http://dx.doi.org/10.1590/1678-4162-13007

Arq. Bras. Med. Vet. Zootec., v.76, n.1, p.167-171, 2024

Communication

[*Comunicação*]

Mineral content and bioactive compounds in cactus cladodes varieties

[*Teor de minerais e compostos bioativos em variedades de palma forrageira*]

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Minerals perform numerous functions in the animal organism: structural, physiological, catalytic, and regulatory action, with involvement in almost all metabolic pathways (Mendonça Júnior *et al*., 2011). Thus, feeds and diets that contain these elements in deficit, excessive amounts or unbalanced proportions can cause damage to the animal productive, reproductive and health indexes.

Cactus cladodes, regardless of genus and species, exhibit disproportionate amounts of mineral elements in its composition (Dubeux Jr *et al*., 2021; Silva *et al*., 2023). Recent studies have reported that diets containing cactus cladodes (*Nopalea* or *Opuntia*) provide excessive intake of minerals, which, in the long-term, can cause negative effects on the organic systems of animals, in addition to cause environmental contamination due to the high excretion of these elements via urine and feces (Silva *et al*., 2021, 2023).

Cactus cladodes are rich in bioactive molecules, which may have beneficial effects on the animal organism or act as anti-nutritional factors (Astello-García *et al*., 2015; Dubeux Jr *et al*., 2021). However, there are still few studies that investigated the phytochemical profile of different varieties of cactus cladodes cultivated in the Northeast region of Brazil.

Thus, it was hypothesized that, regardless of the variety, cactus cladodes have unbalanced mineral relationships and a great diversity of substances with beneficial and/or harmful potential to the organism of production animals. Therefore, the purpose of the present study was to characterize the composition of macrominerals and trace minerals, in addition to quantifying the levels of hydrocyanic acid and performing a qualitative phytochemical screening in three varieties of cactus cladodes cultivated in the Brazilian semi-arid region.

The cactus plants (subterminal cladodes) were manually harvested with about 2 years old, in a private rural property, located in the Agreste region of the state of Pernambuco, Brazil: Miúda (*Nopalea cochenillifera* Salm Dyck), IPA-Sertânia (*Nopalea* sp.), and Orelha de Elefante Mexicana (O.E.M.) (*Opuntia stricta* Haw). The cladodes were processed was carried out in a forage machine (MC1n Laboremus®, Campina Grande, Brazil) and pre-dried in a forced ventilation oven set at 55ºC for 72 h and then processed in a Wiley mill (TE-648–Tecnal®) to pass through a 1-mm screen sieve. The samples were analyzed for dry matter (DM; method 934.01) as described in AOAC (Official…, 1990).

For quantification of minerals, the samples were digested with nitric acid (HNO_3 65% w/w) in a microwave (Mars® Xpress model: Technology Inside, CEM Corporation, Charlotte, NC). Approximately 0.3 g of the sample was weighed and digested with 5mL of HNO_3. The heating program used was reported by Pereira *et al*. (2020), with subsequent dilution (10-100x) using deionized water.

The macrominerals (calcium, Ca; phosphorus, P; magnesium, Mg; sodium, Na; and potassium, K) and trace minerals (copper, Cu; iron, Fe; zinc, Zn; and manganese, Mn) concentrations were quantified with an inductively coupled plasma

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Submitted: February 27, 2023. Accepted: July 27, 2023.

optical emission spectrometer (ICP-OES), model Optima 7000 DV (Perkin Elmer, USA). The multi-element standard solutions (Merck Certipur®) containing 1000mg/L for the ICP-OES analysis were obtained from the dilution of the analyzed elements in HNO_3.

The hydrocyanic acid (HCN) was determined using methodology recommended by Ades Totah and Hernández Luis (1986) adapted by Silva (2015). Qualitative phytochemical screening of bioactive compounds from cactus cladodes varieties was performed by thin layer chromatography, with 0.5g of samples diluted in 10 mL of methanol, by decoction, for 10 minutes on a hot plate. Then, they were filtered into Eppendorf-type tubes.

