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RESEARCH ARTICLE
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Chemical Composition of Foliage and Seed Dormancy in *Desmanthus virgatus* (L.) Willd for Forage use in Dry Tropical Regions of Mexico

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ABSTRACT

Legumes are rich in protein and secondary metabolites and also develop root nodules, all of which contribute to the advancement of more sustainable animal production systems. The nutritive value and seed dormancy of *Desmanthus virgatus* (L.) Willd., a native legume with forage potential, were evaluated in two ecotypes, Copalillo and Pungarabato, from dry tropical zones in Mexico. Foliage and seed samples from two ecotypes of *Desmanthus virgatus* (L.) Willd. were collected in Mexico during autumn-winter 2022 and 2023, and analyzed for dry matter, ash (CEN), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), and acid detergent fiber (ADF) following AOAC methods. Germination tests evaluated the effects of three storage periods, 270, 300, and 350 days, on dormancy. The Pungarabato ecotype had higher CP and EE content (22.25 and 11.94%, respectively) than the Copalillo ecotype (15.95% and 10.20%, respectively), suggesting a superior nutritional value of the Pungarabato ecotype. No significant differences ($P \geq 0.05$) in CEN, NDF, ADF, and crude fiber were detected between the ecotypes. Similar secondary compounds between ecotypes identified by high-performance liquid chromatography were gallic acid, quercetin rhamnoside, kaempferol rhamnoside, quercetin glucoside, and kaempferol glucoside. Both ecotypes had moderate germination rates, with higher germination rates after 300 storage days (40.3% for Copalillo and 34.7% for Pungarabato). The findings suggest that *D. virgatus* could be a viable alternative to improve forage availability in dry seasons and contribute to the sustainability of livestock production systems in the dry tropics. These results contribute to the knowledge of adaptable forages to face seasonality in arid and tropical regions.

Keywords: *Desmanthus virgatus*, Chemical composition, Seed dormancy, Secondary compounds.

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INTRODUCTION

Within tropical regions, several challenges exist regarding the sustainable use of natural resources and the diversity of protein sources for livestock feed. In these areas, livestock production experiences significant improvements when forage with high nutritional value is available that fully satisfies the animal's requirements. Native legumes, due to their high productivity and quality, offer considerable potential for integration into animal production systems (Sosa-Montes et al., 2020). The nutritional value of forage plants varies depending on the species, stage of development, and environmental conditions. In general, legumes have higher protein content, giving them an advantage over grasses (UE, 2023). Furthermore, legumes tend to maintain their nutritional

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value for a longer period, allowing them to be used in animal feed even during dry seasons (Simbaya et al., 2020). The benefits of legumes belonging to the Fabaceae family have been widely documented. These plants stand out for their ability to fix atmospheric nitrogen, thanks to the symbiosis with bacteria present in their roots, which improves soil fertility (Buernor et al., 2022). Furthermore, the use of legumes in animal production systems is associated with reduced production costs and sustainable animal production practices by implementing silvopastoral systems (Muir et al., 2025). Knowing the nutritional properties of legumes is important for selecting them as livestock feed (Sosa-Montes et al., 2020). *Desmanthus virgatus,* a native species distributed in tropical areas and resistant to the prevailing environmental conditions of arid and semi-arid zones (Medeiros et al., 2023). *D. virgatus* primarily reproduces through seed production (Peralta, 2019). Germination is a complex process in which a seed must complete a series of cellular events to achieve root formation and plant growth. *Desmanthus virgatus* seeds remain dormant, which means that, although they are alive and have a favorable environment, they do not initiate the germination process. This occurs because the seed coat acts as a physical barrier, preventing the entry of water and gases essential for germination (Barjasteh et al., 2023). Based on these considerations, the objective of this research was to evaluate the chemical composition of the foliage of *Desmanthus virgatus* (L.) Willd and the dormancy of the seeds of two ecotypes for forage use in animal feed, aspects related to the nutritional value and density of the plant.

