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Original article

Comprehensive phenolic profiling of Australian-grown Progrades™ *Desmanthus* through LC-ESI-QTOF-MS^2 and determination of their antioxidant potential

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Summary *Desmanthus* is a Mimosaceae legume genus that is native to the Americas. Decades ago, the introduction of *Desmanthus* with impressive survival ability in semi-clay soil filled the gap of no sown pasture legume adaptive to the northern Australian environment. In this study, the comprehensive phenolic profile of Progrades™ *Desmanthus* collected across northwestern Queensland was characterised by LC-ESI-QTOF-MS^2. Their antioxidant potential was evaluated by the *in vitro* antioxidant assays and correlatively analysed with the phenolic contents. As discovered, the phenolic contents varied significantly among geographical locations. *Desmanthus* collected from Armreynold showed the highest phenolic content mainly consisting of condensed tannins, whereas the Madison region sample displayed the highest flavonoid content. The mass spectrometry results identified 68 phenolic compounds, highlighting 21 phenolic acids and 36 flavonoids. This study provides academic evidence for the utilisation of *Desmanthus* in food and husbandry industries as an antioxidant ingredient or a potential source of phenolic compounds.

Keywords Antioxidant activities, characterisation, *Desmanthus*, phenolic compounds.

Introduction

Desmanthus is a Mimosaceae legume genus containing more than 24 species, which is native to North, Central and South Americas (Gardiner & Rangel, 1996). Benefiting from its growth habit in extreme environments, *Desmanthus* was considered to be utilised as an economical and suitable choice of pasture plant in northern Australia. Five common species include *Desmanthus virgatus*, *D. leptophyllus*, *D. illinoensis*, *D. icornutus* and *D. pernambucanus* located in tropical/subtropical areas with various climatic and geographical conditions. The first three *Desmanthus* species were mainly used as forage in the Americas, whereas *D. pernambucanus* were commonly used as hedgerows in the alleys of farming systems in Thailand and India due to their taller height (Gardiner & Rangel, 1996). In light of the suitable tropical/subtropical environment, *Desmanthus* was introduced from the Americas decades ago to make it a potential forage legume for Australian agriculture to solve the problem of deficient pasture legumes in northern Australia (Kenny & Drysdale, 2019). Considering the semi-arid clay soils in northern Australia, *D. virgatus* (from Argentina) *D. leptophyllus* (from Cuba) and *D. pernambucanus* (from Mexico) were the three imported *Desmanthus* species. After the abandoned field plots and selected persistent genotypes had been evaluated by the CSIRO and the Department of Agriculture, the new cultivars known as Progardes™ *Desmanthus* were developed and released (Gardiner, 2016).

The latest reports have shown that *Desmanthus* has been applied widely as forage for animals and showing promising results among different land types and sowing techniques across northern Australia such as reducing methane emissions or improving animal productive performance (Suybeng *et al.*, 2020, 2021). Although many graziers utilise *Desmanthus* as a forage for the crude protein content and ability to fix nitrogen, the polyphenol within *Desmanthus* may also be important. This is because a medium level of the

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condensed tannin content naturally released by the self-defence system of legumes can result in enough positive impacts on animal nutrition, protein digestion and animal productivity (Terrill *et al*., 1992; Barry & Mcnabb, 1999). However, in recent studies related to healthy and efficient forage, the phenolic profile has been paid more research attention as a significant factor concerning the productivity and health of ruminants (Verdecia *et al*., 2020). This is because phenolic compounds, especially condensed tannins and lignins, can form resilient covalent bonds with cellulose and hemicellulose, which would hinder the microbial and physical degradation of ingested feed and reduce the nutritional value of the formulated diet (Waghorn & Mcnabb, 2003). As an illustration, flavonoids (such as rutin and quercitrin) and condensed tannins were commonly found in *D. illinoensis* and *D. glandulosus* (Nicollier & Thompson, 1983; Gonzalez-V *et al*., 2005). Therefore, a better understanding of the comprehensive phenolic profile can provide practical and constructive suggestions for the formulation of effective and nutritive *Desmanthus* forage.

Besides its application in husbandry industries, *Desmanthus* was recorded to be used as a traditional Chinese medicinal plant (Wolfe *et al*., 2008). This medical application might be highly associated with its abundant phenolic compounds showing remarkable bioactivities. Polyphenols are a widely distributed group of secondary metabolites in plants (Suybeng *et al*., 2021). Due to their structure with the polyhydroxy group on the aromatic ring, the potential to scavenge free radicals, regulate enzymes, bond with metal ions and other functions have been applied to promote human health and wellbeing (Mukai *et al*., 2005). Polyphenols are most commonly consumed from fruits and vegetables in daily life. *Desmanthus* as a flowering plant contains polyphenols in multiple forms of flavonoids and condensed tannins (Nicollier & Thompson, 1983; Suybeng *et al*., 2021), which could be further considered as a potential source of phenolic compounds or an alternative antioxidant ingredient. Most of today's studies on *Desmanthus* plants focus more on their potential contribution to husbandry, while the diverse bioactive phenolic profile of *Desmanthus* has been neglected. Therefore, this research investigated the comprehensive phenolic profiles of six different accessions of Australian-grown *Desmanthus* and further evaluated the most promising species in terms of phenolic contents and antioxidant performance.

Material and methods

Chemicals and materials

All chemicals were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia) with the exceptions of 99% ethanol from Thermo Fisher (Waltham, MA, USA) and 98% sulfuric acid from RCI Labscan Ltd. (Rongmuang, Thailand). All six samples of *Desmanthus* were collected among different locations in northern Australia (two in the Burketown area and four in the Gregory region), in which edaphic, climatic conditions vary for separate samples. Each sample is assigned with names characterised by their location information and labels: *Desmanthus* collected from Burketown East (17°58′069” S, 159°45′55″ E, adjust bar SE, 24/04/21) was assigned with BURE; *Desmanthus* collected from Burketown West (adjust bar NW, 24/04/21) was assigned with BURW; *Desmanthus* collected from Gregory trip D (18°46′705” S, 139°08′999″ E, D/D plant tips, 23/04/21) was assigned with GTD; *Desmanthus* collected from Gregory trip B (18°39′611” S, 139°69′133″ E, B/B plant tips, 23/04/21) was assigned with GTB; *Desmanthus* collected from Gregory trip C (18°40′475” S, 139°09′066″ E, C/C plant tips, 23/04/21) was assigned with GTC; and *Desmanthus* collected from Gregory trip A (18°38′3905” S, 139°09′719″ E, A/A plant tips, 23/04/21) was assigned with GTA.

