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TECHNICAL REPORT

Proximate composition, mineral content, and antioxidant properties of 14 Mexican weeds used as fodder

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Many wild weeds are used in Mexico as fodder. Due to their economic value, this investigation was undertaken to determine the chemical composition of 14 species. The mineral, crude protein, fiber, and total phenolic content, as well as the antioxidant activity, was determined. There was a significant variation in the mineral content among the 14 weeds, which all had calcium and potassium as the predominant elements. According to the results, some of the weeds might represent possible mineral deficiency sources, making the supplementation of certain minerals advisable. Although five of the species contained a low concentration of magnesium, the overall mineral ratios of the forage weeds were at safe levels, preventing grazing animals suffering from grass tetany, in contrast to the excessive calcium : phosphorus ratios that can affect their normal growth and bone development. The crude fiber and protein content in most of the weeds fell into the recommended range for the maintenance of cattle. The phenolic content of the studied weed extracts was higher than that reported for several Mediterranean forage species. These phenolic profiles correlate well with the radical scavenging activities of the extracts.

Keywords: antioxidant, fiber, fodder weeds, minerals, phenolics, protein.

INTRODUCTION

For farmers, weeds cause more harm than good if not controlled; however, in Mexican traditional domestic ranching, the aerial parts of several weeds are consumed as forage by livestock and play an important role in the traditional household economy. Weeds also are used as human food or construction material. An ethnobotanical study of weeds from the state of Queretaro, Mexico, revealed that most of them belong to Asteraceae, Poaceae, Euphorbiaceae, and Fabaceae. Of the 102 described weed species from Queretaro, 25 are consumed as fodder by cattle, sheep, horses, and pigs (Suárez *et al.* 2004). In addition, some of them are edible and medicinal plants. Previous phytochemical investigations of some of these species have led to the isolation or identification of flavonoids (Saito 1974, 1976; Kaneta *et al.* 1978; Poi & Adityachaudhury 1989; Ragunathan & Sulochana 1994; Saito *et al.* 1995, 1996, 1998; Rochfort *et al.* 2006; Romani *et al.* 2006), phenolic acids (Chitindingu *et al.* 2007), terpenoids (Gupta *et al.* 1977; Ganzinger *et al.* 1981; Singh *et al.* 1990; Kinjo *et al.* 1994; Chhabra *et al.* 1999; Menut *et al.* 2000; Perez-Amador *et al.* 2000; Das *et al.* 2004; Das *et al.* 2006), fatty acids (Cisowski *et al.* 1996; He & Corke 2003), and alkaloids (Wilkinson *et al.* 1986). Malvalic acid, a cyclopropenyl fatty acid isolated from cheeseweed (*Malva parviflora* L.), has been related to mortality in foraging livestock (Shale *et al.* 2005).

The presence of phenolic components in the aerial parts of forage legumes has been associated with the prevention of grazing at the start of the vegetative season (Gutman *et al.* 2000); nonetheless, these phytochemicals

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are recognized for their antioxidant properties (Scalbert *et al*. 2005; Sooberate *et al*. 2005). Although the antioxidant activities of natural compounds have been associated with human health, their roles in animal nutrition are still a topic of current interest. Among the few data available, it has been documented that reactive oxygen metabolites might contribute to mastitis, udder edema, and the suboptimal reproductive performance of dairy cattle (Miller & Brzezinska-Slebodzinska 1993). Furthermore, pneumonia and enteritis in farm animals have been considered to be a consequence of oxidative stress (Lykkesfeldt & Svendsen 2007).

To assess their nutritive and nutraceutical values, this investigation was undertaken to determine the chemical composition and antioxidant properties of the following fodder weeds: rough pigweed (*Amaranthus hybridus* L.), field mustard (*Brassica rapa* L.), Mexican aster (*Cosmos bipinnatus* Cav.), Bermudagrass (*Cynodon dactylon* Pers.), manayupa (*Desmodium molliculum* DC.), morningglory (*Ipomoea purpurea* Roth.), *M. parviflora*, toothed burclover (*Medicago polymorpha* var. *vulgaris* Benth.), tenleaf (*Oxalis decaphylla* H. B. & K), Santa Maria feverfew (*Parthenium hysterophorus* L.), Irish eyes (*Sanvitalia procumbens* Lam.), simsia (*Simsia amplexicaulis* Pers.), Johnsongrass (*Sorghum halepense* Pers.), and *Tithonia tubiformis Cass*.

