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Chemical composition, antioxidant activity and bioactive compounds of vegetation species ingested by goats on semiarid rangelands

H.M. Cuchillo[1,2], D.C. Puga[1,6], N. Wrage-Mönning[3], M.J.G. Espinosa[4], B.S. Montaño[1], A. Navarro-Ocaña[4], J.A. Ledesma[5], M.M. Díaz[1] and R.F. Pérez-Gil[1]

[1] Animal Nutrition Department, National Institute of Medical Sciences and Nutrition "Salvador Zubirán"; D.F, 14000, Mexico
[2] Tropical Forages Program, International Center for Tropical Agriculture (CIAT); Cali, 6713, Colombia
[3] Faculty of Life Sciences, Agricultural Sciences, Rhine-Waal University of Applied Sciences; Kleve 47533, Germany
[4] Faculty of Chemistry, National Autonomous University of Mexico (UNAM); D.F, 04510, Mexico
[5] Direction of Nutrition, National Institute of Medical Sciences and Nutrition "Salvador Zubirán"; DF, 14000, Mexico

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[6] Corresponding author: e-mail: claudia.delgadillop@quetzal.innsz.mx

ABSTRACT. The first goal of this study was to evaluate the chemical composition of vegetation consumed by goats on semiarid rangelands. Secondly, antioxidant activity and identified bioactive compounds of this vegetation were estimated. Twenty-five samples were analysed, including leaves, stems, fruits, or a combination of them. Chemical composition demonstrated large differences among species and smaller differences within species. Some species had high protein contents, e.g. *Acacia schaffneri*, *Celtis pallida* and *Prosopis laevigata*. Methanol:water was the best solution to extract bioactive compounds, which we found in larger amounts in complete plants and stems than in fruits and leaves. *A. farnesiana* pods had the largest polyphenol contents, whereas *A. farnesiana*, *A. schaffneri*, *Leptochloa dubia* and others showed the best antioxidant activity. A positive correlation was observed between antioxidant activity and polyphenol-flavonoid concentration. Based on these results, it seems necessary to study in more detail some species, e.g. the fruits of *Acacia farnesiana* and *A. schaffneri*, to better understand their implications for ruminant feeding and nutrition.

Introduction

In 2009, approximately 270 million goats in the least developed countries around the world were maintained mostly in arid and semi-arid ecosystems based on available local fodder resources (FAOSTAT, 2010). In these developing countries, where the demand for food supplies is growing due to population growth, this kind of farming increases food security. Goats are especially suited for such management as they are able to maintain a reasonable output even when the forage on offer is scarce or when it comprises poor quality feedstuffs (Jiménez-Ferrer et al., 2008). Plant species in these conditions resist herbivore ingestion by biological strategies, principally through production of plant bioactive compounds, which might considerably change animal-plant interrelationships (Villalba et al., 2010; Wrage et al., 2011) and potentially reduce the digestibility of the diet (Rochfort et al., 2008).

Some authors have suggested that pastoral feeding on shrublands may increase the animals' wellbe-

ing (Rochfort et al., 2008; Vasta et al., 2008; Patra and Saxena, 2011). For example, *Acacia pennatula* has been shown to have anthelmintic properties due to its plant bioactive compounds (PBC) (Alonso-Díaz et al., 2010). Complementary effects of PBC, e.g. flavonoids, have been claimed to include anti-inflammatory, antiallergic, antimicrobial, anticarcinogenic activities, cholesterol modulation, and reduction of the incidence of cerebral infarctions. Hydroxycinnamic acids have shown analogous beneficial activities (Vemuri et al., 2008). It is necessary, however, to consider both the benefits and disadvantages of ingestion of PBC. Some inconveniences of PBC ingestion are the detrimental effects on the digestibility of feedstuffs and worse animal performance (Ben Salem et al., 2005).

Nutraceutical properties transferred from plant tissues to ruminants are expected to influence animal products for human consumption (Rochfort et al., 2008); for instance, grazing or browsing have demonstrated to enhance the quality of meat, milk and cheese products more than their basic nutritional parameters (Vasta et al., 2008; Pajor et al., 2009; Cuchillo et al., 2010a,b).

Studies investigating PBC have focussed on temperate forages, legumes and seeds or on tropical plant species (Carnachan and Harris, 2000; Mustafa et al., 2010). The knowledge of feedstuffs from arid and semiarid ecosystems is much more limited with respect to their PBC contents and antioxidant activities. Usually, investigations of arid or semiarid systems have mainly evaluated nutritional characteristics of a few vegetation species, mainly herbs. Moreover, the assessments that included shrub species have mostly tested these for detrimental feed ingredients and their impact on dry matter intake and protein digestibility (Ben Salem et al., 2005; Baraza et al., 2008; García-Winder et al., 2009).

Several studies have highlighted that the medium used to extract PBC can modify the biological activity of the analysed samples (Ruiz-Terán et al., 2008; Mustafa et al., 2010). Although methanol is the solvent most used for plant tissue extractions, other solvent systems have been used sucessfully to evaluate antioxidant activities and phenolic compounds (Alonso-Díaz et al., 2010; Reynaud et al., 2010). Solvents have not been compared for their potential to recover PBC for antioxidant activity assessment in rangeland plants. The goals of the present experiment were to evaluate the chemical composition, antioxidant activity, and bioactive compounds of plants consumed by goats on semiarid rangelands of central Mexico.

Material and methods

Animals and experimental design

The experiment was carried out in Queretaro, Mexico (20°35' N, 100°18' W; 1,950 m.a.s.l.) during the summer of 2008. The area has a dry, semiarid climate with an average annual precipitation of 460 mm with isolated rains in winter. A group of 40 French Alpine goats that weighed 50 ± 5 kg and had a lactation period of 150 days was allowed to graze and browse freely from 08.00 to 17.00 h on 14 ha of rangeland. The animals were kept in overnight confinement and they did not receive any supplementary feeding. The vegetation included the following forbs, leguminous trees and cactaceous species: *Acacia farnesiana*, *A. schaffneri*, *Aristida adsencionis*, *Bouteloua curtipendula*, *B. repens*, *Celtis pallida*, *Chloris virgata*, *Jatropha dioica*, *Leptochloa dubia*, *Mimosa biuncifera*, *Opuntia affasiacantha*, *O. amyctaea*, *O. hytiacantha*, *O. robusta*, *O. streptacantha*, *O. tomentosa*, *Prosopis laevigata*, *Rhynchelytrum roseum*, *Urochloa fasciculata*, *Verbasina serrata* and *Zalazania augusta.* Sampling of grazed or browsed species was performed simulating the goats' bites, following the recommendations of Agreil and Meuret (2004). Briefly, two control animals were selected to be monitored continuously along intervals of 30 min from a very close distance. The selected animals were adapted to the presence of observers to permit them to see the animal's mouth and the chosen plants without modifying the grazing behaviour. Observations of goats were carried out between August and September. Twenty-five rangeland vegetation samples were identified, collected and dried on 3 different days. Sampling was done twice daily. A total of one hundred and fifty samples was collected. The vegetation sampling included leaves, stems (cladodes for *Opuntia* species), fruits or a combination of them, in line with the individual bites of the goats.