MATERIALS & METHODS

Study Area

Samples were obtained from the municipality of Copalillo, located at 18°02′00″NL, 99°07′00″WL, at 900 meters above sea level, with an average annual temperature of 30°C and an average annual rainfall of 900mm; and from the municipality of Pungarabato, located at 18°21′4″NL, 100°40′06″WL, at 246 meters above sea level, with an average annual temperature of 35°C and an average annual rainfall of 703mm, respectively, both entities in the state of Guerrero, Mexico.

Study Design and Variables Measured
Phenological Evaluation

The phenological study of the plants was carried out in a natural environment at a similar phenological age (90 days after the start of the rainy season). During the study (first week of October 2023), 40 plants (Copalillo ecotype) and 30 plants (Pungarabato ecotype) were evaluated; each plant was considered a repetition. In each plant, the following variables were measured: height with a tape measure graduated in cm from the base of the floor to the highest aerial part of the plant, basal diameter with a digital vernier caliper (MOSU-MX®) graduated in millimeters, number of branches, leaves, flowers, pods, pod weight, and biomass supported by an electronic scale with a capacity of 10kg and a sensitivity of 0.01g (Scale: model ESO24).

Chemical Composition

Phenologically mature pods and foliage of the ecotypes were collected manually in their entirety per plant during the fall-winter of 2023. The samples were dehydrated in a forced-air oven at 50°C for 72h until constant weight was reached, and then ground to a particle size <1mm in a Willy mill. Subsequently, the samples by ecotype were analyzed for proximate chemical composition in the Laboratory of the Higher Agricultural College of the State of Guerrero. Dry matter contents, ash (AOAC, 2023), crude protein by micro Kjeldahl method (AOAC, 2023), ether extract, neutral detergent fiber (NDF), and acid detergent fiber (ADF) (AOAC, 2023). Organic matter and crude fiber (CF) were measured, in triplicate samples.

Major Secondary Compounds

For the identification of major secondary compounds, hydroalcoholic extracts (70% methanol and 30% water: the mixed solvent allowed the extraction of compounds from medium polarity to high polarity compounds from the plant) were obtained from the plant leaves in a mass-to-volume (m/v) ratio of 1:10 for 72h, at room temperature and without exposure to light, the liquid solution was filtered with gauze, cotton and Whatman® 42 filter paper and subsequently concentrated under reduced pressure in a rotary evaporator (Buchi R-300, Switzerland) at 45°C for solvent removal (Fig. 1). The semi-solid hydroalcoholic extract (HA-E) was taken to complete dryness by lyophilization processes (Fig. 1) (Olmedo-Juárez et al., 2017).

The hydroalcoholic extract was analyzed by high performance liquid chromatography (HPLC) using a Waters 2695 (Waters Corporation, USA) and a Supelcosil LC-F column (4.6mm x 250mm, i.d., 5µm particle size; Sigma-Aldrich, Bellefonte, PA, USA) for chemical separation. The mobile phase consisted of an aqueous solution with 5% trifluoroacetic acid (Solvent A) and acetonitrile (Solvent B). The gradient system was as follows: 0-1min, 0% B; 2-3min, 5% B; 4-20min, 30% B; 21-23min, 50% B; 24-25min, 80% B; 26-27min, 100% B; 28–30min, 0% B. The retention rate was maintained at 0.9mL/min, with an injection volume of 10µL. Absorbance was measured at 270–330nm. Major compounds were identified based on retention times and the spectra emitted by each peak, using a compound library and reference standards (Mabry et al., 1970; Wagner & Bladt, 2001).

Germination Test

Germination was evaluated in 72-well trays for each ecotype. Three seeds were placed in each well, in a substrate consisting of 50% loamy soil and 50% organic matter. The design was completely randomized with a 2*3 factorial arrangement (two ecotypes: Copalillo and Pungarabato * three post-harvest times: 270, 300 and 350 days) for a total of six treatments by three replicates (three trays per treatment). Germination was evaluated cumulatively and was assessed on days 4, 5, 6, 7 and 8 after sowing in the trays. The germination percentages for each treatment were estimated from the data using the equation:

Fig. 1: Summary of the procedure developed to obtain the hydroalcoholic extract of the plant.

$$Germination(\%) = \frac{Germinatedplants(n°)}{Sownseeds(n°)} * 100$$

Statistical Analysis

In the proximate chemical composition, the comparative effect between means by ecotypes (Copalillo and Pungarabato) for dry matter (DM), ash, crude protein (PC), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), organic matter (OM), crude fiber (FC) and the variables of height, basal diameter, number of branches, leaves, flowers, pods and pod weight and biomass were analyzed in a completely randomized design. The major secondary compounds identified in the HPLC analysis were described by integrating the information of the two ecotypes. The germination percentage by ecotype effect and seed dormancy was analyzed in a 2*3 factorial design. In all cases, the differences were established by analysis of variance (ANOVA) and the comparison of means between treatments was performed with the Tukey test at P≤0.05 (SAS, 2021).