Sample preparation and extraction of phenolics

The *Desmanthus* samples were dried at 60 °C and the whole samples were ground to 2 mm through a cutting mill (Retsch SM300, Germany) for further experimentation. The extraction process was conducted by following Duan *et al.* (2023) with modifications. Specifically, 20 mL of 70% ethanol was used to extract the processed samples (w/v:1/1). Then, the extracts were homogenised by the Ultra-Turrax T25 homogeniser for 20 s (IKA Inc., Wilmington, NC, USA), followed by an overnight incubation in a shaking incubator (ZWYR-240, Labwit) at 120 rpm and 4 °C. After incubation, phenolic extract supernatants collected after the centrifuge (Hettich Rotina 380R) at 5000 rpm for 15 min at 4 °C and filtration using 0.45 μm syringe filters were stored at −20 °C for further analyses.

Estimation of polyphenols and antioxidant assays

Total polyphenol content (TPC)

The TPC of *Desmanthus* samples were measured by the Folin–Ciocalteu method following the previous antioxidant research article published by Ma *et al.* (2019). The detailed protocol was described in Method S1.

Total flavonoid content (TFC)

The TFC of *Desmanthus* samples was measured by the modified aluminium chloride method according to the protocol reported by Duan *et al.* (2023). The detailed method is shown in Method S2.

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Total condensed tannins (TCT)
The TCT of *Desmanthus* samples were measured by colourimetric content based on the research article from Wu *et al.* (2022). The detailed procedures were described in the Method S3.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay
The DPPH assay was used to determine the radical scavenging activity of *Desmanthus* samples by following the method reported by Peng *et al.* (2019) with some modifications. The detailed method was shown in the Method S4.

Ferric Reducing-Antioxidant power (FRAP) assay
The FRAP assay was conducted by following the method from Benzie & Strain (1996) with some modifications. The detailed protocol was reported in the Method S5.

2,2′-Azinobis-(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS) radical scavenging assay
The ABTS assay was used to evaluate the free radical scavenging activity of *Desmanthus* samples by following the method reported by Re *et al.* (1999). The method was detailed in the Method S6.

Hydroxyl radical scavenging activity (•OH-RSA)
The determination of •OH-RSA was based on the Fenton-type reaction method developed by Smirnoff & Cumbes (1989) with some necessary modifications. The detailed procedures are shown in Method S7.

Ferrous ion chelating activity (FICA)
According to Amrit *et al.* (2023), the ferrous ion chelating activities of *Desmanthus* samples were measured with necessary modifications. The detailed protocol was reported in the Method S8.

Reducing power assay (RPA)
By following the method reported by Peng *et al.* (2019) with the necessary modification, the reducing power activities of *Desmanthus* samples were determined. The detailed method was reported in the Method S9.

Total antioxidant capacity (TAC)
The phosphomolybdate method was used for the determination of total antioxidant capacity (Peng *et al.*, 2019). The procedures were detailed in the Method S10.

Characterisation of phenolic compounds by LC-ESI-QTOF/MS analysis

The phenolic profiles of *Desmanthus* samples were characterised by Agilent 1200 series HPLC equipped with an Agilent 6520 Accurate-Mass Q-TOF MS (Agilent Technologies, U.S.A.) by following the method reported by Duan *et al.* (2023). The specific parameters were recorded in the Method S11.

HPLC-PDA analysis

An Agilent-1200 HPLC system (Agilent Technologies, U.S.A.) equipped with a photodiode array detector (Agilent Technologies, U.S.A.) was employed to quantify the phenolic contents in *Desmanthus* samples. A reversed-phase C18 analytical column (3 μm, 150 mm × 4.6 mm Synergi Hydro-RP 80A) was used for separation. The mobile phase A was 1% acetic acid in water and mobile phase B was acetonitrile. The elution gradients were set according to Peng *et al.* (2019). The column temperature was maintained at 25 °C and the injection volume was 20 μL. The flow rate was 0.4 mL min^{-1} and the wavelength detection range was set to 200–600 nm.

Statistical analysis

The data for antioxidant activities and phenolic contents were represented as means ± standard deviation. One-way analysis of variance (ANOVA) was used to test differences in terms of mean values when comparing different samples. Tukey's honest significant differences (HSD) were used to run multiple rank tests at $P < 0.05$.

Results and discussion

Polyphenols estimation of *Desmanthus* samples

Desmanthus from South America was found to be surviving and thriving in semi-arid clay soil. Therefore, Desmanthus as a potential forage choice was investigated in terms of its polyphenols content and antioxidant potential, and results were summarised in Table 1.

For TPC, Desmanthus sample BURW had the highest value (71.69 mg GAE g^{-1}) with sample GTA (67.85 mg GAE g^{-1}) and BURE (60.89 mg GAE g^{-1}) the next highest. The variation in TPC content could arise due to different geographical origins, soil types and climatic conditions as samples BURE and BURW were collected closer to sea whereas the others were located further inland (Tounekti *et al.*, 2013). Since sample GTA was obtained close to the Gregory River in western Queensland this could be a reason why it had a higher TPC value, which was geographically found to be different from accessions GTD, GTB and GTC. Flavonoids and condensed tannins had previously been found in *Desmanthus illinoensis*, although there were no values for TPC, TFC or TCT values were reported. In comparison, research from China that includes 45 common medicinal plants only had

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Table 1 Determination of phenolic content and their antioxidant activity

