Research Article

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Exploring the biological activity of condensed tannins and nutritional value of tree and shrub leaves from native species of the Argentinean Dry Chaco

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Abstract

BACKGROUND: Tropical tree or shrub leaves are an important source of nutrients for ruminants and a potential source of biologically active compounds that may affect ruminal metabolism of nutrients. Therefore, eight woody species from the native flora of Argentinean Dry Chaco, rich in secondary compounds such as condensed tannins (CT), were assessed for their nutritional value, CT fractions and *in vitro* true digestibility of dry matter, as well as biological activity (BA).

RESULTS: Differences among species were found in contents of total phenol, protein-precipitating phenols (PPP), bound proteins to PPP (BP) and BP/PPP ($P < 0.0001$). The BP/PPP ratio reveals differences among species in potential BA as indicated by protein precipitation. The major CT of each species were isolated and purified for use as a standard. Although *Schinopsis balansae* had the most ($P \leq 0.05$) total CT (19.59% DM), *Caesalpinia paraguariensis* had greater ($P \leq 0.05$) BA with the most PPP (530.21% dry matter). *Larrea divaricata*, at 0.97, followed by *Acacia aroma*, at 0.89, had CT with the highest ($P \leq 0.05$) BP/PPP ratios, followed by *Prosopis alba* (0.59).

CONCLUSION: There were differences in nutritive value and bioactivity among species. Those with the greatest CT were not necessarily those with the most BA. *Caesalpinia paraguariensis, S. balansae* and *L. divaricata* were the most promising species as native forage CT sources. *Cercidiurm praecox* (20.87% CP; 18.14% acid detergent fiber) and *Prosopis nigra* (19.00% CP; 27.96% acid detergent fiber) showed the best ($P \leq 0.05$) nutritive values. According to their nutritive traits, these species might be complementary in grass-based ruminant diets.

Keywords: condensed tannins; *in vitro* true digestibility; protein precipitating phenolic; woody species

INTRODUCTION

In livestock production systems of the semiarid Argentinean Chaco, woody perennial plants represent an important source of supplementary feed during the dry season when fresh forage availability is limited.[1] In general, woody plants have not been widely evaluated as a forage source in Argentina and in many other parts of the world owing to their relatively low forage production, as well as their poor nutritional value compared to herbaceous species (grasses or legumes). However, browse fodders are a naturally existing bank of protein to improve ruminant diets, and are generally rich in condensed tannins (CT).[2] The consumption of tannin-rich plants has been associated with negative nutritional effects,[3] while recent studies[4] observed that consumption of native plants might have several potential benefits for ruminant livestock nutrition, ruminal digestion and fermentation,[5] as well as methane production.[6–8] Woody plants produce a great variety of bioactive compounds that might improve nutrient utilization by modifying feed efficiency and/or nutritional qualities of animal products (e.g. meat and milk). For instance, CT are often found in these plants and might produce negative or positive effects on animal nutrient utilization.[9,10]

Condensed tannin synthesis is associated with cell differentiation and exogenous factors. Seasonal effects, light, temperature and soil fertility are some exogenous factors related to CT synthesis.[11] These CT bind weakly or strongly, depending on their chemical characteristics, with protein, polysaccharides, nucleic acid, steroids, alkaloids and saponins.[12] Condensed tannins can have negative effects on animal nutrition, but there is evidence that some have a beneficial impact on biological

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systems because they act as antioxidants, metal complexants and protein-precipitating agents. In these cases, biological activity is defined as the ability to form complexes with proteins.[13] Depending on ruminant species, low levels of CT (3.4%) protect proteins from rumen degradation.[14] Concentrations of CT between 6% and 12% dry matter (DM) depress voluntary feed intake, digestive efficiency and animal productivity. Forages containing moderate levels of CT (2–4% DM) may exert beneficial effects[14] on protein metabolism in ruminants by reducing the degradation of dietary protein into ammonia by rumen microorganisms and increasing the flow of protein from the rumen, as well as resulting in an increased absorption of amino acids in the small intestine.[15] Therefore, plants containing moderate concentrations of CT may increase the sustainability and efficiency of intensive grazing systems by decreasing urinary nitrogen excretion.[3]