Plants' chemical composition

The samples were ground to a particle size of 1 mm and analysed as follows: moisture (oven-drying at 60°C), fat, crude fibre and ash contents were determined using standard methods (AOAC, 2003). Nitrogen was measured using the Micro-Kjeldahl technique (AOAC, 2003). N-free extractives were calculated as the difference between 100% and protein (nitrogen factor: 6.25), fat, crude fibre, and ash percentages. Gross energy was determined using the calorimetric Parr bomb (Parr Instrument Company, Illinois. USA). All samples were analysed in triplicate.

Extraction

Three subsamples (20 g each) weighed in Erlenmeyer flasks with 150 ml of methanol, methanol:water (80:20), or acetone, were set to shaking for 24 h at room temperature. All extracts were then filtered and washed (50 ml of respective solvent), then the filtrates were concentrated with a vacuum rotary evaporator (Büchi R-205**,** Labortechnik AG, Switzerland) at 30°C and 150 rpm. Further, extracts were frozen at –80°C, lyophilized **(**Labconco Freezone 6, Labconco Corp., Kansas City, MO, USA) and stored at 4°C for later analysis.

Qualitative radical scavenging activity

Fifty milligrams of dry extracts were diluted with 0.5 ml of the respective extraction medium. Afterwards, 20 µl aliquots were applied individually to the baseline of TLC (thin layer chromatography) plates (20 × 10 cm silica gel 60 F254; Merck, Germany) and the sample was allowed to dry. Then, the TLC plates were eluted with a solvent system consisting of methanol:ethyl acetate (70:30). Once dried at room temperature, the plates were tested against $DPPH^+$ (1,1-diphenyl-2-picrylhydrazyl; 200 mg $DPPH^+$ dissolved in 100 ml methanol) spray reagent to determine the qualitative radical scavenging activity (QLRA) of the samples, visible as yellow-on-purple spots due to the decoloration of $DPPH^+$(Sharma et al., 1998). Butylhydroxyanisol (BHA) and α-tocopherol standards were used as references.

Quantitative radical scavenging activity (QRA)

QRA was determined following the method of von Gadow et al. (1997) with some modifications. Briefly, 0.25 ml of each extract solution (200 ppm) were added to 2 ml 0.36 mM $DPPH^+$ solution. The mixture was shaken vigorously and left to stand for 30 min in the dark. Absorbance was measured at 517 nm at $t = 0$ and after 30 min using a Beckman DU-70 spectrophotometer. Quantitative radical scavenging activity was calculated as follows: QRA (%) = $((At_0\text{-}At_{end})/At_0)$*100, where: At_0 is the initial absorbance at time zero and At_{end} is the final absorbance after 30 min. BHA and α-tocopherol standards (100 ppm) were used as references. All determinations were performed three times on each sample.

Total polyphenol content and plant bioactive compounds (PBC)

Total polyphenol content (TPC) in the plant extract (methanol:water (80:20 v/v)) was determined by the Folin-Ciocalteu colorimetric method described by Taga et al. (1984). The concentration was calculated using gallic acid as the standard, and the results were expressed as mg of gallic acid equivalents (GAE) per 1 kg dry matter of plant extract. To determine flavonoids and hydroxycinnamic acid, 20 mg of each dry extract were assessed by HPLC according to Ubando-Rivera et al. (2005). An HPLC 1525 high-pressure binary pump (Waters Milford, USA) and Symmetry C18 column (5 µm steel 3.9 mm × 150 mm; Waters Milford, USA) were employed. Methanol:water (at a ratio of 70:30 v/v) and 0.16 M acetic acid (pH 2.4) were used as carriers at a flow rate of 1 ml · min^{-1}. The oven temperature was held at 45°C, whereas the detection was performed at 280 nm (486 Waters Milford, USA). The following substances dissolved in methanol were used as standards: catechin (2.21 mg·ml^{-1}), epicatechin (1.25 mg · ml^{-1}), gallocatechin (0.032 mg · ml^{-1}), gallic acid (0.030 mg · ml^{-1}), caffeic acid (0.032 mg · ml^{-1}), cinnamic acid (0.038 mg · ml^{-1}) and ferulic acid (0.031 mg · ml^{-1}). Calibration curves were made for each standard using three dilutions (1:1; 1:3 and 1:5). Peaks were identified by the retention times of individual standard flavonoids and hydroxycinnamic acids, using Brezze version 6.30 Waters Software. The concentrations of hydroxycinnamic acids were expressed as g · 100 g^{-1}, while flavonoid concentrations were expressed as mg · 100 g^{-1} dry matter of extract. All analytical reagents and standards were from Sigma-Aldrich, Steinheim, Germany.

Statistical analysis

Chemical composition, total polyphenol content, hydroxycinnamic acids, flavonoids, and quantitative radical scavenging activity (QRA) of plant samples were analysed by ANOVA ($p = 0.05$) using SAS (2003). The days of collection were treated as repeated measurements. The results of the parallel measurements of each individual sample were averaged before further statistical analysis. For qualitative radical scavenging activity we used non-parametric statistics. The Friedman test was used to establish differences among extract responses; further, the Wilcoxon signed ranks test for related samples was used to identify such differences. For each plant portion (complete, fruits, leaves and stems; cladodes for *Opuntia* species) we used the non-parametric statistic of K independent samples. The Kruskal-Wallis test was used to stablish differences among plant portions. Further, the Mann-Whitney U signed ranks test for related pairs of portions was used to identify such differences (SPSS, 2010).