Assays	BURE	BURW	GTD	GTB	GTC	GTA
TPC (mg GAE/g)	60.89 ± 0.95^{b}	71.69 ± 0.37^{a}	48.37 ± 1.41^{c}	49.35 ± 1.13^{c}	59.94 ± 2.79^{b}	67.85 ± 0.84^{a}
TFC (mg QE/g)	4.73 ± 0.05^{b}	4.47 ± 0.14^{b}	4.17 ± 0.20^{b}	4.34 ± 0.29^{b}	6.70 ± 0.27^{a}	4.40 ± 0.38^{b}
TCT (mg CE/g)	0.72 ± 0.04^{c}	3.07 ± 0.09^{a}	0.23 ± 0.08^{d}	0.05 ± 0.04^{e}	0.88 ± 0.04^{c}	1.45 ± 0.20^{b}
DPPH (mg AAE/g)	117.16 ± 2.62^{c}	147.84 ± 2.33^{a}	101.30 ± 2.18^{d}	103.39 ± 0.74^{d}	130.01 ± 3.82^{b}	133.37 ± 9.22^{b}
FRAP (mg AAE/g)	12.82 ± 0.19^{c}	18.65 ± 0.22^{a}	10.48 ± 0.38^{d}	11.44 ± 0.16^{c}	15.46 ± 0.49^{b}	15.75 ± 0.98^{b}
ABTS (mg AAE/g)	96.92 ± 4.38^{d}	158.34 ± 4.90^{b}	103.82 ± 4.97^{c}	107.03 ± 8.31^{c}	100.60 ± 2.03^{c}	170.76 ± 1.87^{a}
•OH-RSA (mg AAE/g)	107.64 ± 1.93^{b}	97.53 ± 2.39^{c}	66.14 ± 3.65^{d}	68.04 ± 6.45^{d}	65.51 ± 5.26^{d}	115.64 ± 1.26^{a}
FICA (mg EDTA/g)	2.43 ± 0.16^{b}	1.81 ± 0.12^{c}	1.98 ± 0.15^{c}	1.92 ± 0.17^{c}	1.98 ± 0.28^{c}	6.09 ± 0.05^{a}
RPA (mg AAE/g)	100.25 ± 12.65^{b}	118.29 ± 8.48^{a}	66.48 ± 3.81^{d}	70.79 ± 4.90^{d}	81.71 ± 5.80^{c}	107.17 ± 8.52^{b}
TAC (mg AAE/g)	12.12 ± 0.32^{a}	6.19 ± 0.23^{b}	5.63 ± 0.15^{b}	10.09 ± 0.35^{a}	11.15 ± 0.63^{a}	13.08 ± 0.80^{a}

Values are mean ± standard deviation per gram powder weight; n = 3 samples per sample. Values within the same row with different superscript letters ($^{a-e}$) are significantly different from each other ($P < 0.05$). "BURE" is from Armreynold (17.58.069 S, 159.45.55 E, adjust bar SE, 24/04/21); "BURW" is from Armreynold (adjust bar NW, 24/04/21); "GTD" is from Madison (18.46.705 S, 139.08.999 E, D/D plant tips, 23/04/21); "GTB" is from Madison (18.39.611 S, 139.69.133 E, B/B plant tips, 23/04/21); "GTC" is from Madison (18.40.475 S, 139.09.066 E, C/C plant tips, 23/04/21); and "GTA" is from Madison (18.38.3905 S, 139.09.719 E, A/A plant tips, 23/04/21).

•OH-RSA, hydroxyl-radical scavenging activity; AAE, ascorbic acid equivalents; ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid assay; CE, catechin equivalents; DPPH, 2,2′-diphenyl-1-picrylhydrazyl assay; EDTA, ethylenediaminetetraacetic acid; FICA, ferrous ion chelating activity; FRAP, ferric reducing antioxidant power assay; GAE, gallic acid equivalents; QE, quercetin equivalents; RPA, reducing power assay; TAC, total antioxidant capacity; TCT, total condensed tannins; TFC, total flavonoid contents; TPC, total phenolic contents.

two plant species that matched the same levels of TPC values as the Desmanthus samples in this report (Li *et al*., 2008). For other legumes, including plants in the same family as Desmanthus, TPC values of *Cicer arietinum* and *Pisum sativum* were found to be in the range of 11.46–19.42 mg g^{-1} extract (Nithiyanantham *et al*., 2012). Among the legumes, lentils, mung beans, red kidney beans and soybeans TPC values had been reported to be in the range of 17.0 to 21.9 mg GAE g^{-1} (Djordjevic *et al*., 2011).

As for TFC values, sample GTC had the highest value 6.70 mg QE g^{-1}, whereas other the five samples had similar between 4.17 and 4.73 mg QE g^{-1}. When it came to TCT values, sample BURW was still the highest with 3.07 mg CE g^{-1}, and sample GTA was the next with a lower value of 1.45 mg CE g^{-1}, whereas the other 4 samples showed values under 1 mg CE g^{-1}. Sample GTB had a much lower TCT of 0.05 mg CE g^{-1}. Compared with other legumes such as *Phaseolus lunatus*, TFC values ranged from 0.81 to 4.47 mg RUE g^{-1}, whereas total condensed tannins were reported to be in the range of 0–8.97 mg CAE g^{-1} (Diniyah *et al*., 2020).

With increased research interests in *Desmanthus* phenolic content, advanced analytical technologies such as LC-ESI-QTOF-MS were used to further characterise the polyphenol composition.

Antioxidant potential of *Desmanthus*

Determination of antioxidant potential was related to the ability of different polyphenol compounds to act as reducing agents, hydrogen atom donors, metal chelators and radical scavengers tested by assays based on different mechanisms. Among these assays, FRAP illustrated electron-donating ability, DPPH, •OH-RSA, FICA and ABTS evaluated the radical scavenging ability of polyphenol compounds, RPA directly showed reducing power from reduction potential and TAC measured the number of free radicals scavenged by a testing solution.