Protein and CT characteristics are both important for protein precipitation. Even though some molecular features, such as the degree of polymerization, the proportion of *cis*- versus *trans*-flavanol subunits or delphinidins *versus* cyanidins among CT have been postulated as factors associated with the formation of protein–tannin complexes, these do not necessarily explain the differences in precipitation capacities. Three-dimensional structures of CT and protein may be more important factors in tannin–protein interaction.[16] Despite the fact that complexation between tannins and proteins has long been recognized, it is not yet possible to predict the nutritive value of plant proteins based on the total CT content in feeds. In addition, the amount of protein precipitated by CT depends on the method used to determine it, besides other factors such as the material used to develop a standard curve, pH solution, tannin molecular size and conformational flexibility.[17]

The evaluation of woody species has indicated their potential advantage in ruminant diets,[18] but to measure the benefit of CT in the diet[5,19,20] it is important to characterize biological activity. Differences in nutritive value of woody species differ according to their provenance,[4,21,22] making it is necessary to evaluate the species in a particular region to consider them as potential sources of feed for ruminant livestock. Therefore, the objective of our study was to purify and characterize CT found in woody species from the Argentinean Dry Chaco in order to determine their content and biological activity as well as *in vitro* true DM digestibility (IVTDMD).

MATERIALS AND METHODS

Plant material

The plant material analyzed consisted of leaves from native woody plants from the Argentinean Dry Chaco: Quebracho Colorado (*Schinopsis balansae*), Algarrobo blanco (*Prosopis alba*), Tusca (*Acacia aroma*), Guayacan (*Caesalpinia paraguariensis*), Brea (*Cercidium praecox*), Jarilla (*Larrea divaricata*), Mistol (*Zizyphus mistol*) and Algarrobo negro (*Prosopis nigra*). Plant leaves were harvested (27° 47′ 3.91″ S, 64° 16′ 2.21″ W) during November and December of 2013, in Santiago del Estero, Argentina. Fresh leaves from three distinct plants for each species were collected. Leaves were lyophilized for 48 h, ground through a 1 mm mesh in No. 4 Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA), and stored at room temperature in a sealed box for subsequent analyses.

Chemical composition of plant material

DM concentrations of leaf samples were determined according to AOAC.[23] Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were measured using an ANKOM 200 Fiber Analyzer (ANKOM Technology, Fairport, NY, USA). Nitrogen content was determined by Dumas' method[24] using an Elementar Vario Macro C:N analyzer (Elementar Americas, Inc., Mt Laurel, NJ, USA). Crude protein (CP) was calculated by multiplying N by a factor of 6.25.[23]

Determination of tannin fractions

Dried plant materials were characterized for extractable condensed tannin (ECT), protein-bound condensed tannin (PBCT) and fiber-bound condensed tannin (FBCT) concentrations using spectrophotometric methods as described by Terrill *et al.*[21] The CT concentration in each fraction was determined based on reaction with 95:5 (v/v) butanol–HCl mixture, and their absorbance recorded at 550 nm according to Porter *et al.*[25] The standards were purified from each individual species as recommended by Wolfe *et al.*[26] using Sephadex LH-20 (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA). Finally, solutions were lyophilized to recover purified CT. Purified CT from each species was used for the butanol–HCl procedure for anthocyanidin determinations.

Anthocyanidin analysis in purified condensed tannins

One milligram of purified CT was mixed with 4 mL of 95:5 (v/v) butanol–HCl mixture. This mixture was heated in a boiling-water bath for 1 h. Anthocyanidins were analyzed by high-performance liquid chromatographic assay using a Symmetry® C18 5 μm 4.6 × 150 mm Waters column (Agilent Technologies, Santa Clara, CA, USA). All separations were performed at 25 °C, and the mobile phases were 0.13% trifluoracetic acid (TFA) in water and 0.1% TFA in acetonitrile at a flow rate of 0.5 mL min^{-1} in gradient mode. The chromatograms were monitored at 550 nm. Relative concentrations of anthocyanidins were calculated based on a calibration curve developed using the corresponding standards of pelargonidin chloride, cyanidin and delphinidin (Aldrich, USA). The different prodelphinidin/procyanidin ratios were calculated.