RESULTS

Phenological Evaluation

Height, basal diameter, total branch count, pod count, pod weight, and biomass weight were significantly higher (P<0.0001) in the Pungarabato ecotype (Table 1). The ecotypes were similar (P=0.52) in the number of flowers produced at the time of the study.

Chemical Composition of the Plant

Dry matter (DM) (93.0%), crude protein (CP) (22.3%), ether extract (EE) (11.9%) (P<0.0001) and organic matter (OM) (85.5%) (P=0.0285) were higher in the Altamirano ecotype (Table 2). The ecotypes were similar (P>0.05) in nutrient content, ash, neutral detergent fiber, acid detergent fiber, and crude fiber. The results indicated that the Pungarabato ecotype has a higher nutritional value compared to the Copalillo ecotype, highlighting its potential for forage use.

Table 1: Phenological characteristics of two ecotypes of *Desmanthus virgatus* evaluated in a natural environment

	Treatments			
Variables	Copalillo	Pungarabato	P-value	Means
Altura (cm)	130.15	179.42	<0.0001	154.78
Basal diameter (cm)	1.036	1.251	<0.0001	1.1435
Total branches (n°)	25.40	47.46	<0.0001	36.43
Compound leaves (n°)	204.30	-	-	-
Flowers (n°)	30.05	26.04	0.5253	28.04
Pods (n°)	29.57	367.08	<0.0001	198.32
Pod weight (g)	8.42	56.17	<0.0001	32.3
Biomass weight (g)	18.92	100.83	<0.0001	59.87

Table 2: Chemical composition (%) of the *Desmanthus virgatus* foliage Copalillo and Altamirano ecotypes evaluated in a natural environment

Ecotypes	DM	Ashes	CP	EE	NDF	ADF	OM	CF
Copalillo	92.0	7.5	16.0	10.2	69.0	54.6	84.5	14.1
Pungarabato	93.0	7.4	22.3	11.9	68.2	53.0	85.5	14.8
p-value	<0.0001	0.9394	<0.0001	<0.0001	0.6196	0.6258	0.0285	0.5367
MSE	0.4447	0.9135	3.1392	0.5299	3.6665	8.0401	0.9943	2.9512

DM: dry mater, CP: crude protein, EE: ethereal extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, OM: organic matter, CF: crude fiber

Major Secondary Compounds

The secondary compounds similar between ecotypes were gallic acid, quercetin rhamnoside, kaempferol rhamnoside, quercetin glucoside, and kaempferol glucoside (Fig. 2 and 3). Methyl gallate, flavonone, and the hydroxycinnamic acid derivatives quercetin and kaempferol glucoside were found only in the Pungarabato ecotype (Fig. 2). Rutin, quercetin, and kaempferol glycosides were identified only in the Copalillo ecotype (Fig. 3). The gallic acid peak 1 in both ecotypes ranged 7.15–7.23min and 216.6–217.8nm. The quercetin rhamnoside peak 8 (Fig. 2) and peak 7 (Fig. 3) ranged 11.73–11.77min and 209.6–210.7nm, and the kaempferol