Table 1. Chemical composition of shrub species browsed or grazed by goats on semiarid rangelands, g · 100 g^{-1} dry-weight basis

Species	Portion	CP	ASH	EE	CF	NFE	TDN	DE, Mcal	ME, Mcal
Aristida adsencionis	complete	7.7^{l} ± 0.35	9.5^{k}± 0.28	1.8^{f}± 0.08	23.2^{k}± 0.27	57.7^{h}± 0.67	64.0^{h}± 0.78	2.8^{cd}± 0.03	2.3^{dc}± 0.03
Acacia schaffneri	complete‡	17.3^{e}± 0.13	4.2^{s}± 0.11	2.0^{e}± 0.06	16.8^{p}± 0.12	59.6^{f}± 0.13	66.7^{b}± 0.78	3.0^{b}± 0.01	2.5^{ba}± 0.01
Bouteloua curtipendula	complete	6.5^{n} ± 0.17	9.2^{l}± 0.11	1.4^{ij}± 0.09	29.1^{f}± 0.11	53.7^{m}± 0.08	62.1^{j}± 0.29	2.7^{d}± 0.01	2.2^{dc}± 0.01
Bouteloua repens	complete	7.3^{m}± 0.06	11.1^{h}± 0.16	1.5^{ih}± 0.1	27.3^{j}± 0.27	52.7^{o}± 0.11	61.1^{n} ± 0.33	2.7^{d}± 0.01	2.2^{de}± 0.01
Chloris virgata	complete	5.9^{o} ± 0.13	9.0^{m}± 0.16	1.5^{ih}± 0.1	27.9^{h}± 0.11	55.6^{k}± 0.1	62.6^{j} ± 0.16	2.7^{cd}± 0.01	2.2^{dc}± 0.01
Jatropha dioica	complete	7.8^{l} ± 0.12	11.1^{h}± 0.13	2.3^{d}± 0.11	16.6^{q}± 0.14	62.1^{d}± 0.12	64.6^{h} ± 0.19	2.8^{cd}± 0.01	2.3^{dc}± 0.01
Leptochloa dubia	complete	9.9^{g} ± 0.12	9.5^{k}± 0.05	1.7^{gf}± 0.11	28.8^{g}± 0.14	50.1^{r}± 0.14	62.3^{m} ± 0.29	2.7^{d}± 0.01	2.2^{dc}± 0.11
Mimosa biuncifera	complete	19.3^{b} ± 0.2	7.4^{p}± 0.14	1.8^{f}± 0.11	21.6^{m}± 0.16	49.8^{u}± 0.13	65.7^{ef}± 0.44	2.9^{cb}± 0.05	2.4^{bc}± 0.05
Rhynchelytrum roseum	complete	5.3^{q} ± 0.16	7.4^{p}± 0.12	1.6^{gh}± 0.13	29.7^{e}± 0.12	55.9^{j}± 0.15	63.5^{h}± 0.50	2.8^{cd}± 0.05	2.3^{dc}± 0.05
Urochloa fasciculata	complete	5.7^{p} ± 0.16	10.5^{j}± 0.17	2.1^{e}± 0.19	24.6^{j}± 0.45	56.7^{i}± 0.15	62.6^{k}± 0.05	2.7^{d}± 0.05	2.2^{dc}± 5.51
Mean complete		**9.27^{A}± 0.16**	**8.89^{A}± 0.17**	**1.77^{A}± 0.19**	**24.56^{A}± 0.45**	**55.7^{A}± 0.15**	**63.76^{A}± 0.05**	**2.8^{A}± 0.05**	**2.3^{A}± 5.51**
Acacia farnesiana	fruits	9.4^{j} ± 0.45	3.1^{u}± 0.18	1.3^{j}± 0.14	8.5^{x}± 0.11	77.8^{a}± 0.18	71.83^{a}± 0.35	3.1^{a}± 0.02	2.6^{a}± 0.05
Opuntia amyctaea	fruits	5.0^{r} ± 0.17	7.2^{q}± 0.14	0.5^{m}± 0.10	23.2^{k}± 0.14	64.1^{c}± 0.11	64.35^{d}± 0.32	2.8^{cb}± 0.05	2.3^{bc}± 0.05
Opuntia hytiacantha	fruits	4.4^{s}± 0.11	7.6^{o}± 0.12	2.8^{c}± 0.21	14.1^{t}± 0.21	71.1^{b}± 0.36	68.4^{c}± 0.63	2.9^{cb}± 0.5	2.5^{bc}± 0.05
Prosopis laevigata	fruits	9.5^{h}± 0.21	3.5^{t}± 0.20	0.9^{l}± 0.10	35.3^{c}± 0.31	50.6^{q}± 0.16	64.2^{f}± 0.42	2.8^{cb}± 0.50	2.3^{bc}± 0.50
Mean fruits		**7.07^{B}± 2.49**	**5.4^{B}± 2.14**	**1.4^{B}± 1.18**	**20.3^{B}± 0.91**	**65.9^{B}± 10.6**	**67.2^{B}± 3.32**	**3.0^{B}± 0.10**	**2.4^{B} ± 0.10**
Celtis pallida	Leaves	18.5^{c}± 0.09	12.4^{g}± 0.11	1.1^{k}± 0.07	7.6^{y}± 0.11	60.3^{e}± 0.08	64.8^{e}± 0.20	2.8^{cb}± 0.05	2.3^{bc}± 0.05
Prosopis laevigata	Leaves	17.6^{d}± 0.26	8.0^{n}± 0.23	2.1^{e}± 0.18	22.6^{l}± 0.82	49.6^{t}± 0.50	65.2^{g}± 0.48	2.9^{cd}± 0.05	2.4^{dc}± 0.05
Verbasina serrata	Leaves	22.8^{a}± 0.11	12.7^{f}± 0.13	1.7^{gf}± 0.16	13.7^{u}± 0.17	49.0^{u}± 0.12	63.64^{h}± 0.48	2.8^{cd}± 0.05	2.3^{dc}± 0.05
Mean leaves		**19.6^{C}± 2.41**	**11.03^{C}± 2.28**	**1.64^{C}± 0.45**	**14.64^{C}± 6.54**	**52.9^{C}± 5.51**	**64.5^{C}± 0.83**	**2.8^{C}± 0.05**	**2.3^{C}± 0.05**
Celtis pallida	stems	8.1^{k}± 0.34	3.1^{u}± 0.17	0.9^{l}± 0.16	36.0^{b}± 0.18	51.9^{p}± 0.11	64.5^{e}± 0.68	2.8^{cb}± 0.05	2.3^{bc}± 0.05
Verbasina serrata	stems	8.0^{k}± 0.17	8.0^{n}± 0.14	1.3^{j}± 0.13	35.1^{d}± 0.13	47.5^{w}± 0.14	61.37^{l}± 0.51	2.7^{d}± 0.05	2.2^{de}± 0.05
Zalazania augusta	stems	5.1^{r}± 0.15	5.0^{r}± 0.17	3.4^{b}± 0.39	38.5^{a}± 0.15	47.9^{v}± 0.19	64.69^{k}± 0.96	2.9^{d}± 0.05	2.3^{dc}± 0.05
Mean stems		**7.1^{D}±0.15**	**5.4^{D}± 2.1**	**1.9^{D}± 1.18**	**36.5^{D}± 1.5**	**49.1^{D}± 2.11**	**63.5^{D}± 1.75**	**2.8^{D}± 0.10**	**2.3^{D} ± 0.10**
Opuntia affasiacantha	cladodes	4.0^{t}±0.11	21.7^{e}± 0.25	1.7^{gf}± 0.14	14.3^{s}± 0.12	58.2^{g}± 0.18	56.6^{p}± 0.48	2.4^{e}± 0.05	2.0^{fe}± 0.05
Opuntia hytiacantha	cladodes	5.1^{r}± 0.12	23.6^{d}± 0.19	1.7^{gf}± 0.18	20.5^{n}± 0.17	48.9^{v}± 1.03	53.6^{s}± 0.97	2.4^{f}± 0.05	1.9^{g}± 0.05
Opuntia robusta	cladodes	6.4^{n}± 0.24	25.0^{a}± 0.77	1.6^{gh}± 0.12	8.8^{w}± 0.29	58.1^{g}± 0.64	55.5^{q}± 0.68	2.4^{fe}± 0.05	2.0^{fg}± 0.05
Opuntia streptacantha	cladodes	5.1^{r}± 0.14	23.6^{c}± 0.37	1.5^{ih}± 0.17	16.1^{r}± 0 .16	53.5^{n}± 0.11	54.5^{r}± 0.22	2.4^{fe}± 0.05	1.9^{fg}± 0.05
Opuntia tomentosa	cladodes	8.9j± 0.18	24.3^{b}± 0.28	1.8^{f} ± 0.14	10.4^{v}± 0.24	54.5^{l}± 1.16	55.8^{q}± 0.98	2.5^{fe}± 0.05	2.0^{fg}± 0.05
Mean cladodes		**5.9^{E} ± 1.75**	**23.6^{E}± 1.21**	**1.7^{E}± 0.17**	**14.0^{E}± 4.31**	**54.6^{E}± 3.6**	**55.2^{E}± 1.25**	**2.4^{E}± 0.18**	**2.0^{E}± 0.18**