Protein precipitation ability of phenolic fractions

The fraction of phenolics from the total phenolic content with protein precipitating ability was determined by the method described by Hagerman and Butler[27] using a crude plant extract and bovine serum albumin (BSA) as standard protein. Protein-precipitating phenols (PPP) and bound proteins to PPP (BP) were determined following the method described by Naumann *et al.*[13] For PPP determination, the pellet was dissolved in 800 μL sodium dodecyl sulfate–Triethanol amine (TEA) solution. Subsequently, an aliquot of 200 μL of ferric chloride solution (0.01 mol L^{-1} $FeCl_3$ in 0.01 mol L^{-1} HCl) was added to this solution and, finally, the mixture was incubated at room temperature for 30 min. The absorbance of this solution was read at 510 nm. PPP content was calculated as the difference considering total phenolic content with and without BSA addition. The standard curves for total phenolics and PPP were prepared from purified CT solution (1 mg mL^{-1}) in deionized water.

The amount of BP was determined based on the method described by Naumann *et al.*[13] The same procedure was followed as the PPP analysis steps up to the pellet formation. After washing, the pellet was dissolved with 500 μL of buffer A. The solution was placed on a pre-weighed foil cup (Vario Macro), allowed to dry in an oven (30 °C, ∼45 min) and the cup was weighed. The dried protein–phenolic residue was analyzed for N using an Elementar

Vario Macro C:N analyzer (Elementar Americas, Inc., Mt Laurel, NJ, USA). Percent N was converted to crude protein (CP) by multiplying it by 6.25.

In vitro true digestibility

In vitro true DM digestibility (IVTDMD) was determined using the DAISY^{II} incubator (ANKOM Technology, Fairport, NY, USA).[28] 500 mg dried and ground species samples was weighed directly into F57 filter bags provided by ANKOM Technology, and the closed bags were heat sealed. Four bags per species were used for each animal. Ruminal incubation was replicated on two different days for a total of 16 bags per species and every run was used as replicate. The buffer solution was prepared by mixing 266 mL Na_2CO_3 (1.5% w/v)–$Na_2S.9H_2O$ (0.1% w/v) and 1330 mL KH_2PO_4 (1% w/v)–$MgSO_4.7H_2O$ (0.05% w/v)–NaCl (0.05% w/v)–$CaCl_2.2H_2O$ (0.01% w/v)–H_2NCONH_2 (0.05% w/v) and the pH was adjusted to 6.8. Rumen fluid was collected from two steers and two goats with ruminal cannulas, just before analysis during the morning. The fluid was transferred to two pre-warmed Thermo flasks, filtered and flushed with CO_2 gas before placing in an incubator. Aliquots of 1600 mL buffer solution, 400 mL inocula and the samples were placed in the DAISY^{II} incubator digestion jar. The temperature was kept at 39 ° C and the digestion jars were purged with CO_2 gas for 30 s before lidding securely. After 48 h, the jars were removed and the fluid drained. The bags were rinsed thoroughly with cold tap water until the water was clear, using a minimum of mechanical stirring. Microbial debris and any remaining soluble fraction were removed using an ANKOM 200 fiber analyzer following the procedure for determining neutral detergent fiber (NDF) and acid detergent fiber (ADF). *In vitro* NDF weights were recorded for the calculation of IVTDMD values. All assays were performed in duplicate and the averages of these were used as data points.

