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A Systematic Analysis of Nutritional and Mineral Composition and Toxicity in *Acacia* Species Leaves

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Abstract: In the present study, the nutritive composition and mineral content of the leaves of eight species of *Acacia* trees were evaluated to assess their potential for different purposes, such as food resources for ruminants and mineral sources in soils. Caco-2 cells were employed to assess cytotoxicity, revealing that the extracts exhibited no cytotoxic effects after cellular incubation, suggesting their suitability as an alternative animal feed. The leaves proved to be a promising source of protein and fiber, offering an alternative to meet the needs of ruminants. The protein content differed among species, ranging from 18.96% in *A. cyclops* to 14.04% in *A. melanoxylon*. Similarly, fiber content varied from 35.52% in *A. melanoxylon* to 16.43% in *A. cyclops*. The species displayed moderate to high levels of minerals, particularly concentrations of Ca, P, and K. These values varied among species, ranging from 8452.90 to 28,465.31 mg/kg DM for Ca, 309.61 to 1293.82 mg/kg DM for P, and 5557.24 to 11,475.85 mg/kg DM for K, respectively. Each species displayed a distinct profile regarding the analyzed parameters, suggesting varied potential for their respective use. Additionally, vibrational spectroscopy analysis proved to be a highly valuable and dependable method for distinguishing between species.

Keywords: *Acacia* species; leaf; cytotoxicity; nutritive values; mineral content

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1. Introduction

The *Acacia* genus encompasses a diverse group of plant species, comprising more than 1350 variants distributed across hot regions worldwide, including arid zones, coastal areas, subalpine regions, and tropical forests [1,2]. These species are indigenous to Australia, where they are perfectly adapted. However, they have become invasive in many countries, outcompeting many native species and diminishing local biodiversity. The widespread proliferation of *Acacia* spp. as invasive species can be attributed to several key factors. These include their remarkable adaptability to diverse ecological conditions, prolific seed production rates, capacity to maintain viable seed banks for prolonged durations, robust reproductive capacities following disturbances such as burning or cutting, and the allelopathic properties exhibited by *Acacia* species. The substantial proliferation of *Acacia* spp. has engendered adverse impacts on ecosystem biodiversity, precipitating alterations in landscape structure, environmental dynamics, species richness, and the availability of natural resources [1–4]. This concern has spurred an interest in valorizing different parts of these invasive species to harness their potential. Scientific research has established

that different fractions of acacia accumulate beneficial compounds, particularly in their bark, flowers, wood, leaves, pods and seeds [1,2,5–8]. These plant components are rich sources of phytochemical compounds, notably polyphenols, flavonoids, tannins, saponins, alkaloids, and others [1,2,5,6,9]. Leaves represent a readily accessible component of *Acacia* spp. and are crucial in releasing allelopathic compounds that significantly contribute to their success as invasive species [1]. These volatile or non-volatile compounds are released through leaching, and ongoing research focused on their identification and confirmation of allelopathic effects has unveiled intriguing potential applications, ranging from bioherbicides to pharmaceutical formulations [1]. Extensive research has explored the extraction of various bioactive compounds from *Acacia* spp. leaves, employing polar or non-polar solvents to recover various hydrophilic and lipophilic components. Remarkably, these studies have demonstrated a spectrum of biological activities, such as potential biopesticidal, antimicrobial, and antioxidant activities across several *Acacia* species, including *A. dealbata* [10], *A. longifolia* [11], and *A. saligna* [12–14]; anthelmintic properties have been demonstrated for *A. melanoxylon*, *A. karroo* [15], and *A. mearnsii* [16]. The increasing demand for and associated high cost of conventional animal feed have highlighted the necessity for sustainable alternatives. The use of *Acacia* species as fodder for ruminants has gained importance in numerous tropical regions due to their ability to provide a valuable source of food for livestock in difficult environmental conditions [17,18]. *Acacia* species, with their adaptability to poor soils and periods of drought, become an attractive alternative in areas where other sources of fodder may be scarce or of poor quality. In addition, acacia have been recognized for their high content of protein and other essential nutrients, contributing to the health and performance of ruminants [19–21]. The leaves of *Acacia* spp., comprising 40% dry matter, 4% minerals, 15% protein, 50% neutral detergent fiber, and 39% acid detergent fiber [22], exhibit potential as forage. Studies evaluating the nutritive and mineral potential of various *Acacia* species [19,20,23,24] have demonstrated that acacia utilization in livestock production presents itself as a valuable feed resource during feed gaps and drought seasons.