MATERIALS AND METHODS

Chemicals

All the solvents were analytical grade. KCl, $CaCO_3$, MgO, H_3PO_4, NaCl, Fe, Zn, Cu, KH_2PO_4 $(NH_4)_2MoO_4$, C_6H_4-1,4-$(OH)_2$, Na_2SO_3, NaOH, HCl, H_2SO_4, HNO_3, La_2O_3, Na_2CO_3, boric acid, methylene blue, methyl red, and copper (II) sulfate were obtained from Mallinckrodt Baker (Phillipsburg, NJ, USA). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), gallic acid, and Folin Ciocalteu reagent were purchased from Sigma (St Louis, MO, USA).

Plant material

Except *C. dactylon* and *S. halepense*, which are perennial, the other plants analyzed are annual wild species growing only during the wettest time of the year in semiarid and uncultivated lands, where cattle usually graze from June to September, the period when weeds grow and become very leafy. These weeds begin to disappear by October. The rainfall in the different zones where the plants were collected is low, varying from 0.1–235 mm, and the temperatures in this period range from 16.3–23.2°C.

The aerial parts of the plants at the mature stage were collected in August 2005 in the following localities of the state of Queretaro: Boye (Cadereyta) (*A. hybridus,* voucher no. 55); San Juan del Rio-Amealco (*C. dactylon*, 13a; *D. molliculum*, 13b; *O. decaphylla,* 836a; *S. halepense,* 13c); Laguna de Servín (Amealco) (*S. amplexicaulis,* 68); Tequisquiapan (*T. tubiformis,* 178; *I. purpurea,* 175; *M. parviflora,* 177); Queretaro city (*S. procumbens,* 840; *P. hysterophorus,* 839); Lagunillas (Huimilpan) (*C. bipinnatus,* 67); and Amealco (*M. polymorpha,* 43a; *B. rapa,* 42a). A voucher specimen of each plant has been deposited in the Ethnobotanical Collection of the Herbarium of Queretaro "Dr Jerzy Rzedowski", located at the School of Natural Sciences, University of Queretaro, Mexico. The plants were dried at 39°C and the ground material was stored and protected from light at 4°C for future use.

Mineral and proximal composition

Once the material was ground, each of the following experiments was performed independently. The minerals, Zn, Cu, Fe, and Mg, were measured by atomic absorption spectroscopy (Analyst 100; Perkin Elmer, Waltham, MA, USA). Sodium, K, and Ca were determined by emission spectroscopy and P was evaluated by a micromethod, according to the standard methods of the Association of Official Analytical Chemists (AOAC) (2000). The mineral ratio of Ca : P was expressed on a percentage basis and the ratio of (K)/([Ca] + [Mg]) was expressed on a mEq basis. The crude protein content was estimated by the micro-Kjeldahl method, as modified by Pearson (1971). The crude protein was calculated by multiplying the total N by a conversion factor of 6.25 g protein/g of N. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) content were determined according to the standard methods of the AOAC (2000).

Determination of the total phenolic content

The ground plant material (10 g) was extracted with 80% aqueous methanol by maceration. The extract was evaporated to dryness under vacuum and stored at 4°C. The total phenolic content of the extracts was determined according to the Folin-Ciocalteu colorimetric method (Dewanto *et al*. 2002). The appropriate dilutions of the extracts were oxidized with 250 μL of 1 N Folin-Ciocalteu reagent. After 5 min, 1.25 mL of a 20% Na_2CO_3 solution was added to neutralize the reaction, allowing the reaction mixture to stand for 2 h. Then, the absorbance was measured against a prepared blank at 760 nm using a spectrometer (UV/VIS Lambda 40; Perkin Elmer). The results are expressed as mg of gallic

acid equivalents (GAE) per g of extract. All the data are reported as the average of three measurements.

1,1-Diphenyl-2-picrylhydrazyl scavenging activity

The radical scavenging activity of the extracts was determined using the stable radical DPPH, according to the method reported by Fukumoto and Mazza (2000). All the reactions were conducted in 96 well microplates (Nalge Nunc International, NY, USA). A 20 μL aliquot of 80% methanolic solution of the extracts at various concentrations was mixed with 200 μL of 150 μmol^{-1} of DPPH in 80% methanol. The controls contained all the reaction reagents, except the extract or positive control substances (trolox and butylhydroxytoluene [BHT]). After a 30 min incubation at ambient temperature in darkness, the resultant absorbance was recorded at 520 nm in a tunable microplate reader (Versa Max; Molecular Devices Company, Sunnyvale, CA, USA). The experiments were carried out by using a randomized block design (three blocks): inside each block, every treatment was independently applied three times. The percentage of absorbance inhibition was calculated according to the equation: % inhibition = ([absorbance of control − absorbance of samples]/absorbance of control) × 100. The radical scavenging activities were expressed as IC_{50} values, calculated from the log-dose inhibition curve obtained by a non-linear regression algorithm (Prism 4.0, GraphPad; GraphPad Software, CA, USA).