DM – dry matter; CP – crude protein (N × 6.25); EE – ether extract; CF – crude fibre; NFE – N-free extractives; TDN – total digestible nutrients (TDN = CF (0.5) + CP (0.75) + NFE (0.75) + (EE (0.75) x 2.25)); DE – digestible energy (TDN x 4409 kcal); ME – metabolizable energy (DE × 0.82).

Means with different superscript letters within columns are significantly different at $p = 0.05$. Complete = mix of leaves and stems; ‡ – mix of leaves, stems and pods; Means with capital letters are significantly different (*p = 0.05,**P = 0.001) within column where:

CP: A≠C**,A≠E**,B≠C**,C≠D**,C≠E**

ASH: A≠B**, A≠C**, A≠D**, A≠E**, B≠C**, B≠E**, C≠D**, C≠E**, D≠E**

EE: A≠B**, B≠E**

CF: A≠C**, A≠D**, A≠E**, C≠D**, D≠E**

NFE: A≠B**, A≠D**, B≠C**, B≠D**, B≠E**,C≠D*, D≠E**

TDN: A≠B**, A≠C*, A≠E**, B≠D*, B≠E*, C≠E**, D≠E**

DE: A≠B*, A≠E**, B≠D*, B≠E**, C≠E**, D≠E**

ME: A≠B*, A≠E**, B≠D*, B≠E**, C≠E**, D≠E**

Table 2. Qualitative (QLRA) and quantitative (QRA) radical scavenging activity by $DPPH^{\cdot}$ radical[§] essay of endemic vegetation species browsed or grazed by goats on semiarid rangelands