This study hypothesizes that Acacia leaves are nontoxic and can be used to develop an alternative for animal nutrition and that they can also function as an effective organic fertilizer, promoting soil fertility and sustainable farming.

The aim of this study is to evaluate the safety and potential applications of acacia leaves in animal feed and agriculture, with the goal of promoting their harvest and reducing the invasive pressure of this species. This includes confirming their nontoxicity, as well as analyzing the nutritional and mineral composition of the most invasive acacia species in Portugal.

This research presents, for the first time, a comparison of the nutritional and mineral compositions, as well as the toxicity, of eight acacia species. The findings aim to guide future studies on enhancing the cultivation of these species and reducing their prevalence. Additionally, a non-invasive technique, FT-NIR, was used to accurately assess the quality of the leaves and improve their selection after harvesting

2. Materials and Methods

2.1. Sample Preparation

Acacia leaves were collected from the same harvesting sites utilized in a previous study [25]. The selected species, namely AMy—*A. melanoxylon*, AD—*A. dealbata*, AC—*A. cyclops*, AR—*A. retinodes*, AMs—*A. mearnsii*, AP—*A. pycnantha*, AL—*A. longifolia*, and AS—*A. saligna*, were chosen to facilitate comparisons between different parts of the plant. In this study, sampling did not take place in the same location due to the distinct distribution of the species across Portugal. However, when more than one species was found in the same place, they were collected as described and identified in the aforementioned study [25].

The leaves were immediately frozen at −80 °C after collection. For the determination of nutritional analyses, the leaves were dried in a forced-air oven at 65 °C (±5 °C) for 24 h to assess moisture content. Subsequently, they were ground using a mill with a 1 mm sieve.

The resulting powder was utilized for all nutritional determinations, except for moisture analysis, where the samples were placed on trays as collected to facilitate dehydration.

For elemental analysis, the samples were freeze-dried for 48 h under a pressure of 0.180 mbar to remove all water content. After drying, the samples were reduced to powder using a mill with a 1 mm sieve. The obtained powder was used for the determination of all mineral elements. Micronutrients were analyzed by first ashing 1 g of the samples in a muffle furnace at 480 °C for 16 h, followed by digestion with 3 mL of 20% (*v*/*v*) hydrochloric acid on a hotplate.

2.2. Cytotoxic Analysis

In this study, the Caco-2 cell line, derived from human colorectal adenocarcinoma, was used because it is widely utilized in toxicological research for its ability to differentiate into cells resembling human intestinal epithelium. This is especially relevant in evaluating the safety of food compounds, or additives as intestinal toxicity is a major concern in food safety assessments.

Lyophilized acacia leaf samples were used, and subsequently, the corresponding masses were weighed to prepare the extracts at 150, 250, and 500 µg/mL in culture medium. The cytotoxicity of the samples was assessed using the MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide). For this assay, a colorectal adenocarcinoma cell line (Caco-2) (ATCC Accession numbers: HTB-37) was cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 1% antibiotic mixture and 10% fetal bovine serum at passages 33-37 and incubated at 37 °C with 5% CO_2. For the MTT assay, cells were seeded in 96-well plates (cat. number 734-2802 Avantor, VWR, Amadora, Portugal) at 0.5×10^4 cells/well. After reaching confluence, cells were exposed to samples for 24 h, with RPMI medium as a negative control. Following incubation, MTT solution (0.5 mg/mL) was added and incubated for 3 h at 37 °C. Formazan crystals were dissolved in DMSO and absorbance was measured at 570 nm in a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA).

2.3. Nutritional Analysis

The samples underwent analysis to determine their ash, protein, fiber, and fat content, following the methods outlined in AOAC [26]. Additionally, the assessment of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) was conducted according to the protocols described by [27].

2.4. Mineral Analysis

The methodology employed for micronutrient assessment entailed post-incineration of the samples following digestion with hydrochloric acid. Quantification of the elements calcium (Ca), magnesium (Mg), potassium (K), manganese (Mn), copper (Cu), lead (Pb), cadmium (Cd), zinc (Zn), iron (Fe), chromium (Cr), nickel (Ni), and sodium (Na) was conducted using atomic absorption spectrophotometry (Thermo Scientific Series iCE 3000, Thermo Scientific, Waltham, MA, USA). Phosphorus (P) was quantified through molecular absorption spectrophotometry (Thermo Electron Corporation evolution 300 LC, Waltham, MA, USA).

2.5. Spectroscopic Analysis

The samples were analyzed directly after harvesting, without any extraction process, using a Fourier Transform Near-Infrared (FT-NIR) spectrometer (MPA Bruker Optics, Ettlingen, Germany). The analysis was performed in transmitted mode with a rotating sample cup. Spectral acquisition was performed with 1 mm quartz cells. Each spectrum was acquired with 32 scans and a spectral resolution of 8 cm^{-1}, covering the spectral region from 12,000 to 4000 cm^{-1}.