The highest value of DPPH was shown to be the BURW sample with 147.84 mg AAE g^{-1}, followed by samples GTC and GTA showed relatively lower levels of 130.01 mg AAE/g and 133.37 mg AAE g^{-1}, accordingly. Whereas samples GTD and GTB only displayed 101.30 mg AAE g^{-1} and 103.39 mg AAE g^{-1}. In comparison, *Prosopis laevigata*, as a legume species in Aridoamerica with similar climatic conditions, had a range of 9.11–9.32 mg AAE g^{-1} DPPH values, which was prominently lower than the *Desmanthus* samples (Díaz-Batalla *et al*., 2018). When it comes to FRAP, the same trend in bioactive performance was also observed, where sample BURW showed the highest potential of 18.65 mg AAE g^{-1}, followed by samples GTC and GTA showed lower values with 15.46 and 15.75 mg AAE g^{-1}, then samples GTD and GTB expressed the lowest abilities with 10.48 and 11.44 mg AAE g^{-1}. Although similar trends were observed for FRAP and DPPH values, ABTS, FICA, and •OH-RSA varied significantly among different samples. GTA sample showed the highest ABTS radical scavenging ability followed by BURW and BURE samples. This trend was in line with the antioxidant performance of all common Australia-grown berries identified previously. As Subbiah *et al*. (2020) discovered,

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the FRAP activities of berries ranged between 121.51 mg AAE g^{-1} and 367.43 mg AAE g^{-1}, whereas the DPPH and ABTS abilities were much lower and all below 5 mg AAE g^{-1}.

Vegetables and fruits from plants are known as healthy food due to their high polyphenol content and antioxidant potential. These compounds help to remove reactive oxygen species (ROSs) that could potentially damage human bodies. Assays like RPA, TAC, $^{\bullet}$OH-RSA and FICA gave clear information in terms of the antioxidant potential. RPA values of *Desmanthus* samples showed sample BURW to be the highest followed by sample GTA and sample BURE, whereas sample GTD showed the lowest RPA value. Meanwhile, $^{\bullet}$OH-RSA values demonstrated that sample GTA was the highest with sample BURE and BURW following next to it. Additionally, the highest FICA values were found in sample GTA and BURE, whereas the other 4 samples had similar values around and below 2.00 mg EDTA g^{-1}. Finally, the TAC of sample GTA was the highest with 13.08 mg AAE/g, and sample BURE, GTB and GTC were next to it with 12.12, 10.09 and 11.15 mg AAE g^{-1}, whereas sample GTD was the lowest with 5.63 mg AAE g^{-1}. However, there were still large research gaps to be filled in concerning the antioxidant and polyphenol profile of *Desmanthus* species. Most of the assays had no direct comparative data with the *Desmanthus* samples in this study. Combined with all the information gathered along with advanced assays including HPLC and LC-ESI-QTOF-MS, the identification and confirmation of the antioxidant compounds can be attained.

Correlation of polyphenols and antioxidant activities

The correlation between the results of the polyphenols and the antioxidant activities from different assays was performed using a Pearson's correlation test, which is shown in Table 2. A highly significant correlation could only be found between DPPH and FRAP values with 0.994 ($P \leq 0.01$), whereas a significant correlation could be identified with RPA correlated with DPPH (0.868 with $P \leq 0.05$), FRAP (0.837 with $P \leq 0.05$) and $^{\bullet}$OH-RSA (0.851 with $P \leq 0.05$). In the meantime, the correlations between the results of the two parts focused on TPC and TCT, with antioxidant results showing no correlation with TFC. Firstly, TPC had a significant correlation with DPPH (0.959 with $P \leq 0.01$), FRAP (0.933 with $P \leq 0.01$), and RPA (0.965 with $P \leq 0.01$). Then, for TCT, a significant correlation could be identified with DPPH (0.923 with $P \leq 0.01$) and FRAP (0.927 with $P \leq 0.01$) along with a significant correlation with RPA (0.868 with $P \leq 0.05$). From articles related to 33 cool season legumes produced in the US (Xu *et al.*, 2007), similar correlations were reported including TPC with TCT (0.93 with $P \leq 0.01$), DPPH with FRAP (0.95 with $P \leq 0.01$), TPC with DPPH (0.94 with $P \leq 0.01$), TPC with FRAP (0.96 with $P \leq 0.01$), TCT with DPPH (0.88 with $P \leq 0.05$), and TCT with FRAP (0.89 with $P \leq 0.05$).

The two principal components (F1 and F2) in Fig. 1c explained 78.91% of the total variation in *Desmanthus* data and showed clear partitioning of GTA and BURW samples from the others, which was also probed from the dendrogram. Meanwhile, GTB and BURE were also shown to be differentiated from the GTD and GTC samples. In the two sides of Fig. 1a, BURE and GTC had a stronger correlation with F1, whereas GTB and GTD were more correlated with F2, and no clear correlation was observed in GTA and BURW with either. In terms of phenolic and antioxidant parameters, F1 and F2 were strongly correlated with RPA, ABTS, TPC and TFC and TAC, respectively, where TFC and TAC were negatively correlated with each other. Even though the other

Table 2 Pearson's correlation coefficients (r) of phenolic content and the antioxidant potential

Variables	TPC	TFC	TCT	DPPH	FRAP	ABTS	RPA	$^{\bullet}$OH-RSA	FICA
TFC	0.115								
TCT	0.897**	−0.027							
DPPH	0.959**	0.282	0.923**						
FRAP	0.933**	0.280	0.927**	0.994**					
ABTS	0.751	−0.334	0.748	0.705	0.707				
RPA	0.965**	−0.088	0.873*	0.868*	0.837*	0.731			
$^{\bullet}$OH-RSA	0.751	−0.325	0.522	0.533	0.471	0.639	0.851*		
FICA	0.414	−0.202	0.122	0.266	0.218	0.659	0.390	0.667	
TAC	0.209	0.324	−0.226	0.087	0.046	0.027	0.183	0.438	0.596

$^{\bullet}$OH-RSA, hydroxyl-radical scavenging activity; ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid assay; DPPH, 2,2′-diphenyl-1-picrylhydrazyl assay; FICA, ferrous ion chelating activity; FRAP, ferric reducing antioxidant power assay; RPA, reducing power assay; TAC, total antioxidant capacity; TCT, total condensed tannins; TFC, total flavonoid contents; TPC, total phenolic contents.
*Significant correlation with $P \leq 0.05$; **Significant correlation with $P \leq 0.01$.