RESULTS

The mineral composition is presented in Tables 1 and 2. There was a significant variation in the mineral content among the 14 weeds. A high Ca, Cu, and Zn content was observed for *M. parviflora*, a high Na and K content for *S. amplexicaulis*, and a high Fe and P content for *O. decaphylla*. *Cosmos bipinnatus* and *S. procumbens* demonstrated the best Mg content.

The NDF, ADF, and crude protein content is given in Table 3. Six of the studied weeds (*M. parviflora*, *A. hybridus*, *I. purpurea*, *M. polymorpha*, *S. procumbens*, and *T. tubiformis*) had <40% and 50% of ADF and NDF, respectively. The ADF and NDF values for *M. parviflora* were the lowest and those for *S. halepense* were the highest. The protein content observed in the plants ranged from 5.7% (*S. halepense*) to 29.8% (*M. parviflora*).

As phenolic substances have been shown to be responsible for the antioxidant activity of plant extracts (Scalbert *et al*. 2005; Sooberate *et al*. 2005), the total phenol content data for the weed extracts were investigated and the results are shown in Table 3. The total phenol content, expressed as GAE, displayed significant

Table 1. Mineral composition of 14 Mexican forage weeds

Plant	Na ($g\ kg^{-1}$ DM)	Ca ($g\ kg^{-1}$ DM)	K ($g\ kg^{-1}$ DM)	Mg ($g\ kg^{-1}$ DM)	(K)/([Ca] + [Mg])
Amaranthus hybridus	1.97 ± 0.0179	13.71 ± 0.0070	9.50 ± 0.0000	1.58 ± 0.0008	0.30
Brassica rapa	2.52 ± 0.0187	9.51 ± 0.0081	9.16 ± 0.0013	1.78 ± 0.0000	0.38
Cosmos bipinnatus	3.38 ± 0.0339	5.68 ± 0.0023	7.24 ± 0.0002	2.12 ± 0.0080	0.41
Cynodon dactylon	3.41 ± 0.0159	4.71 ± 0.0073	6.18 ± 0.0069	0.94 ± 0.0014	0.51
Desmodium molliculum	1.90 ± 0.0065	7.89 ± 00042	7.64 ± 0.0013	0.69 ± 0.0003	0.43
Ipomoea purpurea	2.69 ± 0.0300	9.08 ± 0.0112	9.39 ± 0.0001	0.63 ± 0.0007	0.48
Malva parviflora	3.08 ± 0.0095	19.32 ± 0.0012	9.68 ± 0.0001	1.22 ± 0.0000	0.23
Medicago polymorpha	1.94 ± 0.0250	8.18 ± 0.0064	8.41 ± 0.0005	1.72 ± 0.0013	0.39
Oxalis decaphylla	3.07 ± 0.0058	5.10 ± 0.0050	8.27 ± 0.0005	1.43 ± 0.0014	0.57
Parthenium hysterophorus	1.26 ± 0.0450	12.06 ± 0.0113	9.44 ± 0.0007	0.53 ± 0.0003	0.37
Simsia amplexicaulis	4.03 ± 0.0309	8.18 ± 0.0025	9.64 ± 0.0002	0.83 ± 0.0002	0.52
Sorghum halepense	2.91 ± 0.0145	4.08 ± 0.0046	9.00 ± 0.0007	1.81 ± 0.0020	0.65
Sanvitalia procumbens	3.16 ± 0.0380	7.62 ± 0.0082	9.46 ± 0.0002	2.37 ± 0.0025	0.42
Tithonia tubiformis	3.13 ± 0.0196	19.53 ± 0.0014	8.89 ± 0.0002	0.33 ± 0.0002	0.23
Recommended ($g\ kg^{-1}$)†	0.6–0.8	1.9–4.5	6–7	1–2	<2.2

†Recommended value for the maintenance of beef cattle (NRC 1996). All the values were the mean of three measurements. DM, dry matter.