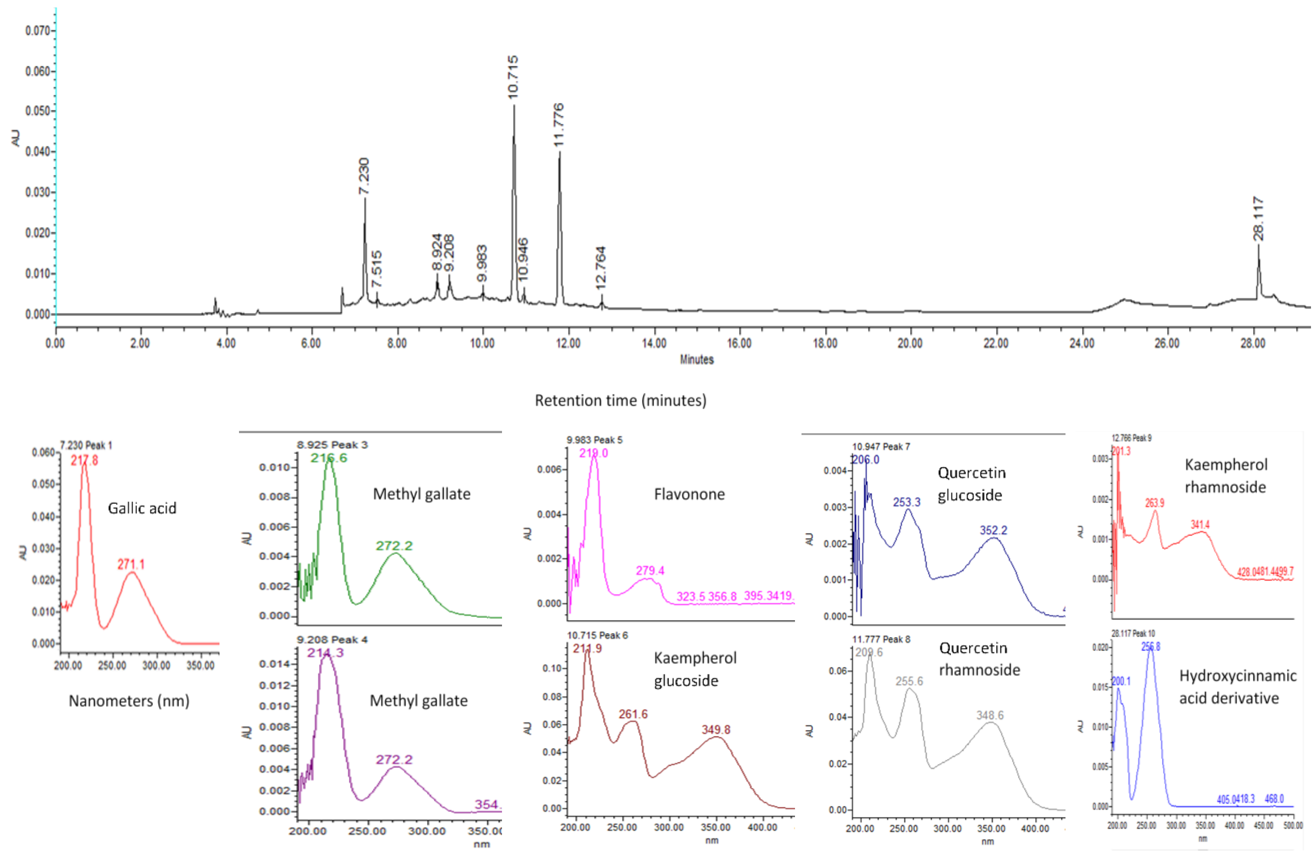

Fig. 2: Major secondary compounds in the foliage of *D. virgatus* ecotype Pungarabato (HPLC: 270 nanometers (nm)).