Statistical analysis

Analysis of variance (ANOVA) was carried out on the chemical composition, fiber content, fraction of condensed tannin and biological activity data using the general linear model. Significance between means was tested using least significance difference. Plant species were the independent variable, individual plants were the experimental unit (three replications) and laboratory analyses were the dependent variables in the model. For IVTDMD determination, individual incubations were considered replicates. Data were analyzed by one-way ANOVA using the InfoStat statistical package InfoStat/P version 1.1 computing program.[29] Differences at a probability level $P \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Chemical composition of plant material

Crude protein and fiber (NDF and ADF) content in leaves differed among species (Table 1). Crude protein content ranged from 13.0% to 20.9%. *Cercidium praecox* had the highest CP, but it did not differ from *P. alba*, *P. nigra* and *A. aroma*. The lowest CP was observed in *Z. mistol*. *Caesalpinia. paraguariensis*, *L. divaricata* and *S. balansae* had intermediate CP content which did not differ from the upper CP group of species or from *Z. mistol*. NDF and ADF content varied from 26.16% to 47.45% and from 15.70% to 36.18%, respectively. From NDF and ADF content, we identified three groups: high fiber (i.e. *P. alba, Z. mistol* and *A. aroma*), intermediates (i.e. *P. nigra* and *S. balansae*) and low fiber (i.e. *C. praecox*, *C. paraguariensis* and *L. divaricata*). The proportion of fiber observed in these leaves was low compared to the basal diet of livestock in tropical areas, i.e. C_4 grasses. The range of fiber among species was also somewhat below the concentration that may limit ruminant DM intake.[30] Rossi *et al.*[31] reported similar CP and fiber content in leaves of native woody *A. aroma* and *L. divaricata*. From the nutritional point of view, CP values observed were well above (7%) the general maintenance CP requirement for adult categories of ruminants[32] and the level at which dry matter intake and digestibility may be limited; thus it revealed the potential contribution of native species to improve CP status in the diet for local livestock production systems.[30] Crude protein in leaves was approximately two to seven times the content typical of C_4 pastures,[32] indicating their potential to raise ruminant dietary CP status during the dry season.

Table 1. Chemical composition (% DM) and *in vitro* true dry matter digestibility (IVTDMD) of leaves of native woody species in the Dry Chaco of Argentina (mean ± SD).

Native species	CP (%)	NDF (%)	ADF (%)	IVTDMD (%)
Prosopis alba	17.5 ± 0.1	47 ± 1	36.2 ± 0.4	49 ± 1
Prosopis nigra	19.0 ± 0.2	38.4 ± 0.6	27.9 ± 0.2	64 ± 1
Cercidium praecox	20.87 ± 0.06	34 ± 5	18.1 ± 0.5	85.4 ± 0.3
Caesalpinia paraguariensis	16.00 ± 0.07	26.2 ± 0.8	15.7 ± 0.3	77.8 ± 1
Larrea divaricata	14.9 ± 0.2	30.9 ± 0.5	22.5 ± 0.3	67 ± 2
Zizyphus mistol	13.0 ± 0.1	43.8 ± 0.6	35.1 ± 0.9	45 ± 3
Schinopsis balansae	13.2 ± 0.2	36.6 ± 0.2	30.4 ± 0.6	56 ± 2
Acacia aroma	18.0 ± 0.2	46.8 ± 0.4	35 ± 5	47 ± 2

*CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; IVTDMD, *in vitro* true DM digestibility.

Determination of tannin fractions

The standard curves of concentration *versus* optical density at 550 nm for purified CT from different species are shown in Fig. 1, which represents the intensity of color development from diverse CT in butanol–HCl. It is important to note that there are differences in performance due to changes in their chemical structure and degree of polymerization among sources, even at similar CT content.[16] For those native plants we tested, our results support what has been reported for other species,[33] namely the need to use a CT self-standard for each species when determining a correct quantification of their CT content. Although quebracho tannins are widely accepted as standards, they can give a misleading idea of actual CT content.[33] In our study we observed that, if quebracho CT is used as a standard, *C. paraguariensis* CT content would be overestimated by 25%, whereas for other species the real content of CT would be underestimated by as low as four times the real value. These results demonstrate the need to extract and purify the major CT from each species for use as standard in subsequent determinations.