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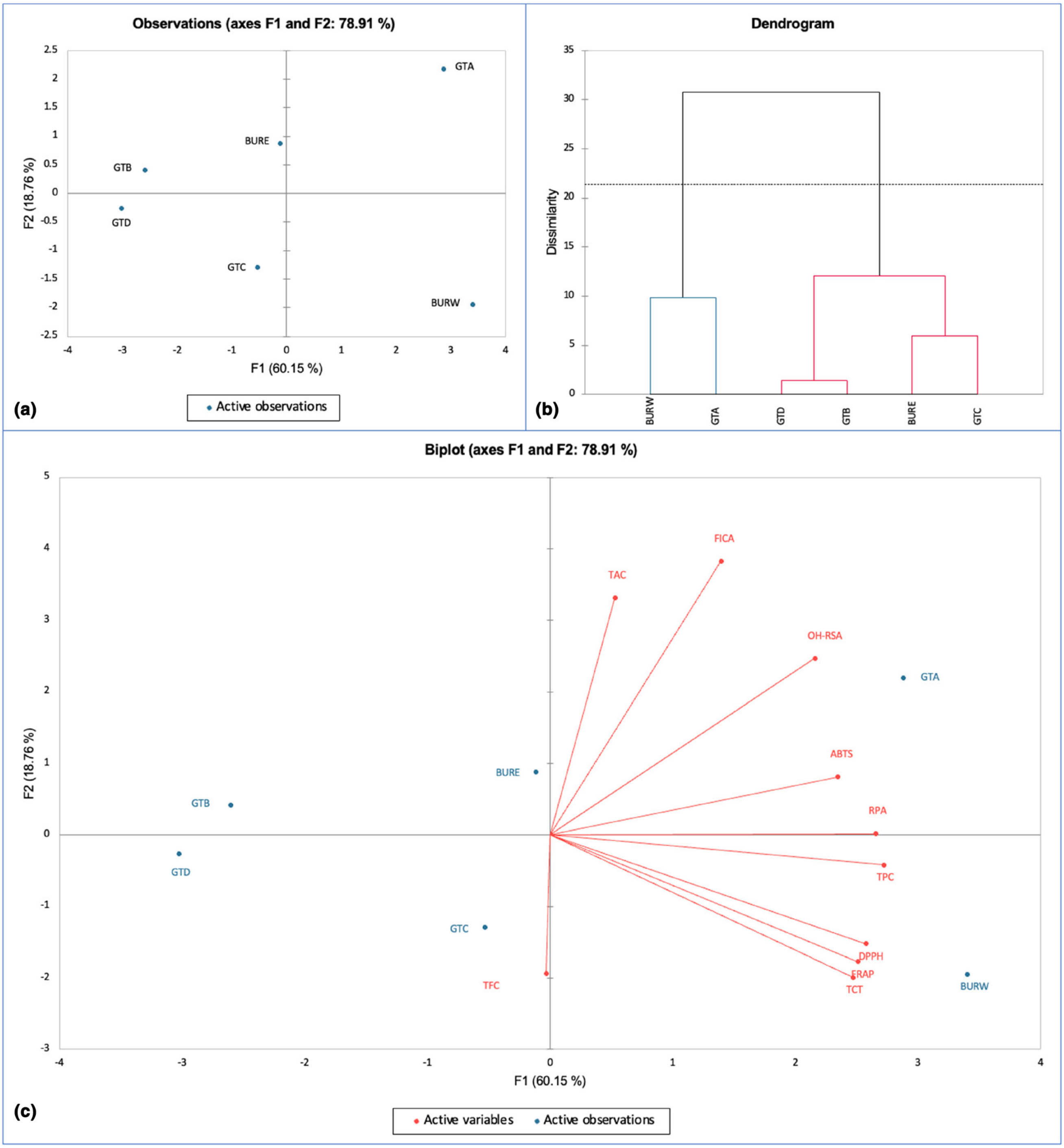

Figure 1 Further analysis graph for correlation determination combining various analysis methods. (a) observation of the six *Desmanthus* samples using biplots, (b) dendrogram of the six *Desmanthus* samples data, (c) biplots data combined with principal components analysis (PCA) graph. "BURE" is from Armreynold (17.58.069 S, 159.45.55 E, adjust bar SE, 24/04/21); "BURW" is from Armreynold (adjust bar NW, 24/04/21); "GTD" is from Madison (18.46.705 S, 139.08.999 E, D/D plant tips, 23/04/21); "GTB" is from Madison (18.39.611 S, 139.69.133 E, B/B plant tips, 23/04/21); "GTC" is from Madison (18.40.475 S, 139.09.066 E, C/C plant tips, 23/04/21); and "GTA" is from Madison (18.38.3905 S, 139.09.719 E, A/A plant tips, 23/04/21).