Table 2. Mineral composition of 14 Mexican forage weeds

Plant	Cu ($\times10^{-3}$ g kg^{-1} DM)	Fe (g kg^{-1} DM)	Zn ($\times10^{-2}$ g kg^{-1} DM)	P (g kg^{-1} DM)	Ca : P
Amaranthus hybridus	9.4 ± 0.0004	0.13 ± 0.0007	1.56 ± 0.0010	3.31 ± 0.0003	4.14
Brassica rapa	6.7 ± 0.0007	0.14 ± 0.0050	2.22 ± 0.0011	7.65 ± 0.0047	1.24
Cosmos bipinnatus	9.7 ± 0.0004	0.12 ± 0.0006	3.28 ± 0.0013	9.12 ± 0.0021	0.62
Cynodon dactylon	10.5 ± 0.0004	0.32 ± 0.0109	4.05 ± 0.0018	7.50 ± 0.0070	0.63
Desmodium molliculum	9.4 ± 0.0004	0.53 ± 0.0131	2.04 ± 0.0027	0.14 ± 0.0003	56.39
Ipomoea purpurea	14.2 ± 0.0004	0.29 ± 0.0018	2.99 ± 0.0026	0.75 ± 0.0009	12.11
Malva parviflora	17.8 ± 0.0007	1.44 ± 0.0002	4.59 ± 0.0005	2.06 ± 0.0001	9.36
Medicago polymorpha	14.2 ± 0.0013	0.79 ± 0.0117	2.04 ± 0.0033	1.37 ± 0.0002	5.95
Oxalis decaphylla	15.3 ± 0.0016	7.05 ± 0.0305	2.76 ± 0.0009	12.55 ± 0.0055	0.41
Parthenium hysterophorus	12.9 ± 0.0004	0.35 ± 0.0086	1.36 ± 0.0010	1.37 ± 0.0051	8.81
Simsia amplexicaulis	17.2 ± 0.0015	0.03 ± 0.0003	2.25 ± 0.0004	0.33 ± 0.0000	24.80
Sorghum halepense	8.9 ± 0.0006	0.27 ± 0.0087	3.59 ± 0.0017	5.33 ± 0.0074	0.77
Sanvitalia procumbens	13.2 ± 0.0009	2.84 ± 0.0191	4.11 ± 0.0006	0.82 ± 0.0004	9.24
Tithonia tubiformis	14.0 ± 0.0012	0.34 ± 0.0186	2.70 ± 0.0039	4.41 ± 0.0017	4.43
Recommended (g kg^{-1})†	0.01	0.05	0.03	1–2	1:1–7:1 (ideal)

†Recommended value for the maintenance of beef cattle (NRC 1996). All the values were the mean of three measurements. DM, dry matter.

Table 3. Total protein, neutral detergent fiber, acid detergent fiber, phenolic content, and antioxidant capacity of 14 Mexican forage weeds

Botanical species	Neutral detergent fiber (% DM)	Acid detergent fiber (% DM)	Protein (% DM)	Total phenolic content (mg of GAE/g of extract)	IC_{50} (μg mL^{-1})
Amaranthus hybridus	45.01 ± 0.70	32.42 ± 0.21	12.28 ± 0.220	49.43 ± 1.40	737.90 ± 1.008
Brassica rapa	63.72 ± 0.97	49.57 ± 0.44	9.89 ± 0.130	40.18 ± 1.20	893.30 ± 1.015
Cosmos bipinnatus	43.55 ± 0.02	41.71 ± 1.93	10.52 ± 0.200	65.01 ± 1.00	365.59 ± 1.023
Cynodon dactylon	70.82 ± 0.23	39.44 ± 1.45	8.48 ± 0.134	47.27 ± 1.80	843.33 ± 1.005
Desmodium molliculum	42.98 ± 0.03	41.67 ± 1.19	16.23 ± 0.080	125.82 ± 0.40	221.30 ± 1.005
Ipomoea purpurea	44.60 ± 0.17	35.41 ± 1.11	20.12 ± 0.200	24.02 ± 0.24	1963.36 ± 1.004
Malva parviflora	26.33 ± 0.46	21.41 ± 0.43	29.86 ± 0.090	21.88 ± 0.52	1577.61 ± 1.022
Medicago polymorpha	44.62 ± 0.52	33.76 ± 1.29	18.59 ± 0.320	38.56 ± 1.29	618.01 ± 1.006
Oxalis decaphylla	51.14 ± 1.02	42.29 ± 0.77	10.46 ± 0.300	59.81 ± 0.78	269.15 ± 1.006
Parthenium hysterophorus	54.80 ± 0.12	43.89 ± 0.35	10.11 ± 0.200	57.13 ± 1.16	289.06 ± 1.012
Simsia amplexicaulis	54.45 ± 0.40	44.76 ± 1.33	11.69 ± 0.100	52.46 ± 1.70	246.03 ± 1.017
Sorghum halepense	73.21 ± 0.65	47.02 ± 0.49	5.72 ± 0.010	69.83 ± 1.80	369.82 ± 1.014
Sanvitalia procumbens	37.37 ± 1.71	32.89 ± 0.82	9.72 ± 0.190	47.60 ± 1.30	862.97 ± 1.021
Tithonia tubiformis	37.73 ± 0.39	36.08 ± 0.08	20.01 ± 0.070	41.58 ± 1.80	671.42 ± 1.016

All the values were the mean of three measurements. DM, dry matter; GAE, gallic acid equivalents; IC, inhibitory concentration.

variation, ranging from 21.88 (*M. parviflora*) to 125.82 (*D. molliculum*) mg of GAE per g of extract.