Total CT concentration (TCT) was different among species (Table 2). For the species analyzed, TCT ranged from 2.59% to 19.59% DM. *Schinopsis balansae* had the highest TCT content (19.59%); *P. alba*, *C. paraguariensis*, *Z. mistol* and *A. aroma* showed intermediate values (6.15–12.03%), whereas *P. nigra*, *C. praecox* and *L. divaricata* had lower contents than the other species

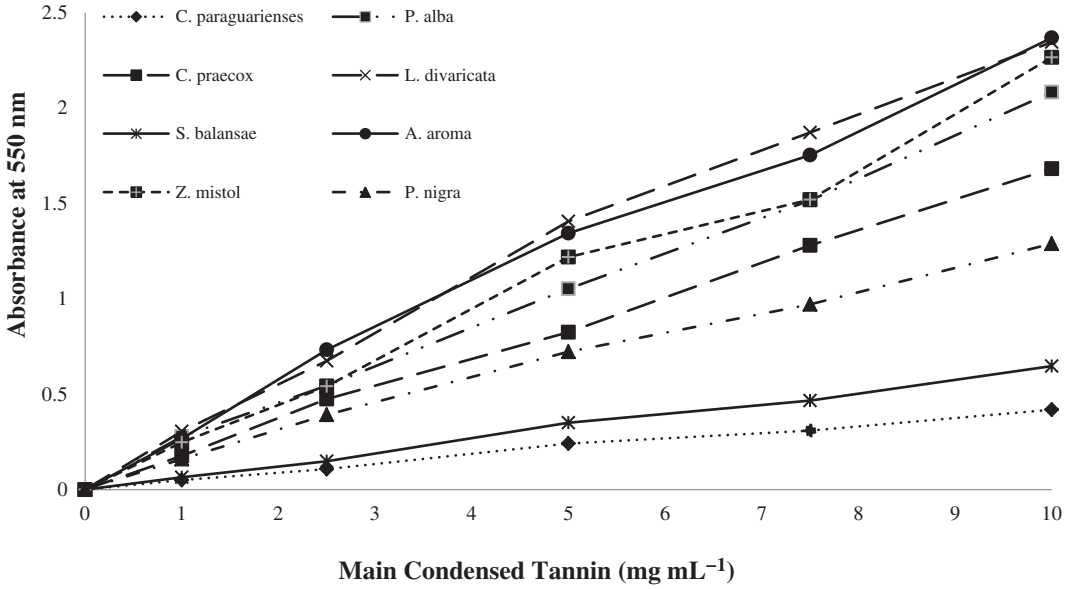

Figure 1. Plot of standard curves for purified condensed tannins from woody native species from the Argentinean Dry Chaco: *Schinopsis balansae*, *Prosopis alba*, *Acacia aroma*, *Caesalpinia paraguariensis*, *Cercidium praecox*, *Larrea divaricata*, *Zizyphus mistol* and *Prosopis nigra*.

Table 2. Condensed tannin composition of leaves of woody species in the Argentinean Dry Chaco

Native species	ECT (%)	PBCT (%)	FBCT (%)	TCT (%)
Prosopis alba	4.19c	1.78a	0.18ab	6.15bc
Prosopis nigra	1.71ab	2.07ab	0.50c	4.27ab
Cercidium praecox	0.35a	1.97ab	0.28b	2.59[a]
Caesalpinia paraguariensis	2.59b	3.96bc	0.93d	7.48c
Larrea divaricata	1.39ab	1.39a	0.01ab	2.80[a]
Zizyphus mistol	5.38 cd	6.56d	0.09ab	12.03d
Schinopsis balansae	6.11d	12.23e	1.25e	19.59e
Acacia aroma	4.50c	5.99 cd	0.54c	11.03d
SEM	0.52	0.73	0.08	0.72
P-value	<0.0001	<0.0001	<0.0001	<0.0001

ECT, extractable condensed tannins; PBCT, protein-bound condensed tannins; FBCT, fiber-bound; TCT, total condensed tannins.
Values in the same column followed by the same letter do not differ according to a least significant difference multiple mean separation ($P \leq 0.05$).