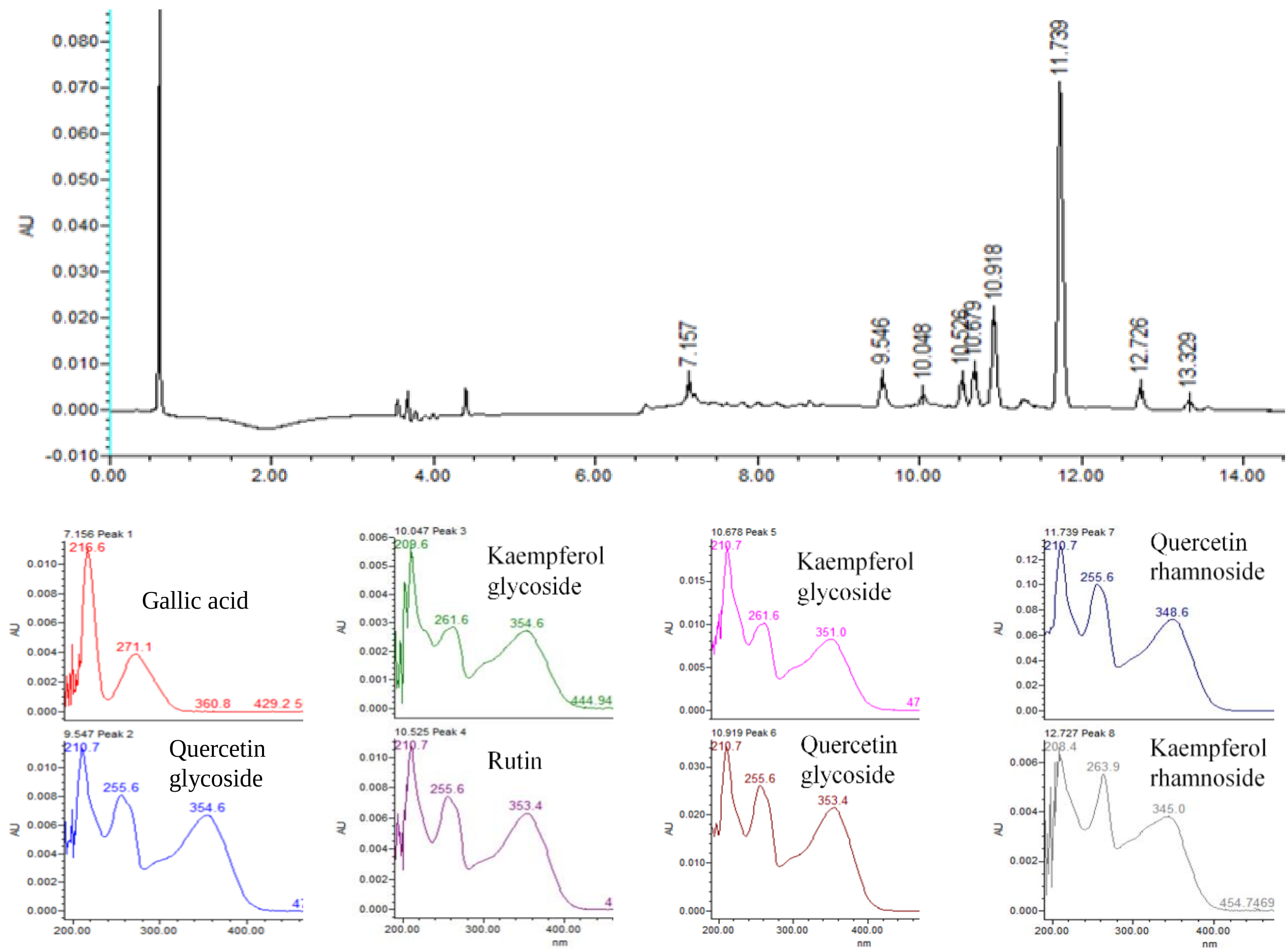

Fig. 3: Major secondary compounds in the foliage of *D. virgatus* ecotype Copalillo (HPLC: 270 nanometers (nm).

rhamnoside peak 9 (Fig. 2) and peak 8 (Fig. 3) ranged 12.72–12.76min and 201.3–208.4nm. The secondary compounds in Fig. 2 showed the methyl gallate peaks 3 and 4 with a retention time of 8.925–9.208min and wavelength 214.3–216.6nm, the flavonone peak 5 with a retention time of 9.983min and 219.0nm wavelength, the quercetin glucoside peak 7 with a retention time of 10.947min and wavelength of 206nm, and the kaempferol glucoside peak 6 with a retention time of 10.715min and wavelength of 211.9nm.

Of the compounds in Fig. 3, rutin peak 4 had a retention time of 10.525min and a wavelength of 210.7nm. Quercetin glycoside peaks 2 and 6 had a retention time of 9.547–10.919min and a wavelength of 210.7nm. Kaempferol glycoside peak 5 had a retention time of 10.678min and a wavelength of 210.7nm.