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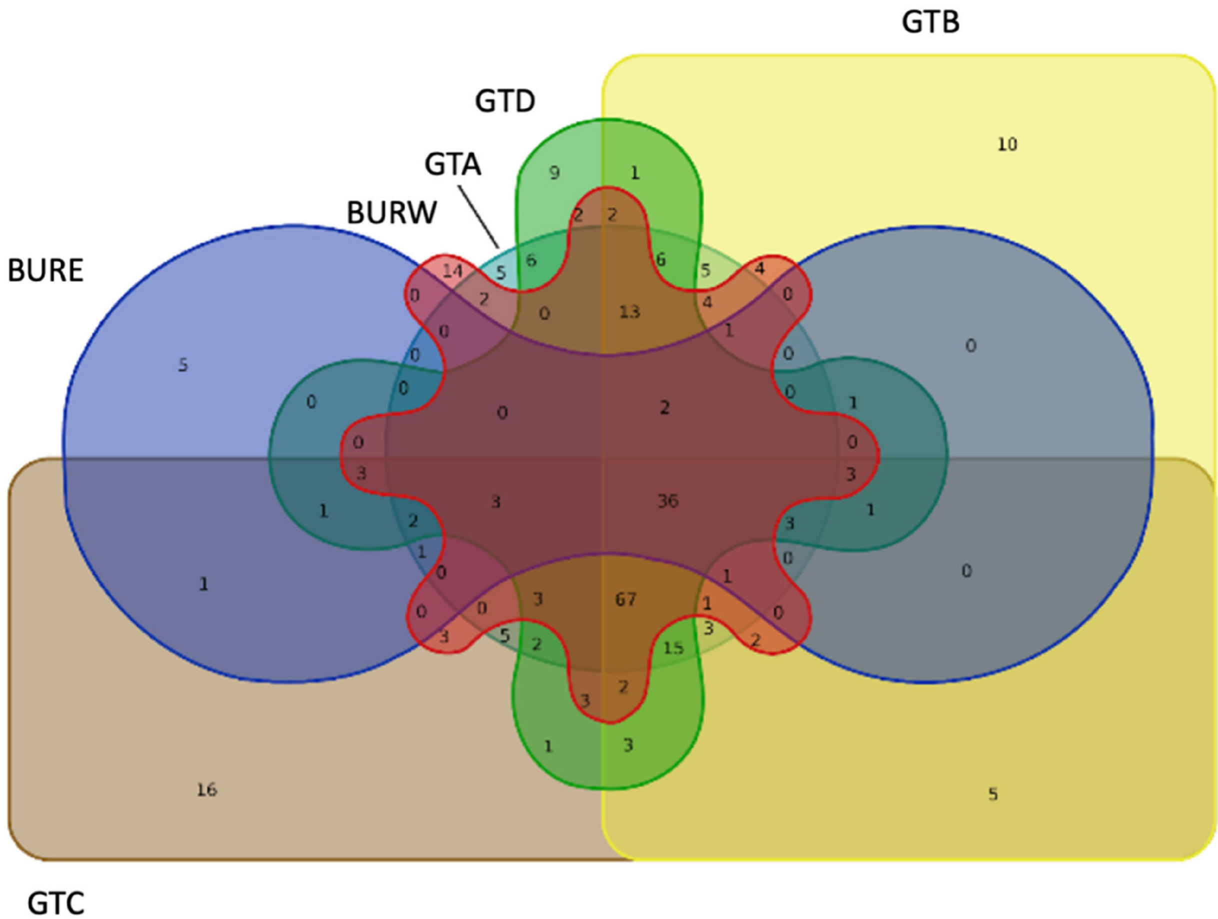

Figure 2 Venn diagram of the LC/MS–MS data visualising the similarities of the contained compounds within six different *Desmanthus* samples. "BURE" is from Armreynold (17.58.069 S, 159.45.55 E, adjust bar SE, 24/04/21); "BURW" is from Armreynold (adjust bar NW, 24/04/21); "GTD" is from Madison (18.46.705 S, 139.08.999 E, D/D plant tips, 23/04/21); "GTB" is from Madison (18.39.611 S, 139.69.133 E, B/B plant tips, 23/04/21); "GTC" is from Madison (18.40.475 S, 139.09.066 E, C/C plant tips, 23/04/21); and "GTA" is from Madison (18.38.3905 S, 139.09.719 E, A/A plant tips, 23/04/21).

parameters were not strongly correlated with either component, DPPH, FRAP and TCT were found to be highly correlated with each other and weakly correlated with F2.

Studies have found that both DPPH and ABTS assays evaluated the free radical scavenging ability, and the ABTS assay could better reflect the hydrophilic, lipophilic and high-pigmented antioxidants in legumes and other similar plants compared to the DPPH assay, which indicated the strong ABTS-reducing ability of positively F2 correlated samples. The high correlation between DPPH, FRAP, and TCT indicated that phenolic compounds present in six *Desmanthus* sample extracts exhibited the strong scavenging ability of DPPH, and ferric ion- and phosphomolybdate ion-reducing abilities, respectively (Peng *et al.*, 2019). Moreover, the dendrogram also clarified that the only similar samples were GTB and GTD of all *Desmanthus* samples with the lowest dissimilarity, which can be observed from the PCA biplot.

LC-ESI-QTOF-MS analysis of the phenolic compounds

Distribution of phenolic compounds in Desmanthus

Various forms of phenolic compounds contained in *Desmanthus* samples were investigated in this study. Considering the complexity of similarities and dissimilarities among compounds that appeared in six *Desmanthus* samples, a Venn diagram was developed and used to visualise the distribution of phenolic compounds, which was shown in Fig. 2 with different shapes and colours to demonstrate the distributions of different samples and combinations.

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Combined with the characterisation of phenolic compounds from six *Desmanthus* samples through LC-ESI-QTOF-MS from Table S1, Fig. 2 showed a more thorough and contrastive view of the phenolic compounds within the investigated *Desmanthus* samples. In total, 278 compounds with similarities were found. Among all six samples, 36 phenolic compounds appeared in all of them. In other words, the maximum overlapping polyphenols were 12.9% shared in all *Desmanthus* samples. Whereas 67 compounds were found within all samples except BURE, which was 24.1% among all phenolic compounds. As for combinations of four samples among all, 13 (4.7%) compounds could be found in BURW, GTA, GTB and GTD. Additionally, 15 (5.4%) compounds could be found in GTA, GTB, GTC, and GTD, which were collected within the same geographical area. Moreover, there were significant numbers of compounds that appeared only in one of the six samples, such as 16 (5.8%) compounds only appeared in GTC, 14 (5.0%) compounds only appeared in BURW, 10 (3.6%) compounds only in GTB, 9 (3.2%) compounds only in GTD, 5 (1.8%) compounds only in BURE and also 5 (1.8%) only in GTA. Unfortunately, with limited similar studies related to phenolic compounds within Desmanthus plants and the investigated PROGARDES samples, no direct cross-reference could be used to compare the phenolic compounds distribution.

LC-ESI-QTOF-MS characterisation of the phenolic compounds

Phenolic acids. In this study, a total of 21 phenolic acids including hydroxybenzoic acids (6), hydroxycinnamic acids (12), hydroxyphenyl acetic acids (2), hydroxyphenyl propanoic acids (1) were identified and characterised in six *Desmanthus* samples.