(2.58–4.27%). Our TCT values were similar to those observed by Jackson *et al.*[22] in leaves of woody Colombian and Australian tropical legumes. These authors suggest that species growing in South America containing from 10% to 24% TCT should only be included in moderate supplementation levels for ruminants. Terrill *et al.*[21] reported that TCT content in forage plants of New Zealand ranged from 1.3% to 12.5% DM. The biosynthesis of these secondary metabolites is dependent on many factors, including climatic conditions and soil fertility, so TCT content can be variable within species from different origins.[12] *Schinopsis balansae* and *Z. mistol* showed the highest ECT content: 6.11% and 5.38%, respectively.

The proportion of ECT relative to TCT varied from 14% to 68%, which is, on average, lower than the values reported by Jackson *et al.*[22] from foliage from South American trees or shrubs. Protein-bound CT also differed among species. For example, *S. balansae* had the highest PBCT content, which was 12.23%, followed by *Z. mistol* and *A. aroma*, with 6.56% and 5.99%, respectively. Generally, the extractable fraction represents the most significant fraction among condensed tannins (>60% of TCT), such as in the case of *P. alba*. However, *P. nigra*, *C. praecox*, *S. balansae*, *Z. mistol*, *A. aroma* and *C. paraguariensis* had higher concentrations of PBCT than those of ECT; this contrasts sharply with *S. balansae* (31.2% of TCT) and *C. praecox* (13.5% of TCT).

The FBCT fraction presented the lowest concentration in all species and its value varied from 0.01% to 1.25%. *Prosopis nigra*, *C. praecox* and *C. paraguariensis* had a higher proportion of FBCT compared to TCT (∼10%), while the proportion of FBCT in the other species varied between 0.4% and 6.4%. The ECT fraction represents about 42% of the TCT content as an average value for all species, while the PBCT fraction corresponds to about 29.03%, and the FBCT fraction is about 6.03%. Terrill *et al.*[21] and Douglas *et al.*[34] reported that ECT comprise the main fraction of TCT in woody or herbaceous legumes: approximately 68%. In our study, the proportion of ECT to TCT was ∼13.5–49.6%, below the values reported for other woody plants, except to *P. nigra* with an ECT content slightly above 68%.

Anthocyanidin analysis in purified condensed tannins

The major CT obtained from all species were exclusively constituted by cyanidin and delphinidin units, whereas pelargonidin units were not detected. *Prosopis alba* had a delphinidin/cyanidin ratio (PD/PC) of 4, followed by *A. aroma* and *Z. mistol* with 3 units of PD per PC (Table 3). This ratio was close to 1 for *C. praecox*, *P. nigra*, *S. balansae* and *C. paraguariensis*, but only 0.5 for *L. divaricata*. However, these Argentinean native species have not been characterized before in terms of their CT composition and input to a ruminant diet and, therefore, this analysis gives useful information. Our data show that species with low ratios of PD/PC were associated with lower dry matter digestibility.

Phenolic fraction with protein precipitation ability

Based on BP values (Table 3), the species in our study can be classified into two groups: those species that presented BP values

Table 3. Protein precipitation ability of phenolic fraction and structural traits of condensed tannins of woody species in the Argentinean Dry Chaco

Native species	PT	PPP	BP	BP/PPP	PD/PC
Prosopis alba	242.74b	93.81c	54.83b	0.59bc	4
Prosopis nigra	213.47b	0a	0a	0a	1
Cercidium praecox	49.79[a]	0a	0[a]	0a	1
Caesalpinia paraguariensis	1187.09e	530.21f	49.98b	0.09a	1.2
Larrea divaricata	399.25c	79.17bc	50.4b	0.97d	0.5
Zizyphus mistol	363.14c	213.89d	56.43b	0.26ab	3
Schinopsis balansae	578.63d	300.93e	50.58b	0.17[a]	0.92
Acacia aroma	190.15b	58.35b	52.05b	0.89 cd	3
SEM	29.79	11.29	7.65	0.13	–
P-value	<0.0001	<0.0001	<0.0001	<0.0001	–

PT, total phenol content (g PT kg^{-1} DM); PPP, protein-precipitating phenolics (g CT bound kg^{-1} DM); BP, protein bound by protein-precipitating phenolics (g protein kg^{-1} DM); BP/PPP, protein bound per gram of protein-precipitating phenolics; PD/PC, prodelphinidin/procyanidin ratio.
Values in the same column followed by the same letter do not differ according to a least significant difference multiple mean separation ($P \leq 0.05$).