Species	QLRA by TLC[†]			QRA %		
	MeOH	MeOH:H_2O (80:20)	acetone	MeOH	MeOH:H_2O (80:20)	acetone
Aristida adsencionis	+++	+++	+	26.43^{aC} ± 0.84	24.34^{bG} ± 0.46	11.22^{cDE} ± 0.19
Acacia schaffneri	++++	++++	++	42.08^{bB} ± 0.14	47.38^{aA} ± 0.17	6.46^{cFGH} ± 2.20
Bouteloua curtipendula	+++	+++	+	25.09^{bC} ± 0.63	35.42^{aD} ± 0.24	18.52^{cAB} ± 2.23
Bouteloua repens	++	+++	+	17.80^{bDE} ± 0.02	30.92^{aBC} ± 0.02	8.55^{cFGH} ± 0.01
Chloris virgata	+++	++	+	27.12^{aC} ± 0.38	27.39^{aI} ± 0.61	16.6^{bBC} ± 1.56
Jatropha dioica	++	++	+++	10.03^{bIJH} ± 0.09	24.13^{aG} ± 0.61	5.79^{cFIGH} ± 1.13
Leptochloa dubia	+++	++	+	47.01^{aA} ± 1.84	21.48^{bH} ± 0.67	14.58^{cDC} ± 0.87
Mimosa biuncifera	++	++	+++	8.93^{bIJ} ± 0.27	28.70^{aFE} ± 2.04	5.67^{cFIGH} ± 0.67
Rhynchelytrum roseum	++++	+++	+	40.08^{bB} ± 0.19	44.63^{aBC} ± 0.24	6.44^{cFGH} ± 0.35
Urochloa fasciculata	+++	++	+	20.11^{bD} ± 0.93	30.19^{aE} ± 0.46	5.95^{cFIGH} ± 0.49
Mean complete				**26.47^{A*} ±12.6**	**31.46^{A*} ± 8.36**	**9.98^{A*} ± 4.87**
Acacia farnesiana	++++	++++	+++	43.22^{bAB} ± 0.15	47.59^{aA} ± 0.13	20.78^{cA} ± 1.64
Opuntia amyctaea	++	+	+	12.11^{bIHG} ± 0.36	20.83^{aH} ± 0.36	4.00^{cIGH} ± 1.59
Opuntia hytiacantha	++	++	+	10.84^{bIHG} ± 0.02	18.30^{aI} ± 0.33	5.57^{cFIGH} ± 0.72
Prosopis laevigata	++	++	++	14.72^{bEGF} ± 4.5	22.92^{aHG} ± 0.95	2.70^{cIH} ± 1.52
Mean fruits				**20.22^{AB*} ± 14.0**	**27.41^{B*} ± 12.3**	**8.26^{B*} ± 7.72**
Celtis pallida	++	++	++	10.41^{bIJHG} ± 0.52	29.49^{aEF} ± 0.29	4.87^{cFIGH} ± 0.51
Prosopis laevigata	+++	+++	++	19.99^{bD} ± 0.28	35.68^{aD} ± 1.24	7.36^{cFG} ± 0.64
Verbasina serrata	+	++	+	6.10^{bJ} ± 0.09	17.04^{aI} ± 0.22	2.50^{cI} ± 0.52
Mean leaves				**$12.17c^{*}$ ± 6.17**	**27.40^{B*} ± 8.25**	**4.91^{D*} ± 2.16**
Celtis pallida	++	++	+	17.06^{bDE} ± 0.90	44.02^{aC} ± 1.54	7.83^{cFE} ± 0.91
Verbasina serrata	++	+	+	12.69^{bIEHGF} ± 1.5	17.78^{aI} ± 0.22	5.66^{cFIGH} ± 1.14
Zalazania augusta	+++	+++	+	27.76^{bC} ± 3.56	46.41^{aAB} ± 0.80	6.97^{cFG} ± 0.84
Mean stems				**19.17^{AB*} ± 7.01**	**36.07^{A*} ± 13.78**	**6.82^{C*} ± 1.59**
Opuntia affasiacantha	++	++	++	16.52^{bEDF} ± 0.50	24.13^{aG} ± 0.86	7.36^{cFG} ± 1.08
Opuntia hytiacantha	++	++	++	14.64^{bEHGF} ± 0.48	46.92^{aA} ± 0.39	6.93^{cFG} ± 0.80
Opuntia robusta	++	++	++	13.53^{bIEHGF} ± 0.67	16.14^{aI} ± 0.91	7.13^{cFG} ± 0.85
Opuntia streptacantha	++	++	++	16.57^{aEDF} ± 0.74	16.39^{aI} ± 0.04	7.95^{bFE} ± 1.24
Opuntia tomentosa	++	++	++	14.26^{bEHGF} ± 2.08	16.77^{aI} ± 0.39	6.21^{cFIGH} ± 1.47
Mean cladodes				**15.10^{C*} ± 1.51**	**24.07^{BC*} ± 11.82**	**7.12^{C*} ± 1.08**
BHA[╬]	+++++	+++++	+++++	90.22	86.36	88.08
Alpha tocopherol	+++++	+++++	+++++	91.07	84.97	89.42

[†] Radical scavenging activity of plant extracts on TLC plates, developed by methanol:ethyl acetate (70:30, v/v). $^{+}$ – weak intensity; $^{++}$ – intermediate intensity; $^{+++}$ – strong intensity, $^{++++}$ – very strong intensity. [§] $DPPH^{\cdot}$=1, 1-diphenyl-2-picrylhydrazyl (200 mg of $DPPH^{\cdot} \cdot 100^{-1}$ ml methanol). [╬] BHA – butylhydroxyanisol. Means with different small letters within the same row are significantly different at p = 0.05. Means with different capital letters within columns are significantly different at p = 0.05; * means values of plants portions within the different extractants are significantly different at P = 0.001, ^{A,B,C,D} means values of plants portions with different capital letters within columns are significantly different at p = 0.05

Table 3. Total polyphenol, hydroxycinnamic acid and flavonoid content of endemic vegetation species browsed or grazed by goats