Hydroxybenzoic acids: All six hydroxybenzoic acid compounds were characterised as Gallic acid 4-*O*-glucoside, Gallic acid, 2,3-Dihydroxybenzoic acid, 4-Hydroxybenzoic acid 4-*O*-glucoside, Protocatechuic acid 4-*O*-glucoside and 2-Hydroxybenzoic acid respectively and observed to be present in negative ionisation mode. Precursor ions *m*/*z* include 331.067 for compound **1**, 169.0142 for compound **2**, 153.0193 for compound **3**, 299.0772 for compound **4**, 315.0721 for compound **5**, 137.0248 for compound **6**. Loss of CO_2 (44 Da) was observed in five of these six compounds, which were characterised by loss of CO_2 from precursor ions to product ions 125 (compound **2**), 109 (compound **3**), 255 (compound **4**), 93 (compound **6**), or between 169 and 152 product ions from compound **1** (Rajauria *et al*., 2016). Meanwhile, a loss of hexosyl moiety (162 Da) was observed in compounds **1** and **5** from the 169 and 153 product ions (Wang *et al*., 2016). Gallic acid 4-*O*-glucoside was observed in samples BURW, GTD, and GTA which were reported to be found in bay and thyme as widely used Australian-grown herbs; gallic acid, as reported in basil and mint, and 2-Hydroxybenzoic acid, as reported in thyme, mint, rosemary, bay, basil, sage, oregano, was observed in all samples; dihydroxybenzoic acid, as reported in rosemary, mint, thyme, basil, was observed in all but sample BURE; 4-Hydroxybenzoic acid 4-*O*-glucoside, as reported in basil, thyme, sage, was observed in sample GTB and GTA; protocatechuic acid 4-*O*-glucoside, as reported in rosemary, thyme, mint, basil, was observed in sample GTC and GTA (Ali *et al*., 2021).

Hydroxycinnamic acids (HQCA) and other phenolic acid derivatives: Twelve compounds with antioxidant potential were observed in hydroxycinnamic acids. Compound **16** was identified as 3-Feruloylquinic acid ($[M-H]^-$ *m*/*z* at 367.1034) observed in a negative mode in sample GTD, GTB and GTA. The product ions were respectively at *m*/*z* 298, *m*/*z* 288, *m*/*z* 192, and *m*/*z* 191 due to the loss of [M-H-C_4H_5O], [M-H-CH_3-O_4], [M-H-$C_7H_{11}O_5$] and [M-H-$C_7H_{12}O_5$] from the precursor molecule, where Feruloylquinic acid (FQA) as a significant kind of hydroxycinnamic acids were predominantly found in coffee beans as an important HQCA source in certain diets (Nagy & Abrankó, 2016). 3-caffeoylquinic acid was a compound found in all Madison samples, with precursor $[M-H]^-$ *m*/*z* at 353.0884 present in *Chrysanthemum coronarium* L. (Wan *et al*., 2017) and also coffee (Gonçalves *et al*., 2017), yielded product ions at *m*/*z* 253, *m*/*z* 190 and *m*/*z* 144 due to the corresponding loss of $HCOOH{\cdot}3H_2O$, $C_6H_5O_2{\cdot}3H_2O$ and $C_7H_{11}O_6{\cdot}H_2O$, respectively, from the precursor molecule. Caffeoyl glucose with the precursor ion at $[M-H]^-$ *m*/*z* 341.088 had been identified only in sample BURE, and the fragment peaks at *m*/*z* 179 and *m*/*z* 161 due to the loss of hexosyl moiety and a water molecule falling off after that, which was further confirmed that the caffeoyl glucose was present in *Annona crassiflora* (Roesler *et al*., 2007) and semen cuscutae (Zhang *et al*., 2018).

Flavonoids. A total of 33 flavonoids were identified including flavanols (9), flavones (4), flavanones (3), flavonols (9), dihydro flavonols (2), anthocyanins (2) and isoflavonoids (7).

Flavanols: Nine compounds as classified in flavonols were identified in this study. (+)-Gallocatechin (Compound **23**, $C_{15}H_{14}O_7$) as found in all six samples was tentatively identified at *m*/*z* 305.0660, which formed the fragment ions at *m*/*z* 261 and 219 *via* the removal of one unit of CO_2 (44 Da) and one unit of C_3O_2 (86 Da) from the precursor ion, respectively. It was well known for its antioxidant and cardiovascular protective effects (Plumb *et al*., 2002), as it was commonly found in tea, red wine and cocoa,

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and Australian-grown herbs such as bay and sage (Ali *et al.*, 2021). 4'-*O*-Methyl-(−)-epigallocatechin 7-*O*-glucuronide (Compound **25**, $C_{22}H_{24}O_{13}$), as found in sample GTD and GTA, was tentatively identified at *m/z* 495.1127, which formed the fragment ions at *m/z* 451 and 313 *via* the removal of one unit of CO_2 (44 Da) and one unit of $C_9H_{10}O_4$ (182 Da) from the precursor ion, which was also reportedly found in Australian-grown thyme from Ali *et al.* (2021).

Flavones, flavanones and flavonols: Four compounds as classified flavones, three as flavanones and nine as flavonols were identified in this study. Apigenin 6-C-glucoside (Compound **31**, $C_{21}H_{20}O_{10}$) as found in all six samples was tentatively identified at *m/z* 431.0992, which formed the fragment ions at *m/z* 413, 341, and 311 *via* the removal of one unit of H_2O (18 Da), one unit of $C_3H_6O_3$ (90 Da) and one unit of $C_4H_8O_4$ (120 Da) from the precursor ion, respectively (March *et al.*, 2006), which was reported to be found in thyme, rosemary, sage, and bay (Ali *et al.*, 2021). Compound **37** was identified as Narirutin, which was found in sample GTD, GTB and GTA, with *m/z* 579.1723 had a fragment ion at *m/z* 271 *via* the removal of $C_{12}H_{20}O_9$ (308 Da), which was also reportedly found in mint (Ali *et al.*, 2021). Quercetin 3-*O*-arabinoside (compound **44**, $C_{20}H_{18}O_{12}$), as found in all but sample GTC in our study, was tentatively identified at *m/z* 449.0736, which formed the fragment ion at *m/z* 317 *via* the removal of one unit of $C_5H_8O_4$ (132 Da), which was also reportedly found in Australian-grown basil from Ali *et al.* (2021).