between 56.43% and 49.98% without differences among them, and those species such as *P. nigra* and *C. praecox* which did not have phenolic compounds with protein-binding capacity. The values obtained for PPP were different among all species. *Caesalpina paraguariensis* showed the highest PPP content, whereas *S. balansae* and *Z. mistol* had intermediate values. Finally, *A. aroma*, *L. divaricata* and *P. alba* had considerably lower values of PPP, being about one-third to one-fifth the values of the other species.

It has been debatable whether BP, PPP or the ratio between them is the most suitable parameter to predict biological activity.[13] The PPP fraction represents those phenolics that precipitate protein, while the amount of protein that has been bound by phenolics is quantified by BP. Naumann *et al.*[13] suggested that the phenolic efficiency to precipitate proteins may be represented as the relation between both magnitudes (BP/PPP ratio) and may be considered as the biological activity of CT; for example, their ability to protect proteins from ruminal degradation. According to this ratio (Table 3), *A. aroma* and *L. divaricata* had the highest values; these ratios were obtained from similar BP values and different values of PPP. *Prosopis nigra* and *C. praecox* presented null values for this ratio, suggesting the lack of biological activity. *Caesalpina paraguariensis* presented a low value for the ratio due to its high PPP content, whereas *Z. mistol* had an intermediate biological activity due to similar values of BP and PPP.

In order to find the most active tannin fraction able to precipitate proteins and to obtain a quantitative parameter of CT biological activity, we evaluated the relation between BP and TCT or ECT. The best correlation was evident between BP and the ECT fraction – higher than that obtained for PB/PPP. This can be explained taking into account that ECT represents the main available portion of tannins capable of interacting with soluble proteins. *Larrea divaricata*, *C. paraguariensis* and *A. aroma* were the species with the highest BP/ECT ratio. By linking the BP/PPP ratio with TCT content, some species with low TCT content, such as *P. nigra* and C. *praecox*, did not have biological activity. However, *L. divaricata,* despite its low TCT, was among the species with the highest biological activity. *Prosopis alba*, *A. aroma* and *Z. mistol*, with intermediate content of TCT, also had high BP/PPP ratios. *Schinopsis balansae* had high TCT content but intermediate biological activity compared with other species. Meanwhile, *C. paraguariensis*, with an intermediate TCT value, had the lowest biological activity. Naumann *et al.*,[13] testing 10 warm-season perennial herbaceous legumes and using the same technique, observed higher BP/PPP ratios than those determined in our experiment. The values reported by these authors ranged between 0 and 1.39 and only one species did not have biological activity.

Schofield *et al.*[35] reported that an increase in PD/PC ratio is associated with enhanced ability to complex proteins. However, comparing the biological activity values of the major CT purified for every plant species with their proanthocyanidin composition, a correlation was not observed between PD/PC ratio and CT precipitating ability. Condensed tannins of *C. praecox* and *P. nigra* with a PD/PC ratio of 1 did not indicate biological activity. By contrast, CT of *A. aroma* and *Z. mistol*, with a PD/PC ratio of 3, had very different biological activity: 0.90 to 0.26, respectively. Schofield *et al.*[35] suggest that increasing PD/PC ratio improve CT protein-precipitating ability. Lorenz *et al.*[16] affirmed that the interactions between proteins and tannins not only depend on the CT concentration and protein characteristics but also on the plant CT origin. Moreover, Naumann *et al.*[13] proposed that CT capacity to precipitate proteins is related to the CT molecular weight, although they finally concluded that molecular weight alone does not totally explain the variation in protein precipitation by legume herbage CT.

On this basis, and considering our results, we can ascribe the CT affinity behavior with proteins to variations on the spatial structure resulting from the proanthocyanidin sequence as well as the secondary intramolecular interactions in the tannin macromolecule. Considering that our objective was to identify biologically active CT of native species, *L. divaricata* and *A. aroma* appear to be stronger protein-precipitating agents from the prospective of ruminant use.