Species	TPC	Hydroxycinnamic acids, g · 100 g^{-1} DM			Flavonoids, mg · 100 g^{-1}		
		gallic	caffeic	cinnamic	gallocatechin	catechin	epicatechin
Aristida adsencionis	160^{lm} ± 11.15	0.162^{gfe} ± 0.001	0.022^{f} ± 0.004	0.272^{f} ± 0.004	11.05^{c} ± 0.865	0.050^{e} ± 0.015	0.353^{d} ± 0.045
Acacia schaffneri	2730^{b} ± 30.2	0.559^{cd} ± 0.016	0.200^{d} ± 0.017	ND	ND	0.064^{e} ± 0.017	1.342^{b} ± 0.111
Bouteloua curtipendula	314^{igh} ± 10.07	0.194^{gfe} ± 0.005	ND	0.023^{h} ± 0.007	8.32^{f} ± 0.840	0.045^{e} ± 0.021	0.180^{e} ± 0.009
Bouteloua repens	364^{g} ± 10.41	0.580^{e} ± 0.171	0.071^{fe} ± 0.020	1.382^{c} ± 0.135	ND	0.870^{c} ± 0.116	0.316^{d} ± 0.008
Chloris virgata	474^{f} ± 2.65	0.175^{gfe} ± 0.016	0.022^{f} ± 0.011	0.034^{h} ± 0.014	6.50^{i} ± 0.555	0.044^{e} ± 0.005	0.077^{feg} ± 0.012
Jatropha dioica	189^{lk} ± 4.51	0.106^{gf} ± 0.16	0.047^{f} ± 0.018	ND	ND	0.070^{e} ± 0.022	0.011^{g} ± 0.001
Leptochloa dubia	146^{nlm} ± 8.5	0.047^{cd} ± 0.013	0.015^{f} ± 0.003	0.023^{h} ± 0.012	8.66^{e} ± 1.149	0.035^{e} ± 0.011	0.008^{g} ± 0.001
Mimosa biuncifera	341^{gh} ± 2.52	0.223^{fe} ± 0.0647	0.020^{f} ± 0.0037	0.422^{e} ± 0.0088	0.07^{n} ± 0.0016	1.105^{b} ± 0.0866	0.009^{g} ± 0.0011
Rhynchelytrum roseum	231^{jk} ± 3.51	0.255^{e} ± 0.0397	0.070^{f} ± 0.0021	0.020^{h} ± 0.0044	8.09^{g} ± 0.1732	0.004^{e} ± 0.0009	0.145^{ef} ± 0.0208
Urochloa asciculata	966^{c} ± 7.09	0.048^{g} ± 0.0173	0.085^{fe} ± 0.0113	ND	ND	0.021^{e} ± 0.0006	0.050^{feg} ± 0.0250
Mean complete	**592^{A} ± 724**	**0.253 ± 0.036**	**0.061 ± 0.010**	**0.311 ± 0.026**	**7.12 ± 0.597**	**0.231^{A} ± 0.030**	**0.249^{A} ± 0.023**
Acacia farnesiana	38170^{a} ± 357	ND	ND	ND	ND	0.004^{e} ± 0.0020	0.003^{g} ± 0.0013
Opuntia amyctaea	587^{e} ± 23.4	ND	ND	ND	1.150^{m} ± 0.1001	0.004^{e} ± 0.0028	0.021^{fg} ± 0.0015
Opuntia hytiacantha	343^{gh} ± 4.58	0.037^{g} ± 0.0080	0.031^{f} ± 0.0010	ND	25.56^{a} ± 2.5494	0.083^{e} ± 0.0095	0.091^{feg} ± 0.0040
Prosopis laevigata	314^{igh} ± 21.5	0.410^{dc} ± 0.0660	0.230^{d} ± 0.0029	0.223^{fg} ± 0.0300	8.20^{fg} ± 0.5519	0.700^{d} ± 0.0366	0.530^{c} ± 0.0502
Mean fruits	**9854^{B} ± 17077**	**0.224 ± 0.0370**	**0.131 ± 0.0019**	**0.223 ± 0.0300**	**11.64 ± 1.067**	**0.198^{B} ± 0.0509**	**0.161^{B} ± 0.0571**
Celtis pallida	280^{ijh} ± 31.01	0.090^{gf} ± 0.0089	0.021^{f} ± 0.0018	0.040^{h} ± 0.0052	ND	0.004^{e} ± 0.0006	0.003^{g} ± 0.0010
Prosopis laevigata	968^{c} ± 35.95	ND	1.065^{a} ± 0.1407	2.860^{b} ± 0.2123	ND	1.400^{a} ± 0.1229	0.545^{c} ± 0.0866
Verbasina serrata	272^{ij} ± 15.53	0.175^{efg} ± 0.0215	0.380^{c} ± 0.0917	ND	10.21^{d} ± 0.5112	0.004^{e} ± 0.0006	0.005^{fg} ± 0.0002
Mean leaves	**507^{C} ± 347**	**0.133 ± 0.0152**	**0.489 ± 0.0781**	**1.45 ± 0.1087**	**10.21 ± 0.5112**	**0.469^{C} ± 0.0413**	**0.184^{C} ± 0.0292**
Celtis pallida	370^{g} ± 21.08	0.090^{gf} ± 0.0089	ND	ND	4.23^{l} ± 0.5323	0.012^{e} ± 0.0025	0.010^{g} ± 0.0025
Verbasina serrata	874^{d} ± 39.58	0.061^{g} ± 0.0171	0.011^{f} ± 0.0014	0.030^{h} ± 0.0086	20.13^{b} ± 5.7160	0.090^{e} ± 0.0091	0.004^{g} ± 0.0007
Zalazania augusta	480^{f} ± 19.29	0.510^{cb} ± 0.090	0.044^{f} ± 0.0040	1.220^{d} ± 0.1819	ND	0.004^{e} ± 0.0008	0.003^{g} ± 0.0009
	575^{D} ± 231.06	**0.220 ± 0.0386**	**0.028 ± 0.0027**	**0.625 ± 0.0952**	**12.18 ± 3.124**	**0.035^{D} ± 0.0041**	**0.006^{D} ± 0.0014**
Opuntia affasiacantha	202^{k} ± 13.5	0.044^{g} ± 0.0045	0.040^{f} ± 0.0056	ND	6.24^{j} ± 0.9103	0.006^{e} ± 0.0002	0.008^{g} ± 0.0010
Opuntia hytiacantha	77^{n} ± 8.0	0.090^{gf} ± 0.0064	ND	ND	4.42^{k} ± 0.0252	0.006^{e} ± 0.0042	0.003^{g} ± 0.0007
Opuntia robusta	537^{ef} ± 21.46	0.276^{de} ± 0.0203	0.190^{de} ± 0.0046	11.150^{a} ± 0.0177	ND	0.070^{e} ± 0.0002	3.300^{a} ± 0.0809
Opuntia streptacantha	147^{nlm} ± 12.29	0.202^{gf} ± 0.0607	ND	ND	ND	0.060^{e} ± 0.0192	0.004^{g} ± 0.0012
Opuntia tomentosa	335^{igh} ± 27.1	0.060^{g} ± 0.0105	0.043^{f} ± 0.0070	ND	6.82^{h} ± 0.8641	0.020^{e} ± 0.0006	0.013^{g} ± 0.0010
Mean cladodes	**259^{E} ± 169.02**	**0.1347 ± 0.0205**	**0.091 ± 0.0057**	**11.15 ± 0.0177**	**5.83 ± 0.5998**	**0.0188^{A} ± 0.0237**	**0.665^{E} ± 0.0837**

TPC – total polyphenol content (mg of gallic acid equivalents (GAE) · kg^{-1} dry matter). ND – no detected; means with different letters within columns are significantly different at $p = 0.05$; Means with capital letters are significantly different (*$p = 0.05$,**$P = 0.001$) within column where:
TPC: A≠B*, A≠D*, B≠E**, D≠E**
Catechin: A≠ E**
Epicatechin: A≠D**, A≠E*, B≠D*

Results

Chemical composition

The chemical composition of the analysed samples demonstrated large differences among plant species and smaller differences within species (Table 1). Shrubs showed large fibre and small crude protein (CP) concentrations. Some species, however, had high crude protein values, e.g. *Acacia schaffneri*, *Prosopis laevigata*, *Celtis pallida, Mimosa biuncifera* and *Verbasina serrata*. Leaves normally have larger crude protein concentrations than fruits, stems, cladodes and complete plant samples. The largest values of metabolizable energy (ME) and total digestible nutrients (TDN) were found in *A. farnesiana* pods, whereas the largest crude fibre content was in *Zalazania augusta* stems. Variable results for ether extract, TDN and energy values were found among plant species and among plant parts. The significant differences between plant portions and pairs of portions found after the Kruskal-Wallis and Mann-Whitney U tests are shown in Table 1.