Germination Test

The seeds of the two *D. virgatus* ecotypes had similar (P>0.6) germination rates, with 40.3% and 34.7% for the Copalillo and Pungarabato ecotypes, respectively (Fig. 4A). However, storage period influenced germination. The germination rate after 300 days of storage was significantly greater than the germination rates after 270 and 350 days of storage (Fig. 4B).

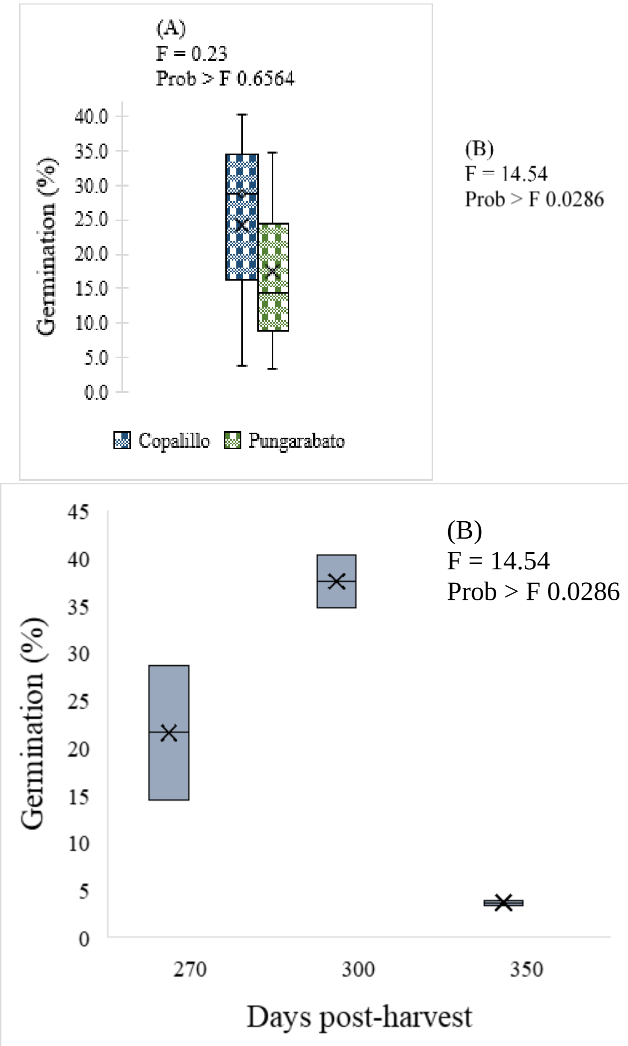

Fig. 4: Germination results in seeds by ecotype (A) and storage days (B).

DISCUSSION

The phenological results demonstrated that the Pungarabato ecotype of *D. virgatus* identified showed greater development with more robust specimens with a greater number of branches, more pod production, and greater weight, which could be related to the plant's ability to compete for space and nutrients.

Furthermore, the development of the two ecotypes in their natural environment was within the dasometry reported by Cunha et al. (2021), who recorded stem diameters between 1 and 3 cm, plant height ≥40 cm, and biomass production between 14 and 222g/plant. Zuber et al. (2023) reported that *D. virgatus* plants (laboratory and field specimens) have the capacity to develop between 4 and 7 nodules per plant root. In our study, nodules were also identified in field specimens (Fig. 5). Etesami & Santoyo (2025) described several of the plant's physiological processes in the nodules, such as increased fixed nitrogen fixation in the soil and plant, improved use of iron and phosphorus in plant nutrition, increased chlorophyll content and photosynthetic activity, and activation of anti-oxidative enzymes that are related to the symbiosis of rhizobic and non-rhizobic bacteria that colonize the nodules. These findings underscore the potential role of *D. virgatus* in establishing sustainable grazing systems.

In the chemical composition analysis, *D. virgatus* Pungarabato ecotype had greater nutritional value by surpassing the Copalillo ecotype in CP, DM, EE, and OM (Table 1). Based on its nutrient content, especially the CP between 16 and 22%, the forage potential of the plant may replace many foods in the diet of animals. Mwangi et al. (2022) reported CP levels between 19 and 29%, similar to those observed in our study, in several *Desmantus* cultivars. Mwangi et al. (2022) also reported neutral detergent fiber of 37–51% and acid detergent fiber of 14–32%, which are lower percentages than those observed for the Copalillo and Pungarabato ecotypes.