In vitro true digestibility

The presence of CT in woody species used as forage is an antinutritional factor, since these inhibit ruminal enzyme action and microbial activity, limit nutrient digestion and reduce fatty acid production as final degradation products.[36,37] However, their importance is based on their effects on digestibility and intake and, therefore, on animal behavior.[38] Adequate CT levels in a ruminant diet can act as protein-precipitating agents, thereby protecting N from ruminal degradation and favoring a more efficient use of N in the lower gastrointestinal tract.[16] On the other hand, dietary CT can modify N excretion, decreasing the amount eliminated in urine and increasing that excreted in feces, with a consequent beneficial effect on soils.[6] To evaluate the forage aptitude of woody species, it is important to determine the CT effect on DM digestibility for *in vitro* systems.

On the other hand, the beneficial impact of CT on ruminants not only depends on plant material per se but also on the ruminant species. Previous studies[39,40] suggest that goats have differences over cattle and sheep when they consume woody plant leaves with high CT content. For instance, Robbins *et al.*[39] and Austin *et al.*[40] mentioned that some browsing species (such as goats) are able to produce proline-rich saliva to block the negative effect of tannins on palatability, nutrient utilization and ruminal fermentation. In cattle, however, no increase in the production of such proteins

has been observed in response to CT ingestion, although other proteins with high affinity for these polyphenols have been found in their saliva.[41]

In vitro DM true digestibility values for the studied species differed, ranging from 45.16% to 85.40%, with *C. praecox* having the highest value (Table 1). We measured the correlation between chemical composition and IVTDMD and found that higher values of IVTDMD were observed when plant material had lower NDF, TCT and ECT contents. This indicates an antinutritional activity for these fractions, with ECT a better predictor of IVTDMD than TCT. The species with the lowest TCT content was *C. praecox*, which also showed higher rates of digestibility (85.40%), while *Z. mistol* and *S. balansae*, with high values of TCT, had the lowest percentages of digestibility: 45.16% and 47.10%, respectively. The exception was *C. paraguariensis*, which had intermediate values of TCT and high percentage of IVTDMD, 77.78%. In contrast, *S. balansae* had intermediate values of IVTDMD despite its high TCT content.

The origin of rumen liquor – steer or goat – did not affect IVTDMD of the native woody species we studied. Molina *et al.*[42] reported that IVTDMD was not influenced by using ruminal liquor from goats or sheep. Gordon *et al.*[43] also found no differences between ruminal liquor of sheep or red deer. Wambui *et al.*,[44] however, found that diet greatly affected browser ruminant microbial activity on NDF and fiber-bound CT *in vitro* organic matter disappearance of some but not all woody browse species in their study. Overall, when goats consumed browse with CT, fiber-bound CT disappearance in forage containing CT decreased. Likewise, studies by Jabari *et al.*[45] suggest that digestibility of DM by micro-organisms in water buffalo (*Bubalus bubalis*) was higher than those in a cow (*Bos* spp.), when fed the same diet, indicating that rumen activity and microorganism population can differ among ruminant species. Taken as a whole, these findings indicate that, when reporting *in vitro* rumen disappearance assay results, authors should report the species and diet that gave origin to the rumen liquor.

CONCLUSION

We observed differences in nutritional components and bioactivity among the eight Argentinean woody species in our study. *Acacia aroma* and *L. divaricata* had the highest protein-precipitating activity (BP/PPP), followed by *P. albadivaricate*. However, this BA did not necessarily correlate to TCT content since *S. balansae* and *Z. mistol* had higher content. Further research is needed to determine what affects ruminant production more: BA or TCT. These species were also not the highest in CP or lowest in fiber content – both indicators of IVTDMD. For example, *C. praecox* contained among the highest CP and lowest ADF content, reflected in 81% greater IVTDMD than, for example, *A. aroma*. According to which nutritive traits are most important to ruminant production, these species might be complementary components in grass-based ruminant feeding systems. They can improve diet quality, increase plant biodiversity, and provide additional ruminant feed during the dry season for cattle grazing the Argentinean Dry Chaco.

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