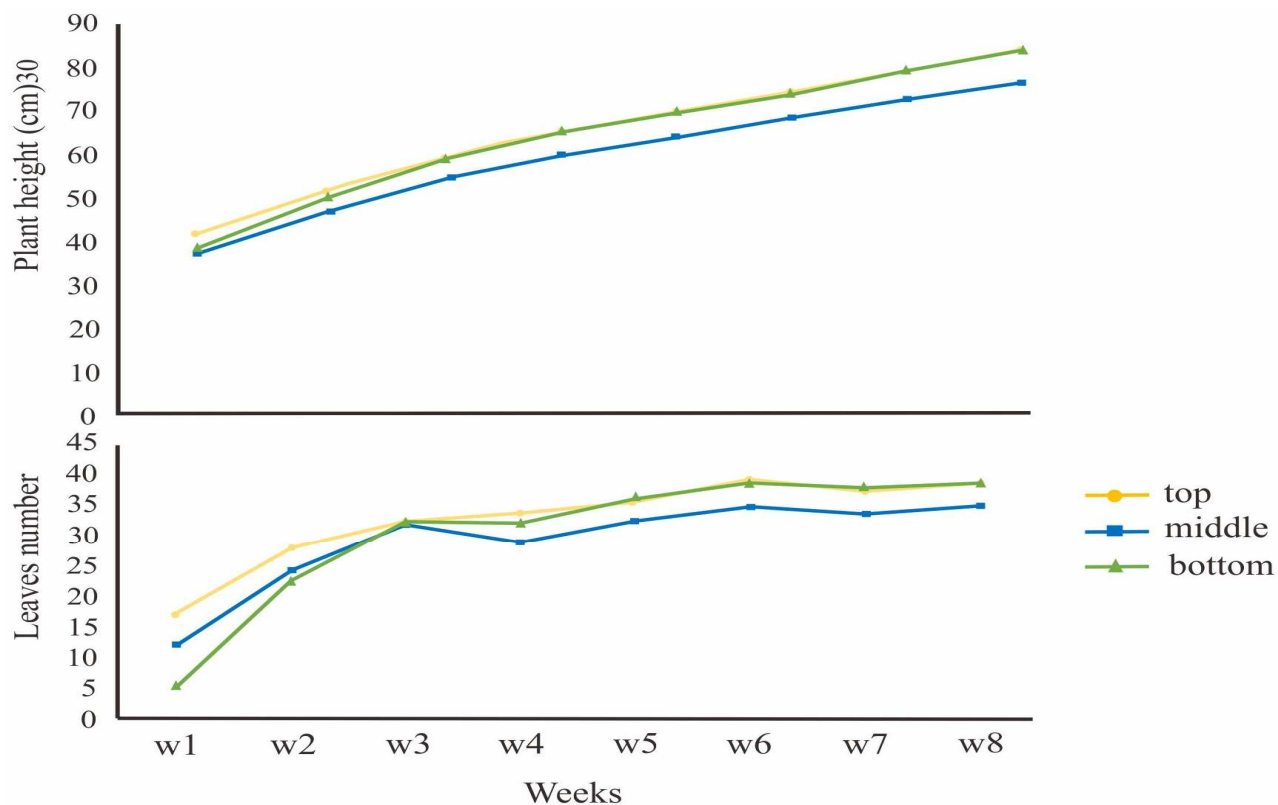


Figure 1. Growth pattern of *G. procumbens* in 8 weeks

**Conclusions:** *G. procumbens* could grow well from all the different cutting diameters (top, middle, and bottom) as plant cultivation materials. Based on biomass production, nutrient content, and palatability degree, *G. procumbens* from the bottom stem cutting had great potential as a forage resource in livestock production, especially the leaves. In addition, the quantitative anti-nutritional component of *G. procumbens* needed to be observed as the limiting factor in utilizing this plant for animals.

**Conflict Of Interest:** The authors declared that there was no conflict of interest including financial or personal relationships, that could influence the current study.

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