Dihydroflavonols, anthocyanins and isoflavonoids: Two compounds as classified dihydroflavonols, two as anthocyanins and seven as isoflavonoids were identified in this study. Dihydromyricetin 3-*O*-rhamnoside (compound **47**, $C_{21}H_{22}O_{12}$) as found in all but sample BURE and GTC in our study, was tentatively identified at *m/z* 465.1042, which formed the fragment ion at *m/z* 301 *via* the removal of one unit of $C_6H_{12}O_5$ (164 Da), which was also reportedly found in Australian-grown thyme from Ali *et al.* (2021). Cyanidin 3-*O*-galactoside (compound **57**, $C_{21}H_{21}O_{11}$) as found in all but sample BURE in our study, was tentatively identified at *m/z* 450.1163, which formed the fragment ion at *m/z* 287 *via* the removal of one unit of $C_6H_{11}O_5$ (163 Da), which was also reportedly found in pistachio (Bellocco *et al.*, 2016) and blueberries (Long *et al.*, 2014). Equol (compound **55**, $C_{15}H_{14}O_3$) as found in sample BURE and GTC in our study, was tentatively identified at *m/z* 243.1019, which formed the fragment ions at *m/z* 225, 211 and 197 *via* the removal of one unit of CH_2.

Other polyphenols. Demethoxycurcumin as a curcuminoid (compound **60**, $C_{20}H_{18}O_5$) found in sample GTB and GTC in our study, was tentatively identified at *m/z* 337.1087, which formed the fragment ion at *m/z* 217 *via* the removal of one unit of C_8H_8O (120 Da) from the precursor ion, which was also reportedly found in *Curcuma longa* in Thailand (Pothitirat & Gritsanapan, 2005). 3,4-DHPEA-AC as a tyrosol (compound **63**, $C_{10}H_{12}O_4$) found only in sample BURW in our study, was tentatively identified at *m/z* 195.0654, which formed the fragment ion at *m/z* 135 *via* the removal of one unit of $C_2H_4O_2$ (60 Da) from the precursor ion, which was also reportedly found in Australian-grown oregano (Ali *et al.*, 2021). The only hydrobenzoketone 2-Hydroxy-4-methoxyacetophenone 5-sulphate (compound **64**, $C_9H_{10}O_7S$) found only in samples GTB and GTA in our study, was tentatively identified at *m/z* 261.0082, which formed the fragment ion at *m/z* 181 and 97 *via* the removal of one unit of SO_3 (80 Da) from the precursor ion and C_5H_5O after the removal of SO_3, which was also reportedly found in Australian-grown rosemary and thyme from Ali *et al.* (2021).

Lignans. There is only one lignan found in this study, which is Todolactol A (compound **68**, $C_{20}H_{24}O_7$) found only in sample BURW in our study, was tentatively identified at *m/z* 375.1439, which formed the fragment ion at *m/z* 313 and 137 *via* the removal of one unit of $C_2H_6O_2$ (62 Da) and one unit of $C_{12}H_{14}O_5$ (238 Da) from the precursor ion, which was also reportedly found in Norway spruce knot wood along with berries and oilseed species (Smeds *et al.*, 2012).

Conclusion

In conclusion, most of the collected *Desmanthus* samples showed remarkable antioxidant potential, which could be attributed to their diverse and abundant phenolic profile. These research outcomes could not only provide animal frames practical suggestions in *Desmanthus* forage formulation from the animal nutrition point of view but also indicate that *Desmanthus* can be further utilised as a promising source of phenolic compounds or antioxidant ingredients from the food perspective. Notably, different assays used in this study showed comprehensive but variable data when testing the samples within the same geographical area. There were not only obstacles in comparing phenolic profiles and antioxidant potential among similar *Desmanthus* species but also a lack of solid data and supportive cross-reference to validate the variances among similar species growing from different geographical locations and climate conditions due to lack of research attention. Future studies can focus on the interspecific and regional differences to develop a more comprehensive phenolic profile and antioxidant potential of *Desmanthus* species.

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Author contributions

Xi Kang: Conceptualization; methodology; investigation; validation; formal analysis; writing – original draft; software. **Siwei Guo:** Software; conceptualization; investigation; writing – original draft; validation. **Cundong Xie:** Software; formal analysis; writing – original draft; writing – review and editing; investigation. **Christopher Gardiner:** Conceptualization; validation; writing – review and editing; resources. **Nick Kempe:** Conceptualization; validation; writing – review and editing; resources. **Hafiz A. R. Suleria:** Conceptualization; methodology; supervision; writing – review and editing; funding acquisition; resources; validation. **Frank R. Dunshea:** Conceptualization; methodology; validation; funding acquisition; writing – review and editing; resources; supervision.

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Conflict of interest statement

The authors declare no conflict of interest.

Ethical guidelines

Ethics approval was not required for this research.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

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This study highlighted that condensed tannins in ruminant diets can benefit the animal in a nutritional way such as by increasing weight gain and boosting reproductive performance or in a medicinal way like reducing bloat. The relevant research outcomes recognised the importance of investigating the phenolic profile and antioxidant activities, which could be further correlatively analysed and explore the contribution of phenolic compounds to the bioactive performance of forage plants such as Desmanthus.
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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Supplementary methods.
Method S1. Total Polyphenol Content (TPC).
Method S2. Total Flavonoid Content (TFC).
Method S3. Total Condensed Tannins (TCT).
Method S4. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Antioxidant Assay.
Method S5. Ferric Reducing-Antioxidant Power (FRAP) Assay.
Method S6. 2,2'-Azinobis-(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS) Radical Scavenging Assay.
Method S7. Hydroxyl Radical Scavenging Activity ($^{\bullet}$OH-RSA).
Method S8. Ferrous Ion Chelating Activity (FICA).
Method S9. Reducing Power Assay (RPA).
Method S10. Total Antioxidant Capacity (TAC).
Method S11. Characterization of phenolic compounds by LC-ESI-QTOF/MS Analysis.
Table S1. Characterization of phenolic compounds from six Desmanthus samples through LC-ESI-QTOF-MS^2.

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