The free radical scavenging properties of the extracts were determined by the DPPH assay, where this radical is reduced by the antioxidant compounds to its hydrazine derivative (Prior *et al.* 2005). The extracts were capable of scavenging DPPH radicals in a concentration-dependent fashion. The estimated medium inhibitory concentration (IC_{50}) values are shown in Table 3. *Desmodium molliculum* showed the strongest DPPH radical scavenging activity, with an IC_{50} of 221.30 μg mL^{-1}, compared to *I. purpurea*, which presented the least DPPH radical scavenging activity, with an IC_{50} of 1963.36 μg mL^{-1}.

DISCUSSION

Although the Ca concentrations of the studied weeds are higher than the reported values for forage cultivars (Nashiki *et al.* 2005), those of Mg and K are lower. The levels of Na, Ca, and K required for cattle maintenance (NRC 1996) are met by all the weeds; however, five species (*P. hysterophorus*, *S. amplexicaulis*, *T. tubiformis*, *I. purpurea*, and *D. molliculum*) do not reach the recommended value for Mg. It has been suggested that forage (K)/([Ca] + [Mg]) molar charge ratios >2.2 increase the risk of grass tetany in grazing animals (Underwood 1981). Although some species contain a low concentration of Mg, the mineral ratios of the forage weeds are at safe levels. Four weeds have both a low concentration of P (<1.2 g kg^{-1} dry weight material) (NRC 1996) and a high Ca : P ratio.

Ideal Ca : P ratios of between 1:1 and 7:1 for growth and bone development have been recommended (NRC 1996); therefore, the Ca : P ratios of *P. hysterophorus*, *S. procumbens*, *M. parviflora*, *I. purpurea*, *S. amplexicaulis*, and *D. molliculum* are excessive. Although *M. parviflora*, *O. decaphylla*, and S. *procumbens* demonstrate Fe values higher than the maximum tolerable concentration (1 g kg^{-1}), *A. hybridus*, *B. rapa*, *P. hysterophorus*, *S. amplexicaulis*, *D. molliculum*, and *M. polymorpha* do not meet the required concentration of Zn (0.03 g kg^{-1}) (NRC 1996).

Similar values to those reported for the protein content in forage legumes (Karachi *et al.* 1997; Nashiki *et al.* 2005) have been obtained in the present study. It has been suggested that 8.9% of the available crude protein in plant material is required for the maintenance of cattle (NRC 1996). This level is met by all the edible plants, except *C. dactylon* and *S. halepense*.

The phenolic content of the weed extracts is high, compared with those of several Mediterranean forage species (11–12 mg g^{-1}) (Pecetti *et al.* 2007). *Desmodium molliculum* shows the highest content of phenols and the best antioxidant capacity; however, this extract displays less antioxidant activity compared to the reported value (Lock *et al.* 2005). The *B. rapa* extract yields a better phenolic content and antioxidant capacity compared to the reported value of 2.50 mg of GAE per g of extract and an IC_{50} value of 550 mg of extract per mg of DPPH, respectively (Romani *et al.* 2006). The evaluated extracts exhibit less antioxidant power than BHT (IC_{50} = 56 μg mL^{-1}) and trolox (IC_{50} = 115 μg mL^{-1}).

CONCLUSIONS

From the above results, we can deduce that weeds, such as *C. bipinnatus*, *M. polymorpha*, *T. tubiformis*, and *A. hybridus* are good-quality forage plants as they meet most of the recommended values for cattle maintenance. In spite of the low value of Ca : P, *C. bipinnatus* demonstrated not only good levels of minerals, fiber, and protein, but also a high content of antioxidant phenolic compounds. However, some of the weeds might represent possible mineral deficiency sources and the supplementation of certain minerals is required. On account of the high content of phenolic components and the good free radical scavenging properties of *C. bipinnatus*, *P. hysterophorus*, *S. amplexicaulis*, *O. decaphylla*, *D. molliculum*, and *S. halepense*, these species represent a natural source of antioxidant agents. Further work is needed to determine the vitamin content and antinutritional components.

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