Radical scavenging activity

All of the extracts tested displayed qualitative radical scavenging activity (QLRA). Acetone extracts resulted in poorer responses than methanol ($P < 0.003$) and methanol:water ($P < 0.001$) extracts according to the Wilcoxon test. No difference was observed between methanol and methanol:water extracts when the Friedman test was employed ($p = 0.157$). Extracts of complete plants had a larger QLRA than fruits, leaves, stems, and cladodes, with some exceptions, e.g. *Acacia* fruits (Table 2). For QRA, whole plants yielded the best activity, followed by fruits, stems, and leaves. Differences were also observed, however, due to the extraction media employed; e.g., stems yielded higher radical protection measured in methanol:water than that observed in methanol and acetone extracts ($p < 0.05$). Cladodes tended to have the lowest mean for QRA for the three extractants. Our results revealed a close Pearson's correlation of the QRA measured by the $DPPH^+$ radical with total flavonoid (r = 0.890) and TPC (r = 0.948) contents.

Plant bioactive compounds

Tests for PBC were performed only in methanolic extractions, since during the first QLRA screening, this alcoholic media showed a similar QLRA as methanol:water. Acetone extracts were discarded because they gave the lowest QLRA response among the three extractants. The analysis of different plant portions (complete plants, fruits, leaves, stems) resulted in different concentrations of PBC (Table 3). Fruits had the highest mean value for total polyphenol content (TPC), whereas cladodes, the lowest, and this difference was significant ($P < 0.001$). This same relationship was observed when stems and cladodes were compared ($P < 0.001$). At single species level, *A. farnesiana* pods had the largest TPC (38,170 mg of GAE · kg^{-1} DM) of the shrubs (Table 3). The remaining extracts ranged from 77 (for *O. hyatacantha* cladodes) to 2730 mg of GAE/kg MD (for *A. schaffneri*).

The results for hydroxycinnamic acids were variable among plant species and plant portions. For example, the analysis showed that the extract of *Bouteloua repens* (complete plant) had the largest gallic acid content (0.580 g ·100 g^{-1} DM), whereas the leaves of *P. laevigata* contained the most caffeic acid (1.065 g ·100 g^{-1} DM). *O. robusta* cladodes showed the highest cinnamic acid average (11.15 g ·100 g^{-1} DM). For flavonoids, gallocatechin values of *O. hyatacantha* fruits (25.56 mg ·100 g^{-1} DM) and epicatechin of *O. robusta* cladodes (3.3 mg ·100 g^{-1} DM) were the largest values measured. The complete plant mean epicatechin content was significantly different ($p < 0.05$) from the cladode mean; the same was found in epicatechin means between complete plants and stems. Complete plant and cladodes, and fruits, and stems were significantly different ($p < 0.05$). No significant difference was found in gallocatechin mean values after statistical analysis.

Discussion

In order to understand the potential benefits of rangeland plant communities for animal husbandry and production in Central Mexico, our first goal was to investigate the chemical composition of the vegetation. Results on composition indicated that the shrubland species are characterized by high fibre contents and poor protein values; these results are in line with other vegetation assessments from low precipitation areas (Baraza et al., 2008; García-Winder et al., 2009). Although *Acacia*, *Mimosa*, *Prosopis* and *Verbasina* species had high crude protein contents, the use of some of these resources might be limited due to the presence of large tannin contents, which are a part of PBC (Ben Salem et al., 2005; Jiménez-Ferrer et al., 2008). The threshold for tannins with no negative effects is about 50 g · kg^{-1}, whereas larger ingestion would signify negative effects on intake. An intake of 20 g · kg^{-1}, however, may have positive effects on protein metabolism, rumen eficiency, and animal performace (Patra and Saxena, 2011).

Other researchers have pointed out that PBC contents are not the key factor for their consumption, instead, a high fibre content of feedstuffs seems to cause greater detrimental effects on intake (Alonso-Díaz et al., 2010). Nonetheless, at a moderate degree of consumption, such resources can be a valuable nutrient supply where seasonality and forage scarcity are the main limitations for low-input farming production. For example, the concentration of protein offered in the diet increased linearly with the inclusion of pods of the leguminous shrub *A. farnesiana* (0%, 12%, and 24%) in pelibuey lamb rations (García-Winder et al., 2009). Velázquez-Avendaño et al. (2005) concluded that the inclusion of 40% *A. farnesiana* in wool-sheep diets based on maize straw is recommended as a valuable protein source.

Another important fodder resource, despite their low protein content (from 4.0 to 8.9%), seemed to be *Opuntia* species, as their CP can be almost completely used to form microbial protein in the rumen. This is possible due to the readily available energy released from cladodes into ruminal fluid, which is essential for microbial protein synthesis (Parveen et al., 2010). The CP values of the *Opuntia* species in our study were around the 6% previously reported (Baraza et al., 2008; Parveen et al., 2010). Despite the good CP results of some of the shrub species, their exclusive use for goat feeding could cause temporary nutritional imbalances or permanent undernourishment, especially during dry seasons when grazing pressure is higher and the availability and nutritive value of forages decreases dramatically (García-Winder et al., 2009). To cope with this risk, proper supplementation during dry seasons is highly recommended (Galina et al., 2007).

In this context, food selection plays a key role in managing the nutritional demands for maintenance and productivity. The intake of specific plants and particular food items is regulated by the availability of the desired food and the capability of grazers to ingest and digest it (Reynaud et al., 2010; Villalba et al., 2010). Palatability, poisonous threshold, and the presence of alternative food choices are complementary causes that alter grazing behaviour; e.g., it is argued that plant bioactive compounds (PBC) modify animal behaviour and forage selection to maintain rumen functioning aimed at avoiding intoxication or metabolic disorders (Villalba et al., 2010). Therefore, the low content of PBC in leaves and cladodes may encourage their intake, whereas the high fibre and PBC contents of stems and complete plants may discourage their consumption (Patra and Saxena, 2011). Consequently, the total polyphenol content (TPC) measured for *A. farnesiana* should be considered with special interest because of its large value (38.2 g of GAE · kg^{-1}). Moreover, this concentration is similar to that reported by Ben Salem et al. (2005), who published values ranging from 37.3 to 69.8 g GAE· kg^{-1} for *A. cyanophylla* leaves. In contrast, Alonso-Díaz et al. (2010) reported larger values for *A. pennatula* leaves (97.2 g GAE · kg^{-1}). Specific geographic conditions and the age of the sampled plants could play an important role in these discrepancies, as suggested by Baraza et al. (2008).