Fig. 5: Image of the *Desmanthus virgatus* plant (average height range in cm) and assessment of the presence of root nodules (arrows on the right image).

Despite the variation in nutrient content, *D. virgatus* has dry matter digestibility reaching 58–85% (Mwangi et al., 2022). Although the CP content is high, Mwangi et al. (2022) reported low ruminal digestion of the CP and related it to possible direct effects on the formation of tannin-protein complexes (Olivares-Pérez et al., 2019) attributed to the secondary compounds in the plant. The implications of the formation of these complexes have been experimentally investigated. Manuel-Pablo et al. (2020) reported that inclusion levels of 1.5, 3.0, and 4.5% of tannins from the fruit of cascalote, *Caesalpinia cacalaco* Bonpl. (Fabaceae), added to the diet of male goats did not affect their growth parameters and ruminal fermentation. Besharati et al. (2022) suggested that this may be due to tannin–protein complexes formed in the rumen dissociating under the acidic conditions of the stomach, allowing the released protein to be digested into amino acids in the small intestine for improved utilization.

The HPLC analysis identified several secondary compounds, including gallic acid, quercetin rhamnoside and glucoside, kaempferol rhamnoside and glucoside, rutin, methyl gallate, flavanones, and a hydroxycinnamic acid derivative. These findings suggest that, in addition to its valuable protein content, this legume can offer nutraceutical benefits due to the diverse range of beneficial secondary compounds in its foliage. Olmedo-Juárez et al. (2025) demonstrated that hydroxycinnamic acid derivatives and flovone were some of the most active compounds to inhibit the eggs of the nematode *Haemonchus contortus* and thus break the cycle of the ruminant parasite. Velazquez-Antunez et al. (2023) also reported the effect of hydroxycinnamic acid extracted from the leaves of *Guazuma ulmifolia* Lam. (Malvaceae) in inhibiting the fertility of *H. contortus* eggs. De Jesús-Martínez et al. (2024) reported that gallic acid and methyl gallate kill *H. contortus* larvae. The aforementioned information suggests that several compounds in the foliage of *D. virgatus* ecotypes Copalillo and Pungarabato, native to Guerrero, Mexico, may possess biological activity capable of inhibiting pathogens harmful to animal health and positively influencing digestion and metabolic processes in ruminants.

The seed germination rates of 40.3% for the Copalillo ecotype and 34.7% for the Pungarabato ecotype can be considered moderate. However, they are relatively low compared to the findings of Olbana et al. (2023), who reported over 80% germination following scarification in boiling water for up to 3min, and over 90% germination using sulfuric acid treatment for 20–30min. We recommend that further germination trials using various pre-germination treatments to break seed dormancy and enhance germination rates be conducted.

Conclusion

Significant differences in CP and EE exist between the Copalillo and Pungarabato ecotypes of *D. virgatus*. Protein levels ranging 15–22% and EE values of 10–12% are considered high for *D. virgatus* as a forage species. The primary secondary compounds identified included gallic acid, quercetin rhamnoside, kaempferol rhamnoside, quercetin glucoside, and kaempferol glucoside, phytochemicals that may offer valuable nutraceutical benefits in animal nutrition. Seed dormancy in *D. virgatus* exceeded 300 days of storage. As a legume capable of forming root nodules, *D. virgatus* contributes to atmospheric nitrogen fixation, supporting sustainability in extensive animal production systems.

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Data Availability: All the data generated during the study are present in the article.

Ethics Statement: The authors declare that they have no conflict of interest with the project.

Author's Contribution: Validation of information and supervision of the study S. Rojas-Hernandez, E. Hernandez Castro and R. Jimenez-Guillen; Methodology and resources R. Bueno-Dirzo, A. Villa Mancera and A. Olmedo-Juarez; Software, Supervision, Writing - review & editing, Writing - original draft J. Olivares-Perez, M.A. Damian-Valdez and F. Quiroz-Cardoso; guided research for undergraduate students L. Sarabia-Salgado, M. Cipriano-Salazar and E. Robledo-Reyes.

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