All standards (Table 1) were used at a concentration of 1.00mg/mL. Samples and standards were applied manually on silica gel 60 - F_{254} chromatographic plates (Macherey-Nagel®, Germany). The plates were developed in vats after saturation with the mobile phase (Table 1). The tank was saturated for about 15 minutes at room temperature (25°C). The bands were applied with a width of 0.5cm, this same value corresponding to the distance between them and the edges of the plates and the size of the width and length of the chromatographic plates. The samples were applied 5 mm from the origin and ending 5mm from the end of the plate.

After the elution of the plates, they were dried at room temperature, observed under ultraviolet light of 254 and 365nm and visible light, and then digitalized. Next, they were revealed with specific reagents for each metabolite (Table 1). The obtained bands were compared to the bands of the corresponding standards.

Table 1. Development systems and developers used for analysis of phytochemicals in extracts of cactus cladodes varieties by thin layer chromatography

Metabolite Class	System	Developer	Standard
Hydrolyzable tannins		$FeCl_3$	Gallic acid
Condensed tannins		VC	Catechin
Flavonoids	90:5:5		Rutin and Quercetin
Cinnamic Derivatives		$AlCl_3$	Chlorogenic acid and Caffeic acid
Terpenes and Steroids	70:30	LB + Δ	β-Sitosterol
Coumarins	50:50:50	KOH + Δ	Coumarin
Saponins	100:11:11:26	LB + Δ	Escina
Quinones	50:6.75:5	HNO_3 + KOH 10%	Senoside A
Alkaloids	50:6.75:5	Dragendorff	Atropine
Reducing sugars	50:20:10:10	Thymol + H_2SO_4 10% + Δ	D-maltose

Systems: 90:5:5 – Ethyl acetate: formic acid: water; 70:30 - Toluene: acetate; 50:50:50 – Ethyl ether: ethyl acetate: 10% acetic acid (saturation); 100:11:11:26 - Ethyl acetate: acetic acid: formic acid: water; 50:20:10:10 – Ethyl acetate: acetic acid: formic acid: water; 50:6.75:5 – Ethyl acetate: methanol: water. Source: Laboratory of Pharmacognosy/CB/UFPE.

The average Ca levels varied from 21.15 to 34.02g/kg DM, in the IPA-Sertânia and Miúda varieties, respectively. On the other hand, all cactus varieties had a low P concentration, with an average value of 5.02g/kg DM (Table 2). High levels of Mg and K were observed in different varieties of cactus cladodes. However, the Na concentration was 0.13g/kg DM, regardless of the variety (Table 2).

The concentrations of macrominerals obtained in the present study are consistent with the results reported in the literature (Santos *et al*., 2009; Silva *et al*., 2022, 2023). According to Santos *et al*. (2009), the imbalance in the Ca:P ratio can cause reduction in DM intake and kidney stones in goats.

Cu and Fe concentrations were 30.98 and 555.89mg/kg DM in the O.E.M. variety. In turn, the Miúda variety presented 52.74 and 605.60mg/kg DM of Zn and Mn, respectively (Table 2). Similar results were reported by Silva *et al*. (2022), who observed that diets with cactus cladodes cause excessive serum and hepatic accumulation of trace minerals in sheep and goats.

Table 2. Mineral content of cactus cladodes varieties

Mineral element	Varieties		
	Miúda cactus cladodes	IPA-Sertânia cactus cladodes	O.E.M.[a] cactus cladodes
Macrominerals (g/kg dry matter)			
Calcium (Ca)	34.02	21.15	23.02
Phosphorus (P)	5.05	5.01	5.01
Ca:P	6.73	4.22	4.59
Magnesium	12.18	9.66	9.92
Sodium	0.13	0.13	0.13
Potassium	18.61	10.38	22.44
Trace minerals (mg/kg dry matter)			
Copper	18.19	16.46	30.98
Iron	189.14	127.99	555.89
Zinc	52.74	25.60	29.16
Manganese	605.60	151.76	61.97

[a] orelha de elefante mexicana.