The ingestion of large quantities of such fodders may involve important changes in rumen physiology, for example, lower degradation rates of protein compromising rumen homeostasis or even animal health (Rochfort et al., 2008). Risky concentrations of TPC for animal consumption can be diminished by chopping or water spraying the feedstuffs before they are offered (Ben Salem et al., 2005). Generally, the efforts to avoid rumen health disorders or toxaemia are less important where diets include diverse botanical choices with variation of toxicity (Villalba et al., 2010). For example, Garcia-Winder et al. (2009) demonstrated that elevated contents of *Acacia* in lamb rations (24%) can inhibit intake, while up to 12% of this shrub legume can be well tolerated and digested in combination with lucerne, maize, and soya-based feeding. In the present study, not all analysed plants showed high TPC values. In some species, these levels were similar to or lower than in species from temperate regions: e.g., *A. adsencionis*, *J. dioica*, *L. dubia* and *O. streptocantha* species had smaller TPC contents (from 0.147 to 0.189 g of GAE · kg^{-1}) than three mixtures of French pasture plants that averaged 9.5 g of GAE · kg^{-1} (Reynaud et al., 2010). Dudonné et al. (2009) reported that the concentration of total phenolic compounds in 30 aqueous plant extracts ranged from 6.86 to 397.03 mg GAE · g^{-1} of sample measured by the Folin-Ciocalteu method, meanwhile we found values from 77 to 38170 mg GAE · kg^{-1} of sample DM. These differences could be a consequence of the extractant employed and nature of the plants, since these factors determine the potential antioxidant activity and PBC profile (Ruiz-Terán et al., 2008; Mustafa et al., 2010).

It is well known that the concentration of bioactive compounds alters the palatability of feedstuffs and, consequently, the feed intake. Nonethless, the significance of bioactive compounds has included benefical aspects such as the improvement in the yield and quality of ruminant-derived feeds (higher CLA content), control of parasites, lower ruminal amonnia N concentration, enhancement of microbial protein synthesis, lower incidence of pasture

bloat, better live-weight gains, and mitigation of methane emisions (Rochfort et al., 2008; Alonso-Díaz et al., 2010; Patra and Saxena, 2011). In short, the inclusion of low amounts of PBC in animal diets has shown a positive impact on animal performance, milk and yield, without compromising dry matter intake or diet digestibilty. Furthermore, these PBCs are frequently cited as being the key to the underlying prevention and/or reduction of oxidative stress-related disorders (Quideau et al., 2011). Nevertheless, viewing plant polyphenols only as antioxidant agents must be considered with a great deal of caution, since the inclusion of high concentrations, which are still subjective values, affects palatability and feed intake and may cause poorer animal performance (Vasta et al., 2008). According to Quideau et al. (2011), the amount of PBC that can be found in each species depends on factors such as: plant resistance to microbial pathogens, resistance and tolerance to ingestion by herbivores, protection against solar radiation, reproduction phase of vegetation, plant nutrition, and interaction with other plants and organisms (insects, symbiotic fungi and bacteria).

The radical scavenging activity of PBCs is, in general, attributed to their hydroxyl groups, which determine their capabilities as effective antioxidants. According to the results of the present study, a positive trend between radical scavenging activity and bioactive compounds was confirmed (for TPC $r = 0.948$, and for flavonoids, $r = 0.890$). It has been suggested that a high correlation between these components is likely when complementary radical scavengers are absent and, at the same time, significant contents of TPC and flavonoids are present. In agreement with this finding, Mustafa et al. (2008) analysed plants by their antioxidant activity and their PBC, demonstrating that such compounds contribute directly to this claimed benefit.

The capability of the extractants to recover PBC is also an important factor that largely determines the assessment of biological activities of extracts. In the current experiment, the qualitative radical scavenging activity (QLRA) and the quantitative radical scavenging activity (QRA) values for the same forage sample varied depending on the solvents used. Methanol:water generally showed higher QRA than methanol and acetone extracts, with a few exceptions; e.g., *A. adsencionis* and *L. dubia*. The results suggest that more polar components present in the methanol:water extracts contributed towards their increased measured scavenging activity. Water coupled with methanol could have an additive effect on hydrophilic antioxidants that methanol or acetone could not extract, thus being less capable to recover radical scavengers. In line with these results, Ruiz-Terán et al. (2008) evaluated plant matrices by TLC and colorimetric assays, obtaining important variations in the responses when the solvent extract differed.

Our findings related to hydroxycinnamic acids showed that caffeic and cinnamic acids were not detected in any extract. A reason for this outcome could be that cell walls represent distinct and specific bound structural components of each plant (Komprda et al., 1999). Therefore, different cell wall components may change the digestibility of feedstuffs, since the hydroxycinnamic acids are in part responsible for the linkages between lignin and hemicellulose (Casler and Jung, 2006). The high value of cinnamic acid in *O. robusta* (11.15%) may be related to this effect.

Recent findings suggest that a high concentration of plant metabolites in the rumen may modify the cellulolytic activity of bacteria by the formation of a complex among plant material, cell and bacteria membranes, and by diminishing protein degradation (Patra and Saxena, 2011). Rochfort et al. (2008) have indicated that a large presence of bioactive compounds is capable of causing direct depression of feed intake, which inhibits ruminal microorganisms and, therefore, causes lower weight gain in lambs. Parveen et al. (2010) proposed that rapid degradation of forages can lead to surplus excretion of nitrogen through urine due to a limited capacity of forming new microbial protein. In that case, the modulator effect of PBC could be beneficial in reducing nitrogen excretion while enchancing by-pass protein availability (Rochfort et al., 2008; Patra and Saxena, 2011). Despite most of the plants analyzed here having high fibre contents, the results permitted identification of valuable protein sources and contributed to filling in the gap in the knowledge about the bioactive compounds of scrubby vegetation and their significance in the diet of goats on semiarid areas. Furthermore, the selection activity of the goats and their ability to mix and match distinct feed items with different PBC contents indicate that they balance their PBC intake below the limit of harmful ingestion (Rochfort et al., 2008; Villalba et al., 2010). The chemical composition of the plant species and plant portions browsed and grazed by goats on semiarid rangelands, explains the presence of some PBCs in goat products (Vasta et al., 2008; Cuchillo et al., 2010a,b).

Conclusions

Large protein contents of *Acacia schaffneri, Celtis pallida, Mimosa biuncifera*, and *Prosopis laevigata* lead these plants to being considered more valuable protein resources than before. Methanol:water was found to be the best solvent medium among those tested for extraction to assess radical scavenging activity. The antioxidant activity and plant bioactive compounds contents of rangeland vegetation are largely dependent on the plant species and plant part. Higher mean values of plant bioactive compounds were found in complete plants and stems than in fruits and leaves. A positive correlation was observed between radical scavenging activity and polyphenol-flavonoid concentration.

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