The varieties IPA-Sertânia and O.E.M. showed the following hydrocyanic acid (HCN) contents: 57.62 and 53.47mg/kg DM, respectively (Table 3). Ferraz *et al*. (2018) reported a similar concentration of HCN when using the Miúda variety in goat kid diets: 50.98mg/kg DM. The presence of HCN in cactus cladodes is an important finding for this plant species, demonstrating that this feed resource widely used in the diet of ruminants in drylands of the world can contribute to animal poisoning, especially when associated with known cyanogenic plants, such as of the *Manihot* genus.

Table 3. Concentration of hydrocyanic acid and qualitative phytochemical screening of bioactive compounds in cactus cladodes varieties

Substance	Varieties		
	Miúda cactus cladodes	IPA-Sertânia cactus cladodes	O.E.M.[a] cactus cladodes
Hydrocyanic acid (mg/kg dry matter)	-	57.62±0.01	53.47±0.18
Reducing sugars	+	+	+
Alkaloids	-	-	-
Anthraquinones	-	-	-
Coumarins	-	-	-
Flavonoids and Cinnamic acid derivatives	+	+	+
Saponins	+	+	+
Condensed tannins	-	-	-
Hydrolyzable tannins	-	-	-
Terpenes and Steroids	+	+	+

[a] orelha de elefante mexicana, (-) absence of the phytochemical, (+) presence of the phytochemical.

The results from the phytochemical screening of the studied cactus cladodes extracts have shown the presence of a wide diversity of bioactive compounds, in all varieties: reducing sugars, flavonoids, cinnamic acid derivatives, saponins, terpenes and steroids (Table 3). These and other phytochemicals were also detected in several studies with genotypes of cactus cladodes cultivated in various parts of the world (Astello-García *et al*., 2015; Saad *et al*., 2017), reinforcing that this Cactaceae represents an important source of bioactive components, which have antioxidant properties and other biological activities. On the other hand, substances such as saponins can cause negative effects on the animal organism and have already been associated with hepatotoxicity in sheep (Silva *et al*., 2021), which demonstrates the need for caution regarding the use of diets containing cactus cladodes in long-term.

In conclusion, cactus cladodes (*Opuntia* and *Nopalea*) have macromineral imbalance and high levels of trace minerals in their composition. Additionally, they present various bioactive substances, some with antioxidant capacity and others with the potential to cause poisoning in animals, which requires caution regarding their use as a dietary ingredient.

Keywords: antinutritional factors, bioactive molecules, cacti, nutritional characterization, semiarid

RESUMO

O objetivo do presente estudo foi caracterizar a composição de macro e microminerais, além de quantificar os teores de ácido cianídrico e realizar uma triagem fitoquímica qualitativa em três variedades de palma forrageira cultivadas no semiárido brasileiro. Os cladódios subterminais das variedades Miúda, IPA-Sertânia e Orelha de Elefante Mexicana (OEM) foram colhidos com aproximadamente dois anos de idade, no Agreste de Pernambuco, Brasil. Os teores médios de cálcio variaram de 21,15 a 34,02g/kg de matéria seca (MS), nas variedades IPA-Sertânia e Miúda, respectivamente. Por outro lado, todas as variedades de palma apresentaram baixo teor de fósforo. Níveis elevados de magnésio e potássio foram observados nas diferentes variedades de palma forrageira. No entanto, a concentração de sódio foi de 0,13g/kg de MS, independentemente da variedade. As concentrações de cobre e ferro foram de 30,98 e 555,89mg/kg de MS na variedade OEM. Já a variedade Miúda apresentou 52,74 e 605,60mg/kg de MS de zinco e manganês, respectivamente. Foram detectados níveis de ácido cianídrico, e a triagem fitoquímica demonstrou presença de grande diversidade de compostos bioativos. A palma forrageira (*Opuntia* e *Nopalea*) *apresenta desequilíbrio macromineral e altos teores de microminerais. Além disso, contém diversas substâncias bioativas, com diferentes capacidades.*

Palavras-chave: cactáceas, caracterização nutricional, fatores antinutricionais, moléculas bioativas, semiárido

ACKNOWLEDGEMENTS

This research was supported by Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) – Recife, PE, Brazil (Grant: APQ-0425-5.01/14; Grant: APQ-0493-4.03/14), and by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - 405297/2018-1; CNPq - 307110/2018-4). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

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