2.6. Statistical Analysis

This study employed one-way ANOVA to explore differences among analyzed parameters across different species. Using the LSD test ($\alpha = 0.05$), individual parameter means were assessed for significance. Heat maps were used to visually group similar clusters, aiding in organization. The goal was to understand parameter behavior across species and identify consistent patterns. Principal component analysis (PCA) was performed on FT-NIR spectral data using various preprocessing techniques including Savitzky–Golay derivatives, standard normal variable transformation (SNV), scatter correction multiplicative (MSC), and combinations thereof. PCA was conducted with STATISTICA 7 for analytical data and UnscramblerX 10.5 and OPUS version 7.5.18 for spectral data. This study reveals the discriminative potential of parameters in characterizing *Acacia* species via spectral analysis.

3. Results and Discussion

3.1. Cytotoxicity Analysis

Cytotoxicity was evaluated using the MTT assay, testing three concentrations of acacia leaf extract prepared in culture medium (150, 250, and 500 µg/mL). This method detects cellular changes at the metabolic level (Kumar et al., 2018). The MTT assay involves the conversion of the yellow dye [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to the purple dye formazan, catalyzed by mitochondrial reductase [28,29]. No cytotoxicity was observed (Table 1). The increase in viability was not sufficiently statistically significant for us to be able to state that cell viability percentage increases with the increase in concentration of extracts, except for the AL species.

Table 1. Cell viability after exposure to extracts. The values are expressed as (mean ± standard deviation).

Species	Cell Viability (%)		
	125 µg/mL	250 µg/mL	500 µg/mL
AR	87.38 ± 16.81 ^{b}	89.66 ± 13.57 ^{a}	113.19 ± 12.65 ^{bc}
AC	79.51 ± 11.23 ^{ab}	100.51 ± 15.98 ^{a}	101.28 ± 9.67 ^{abc}
AD	80.24 ± 20.12 ^{ab}	87.83 ± 19.30 ^{a}	93.44 ± 4.58 ^{ab}
AL	52.65 ± 17.04 ^{a}	95.52 ± 16.77 ^{a}	122.73 ± 12.73 ^{c}
AMs	73.76 ± 10.49 ^{ab}	88.18 ± 19.33 ^{a}	101.33 ± 14.82 ^{abc}
AP	73.33 ± 8.50 ^{ab}	90.20 ± 13.86 ^{a}	99.08 ± 2.86 ^{ab}
AS	77.19 ± 5.64 ^{ab}	101.77 ± 20.98 ^{a}	99.58 ± 6.34 ^{ab}
AMy	78.03 ± 4.98 ^{ab}	78.26 ± 10.39 ^{a}	87.87 ± 0.06 ^{a}

AMy—*A. melanoxylon*, AD—*A. dealbata*, AC—*A. cyclops*, AR—*A. retinodes*, AMs—*A. mearnsii*, AP—*A. pycnantha*, AL—*A. longifolia*, AS—*A. saligna*. Different letters in the same column indicate statistically significant differences ($p < 0.05$).

As far as we know, no study has evaluated cytotoxicity in acacia leaves, so it is impossible to establish a comparison. It was observed that the extracts are not cytotoxic, which indicates that they have no adverse effects on cell viability or cell structures. This observation suggests a potential safety profile for the extracts tested in the context of cytotoxicity, which is an encouraging finding for their potential applications in various contexts.

3.2. Nutritional Analysis

Figures 1 and 2 show the results of the chemical composition of the leaves of *Acacia* species based on dry matter (%DM). These figures illustrate that the protein content ranged from 14.04 to 18.96% for AMy and AC, respectively. The results demonstrated statistically significant differences ($p < 0.05$) among the studied species, which is consistent with values reported in previous studies involving different species, such as 17.6% for *A. nilotica*, 18.9% for *A. tortilis*, and 14.5% for *A. senegal* [24]. Several studies have observed high levels of protein, minerals, and fatty acids in acacia leaves, making them suitable animal protein supplements [30,31]. The protein content in acacia leaves is efficiently utilized by ruminant animals during dry seasons [32] and has been reported to meet the protein requirements

of broiler chickens [30,32]. Furthermore, it has been reported that ruminant animals can effectively utilize the protein content of acacia leaves during dry seasons. Research has also shown that tree species are rich in natural polyphenolic compounds, such as tannins, and generally have higher protein levels [33].

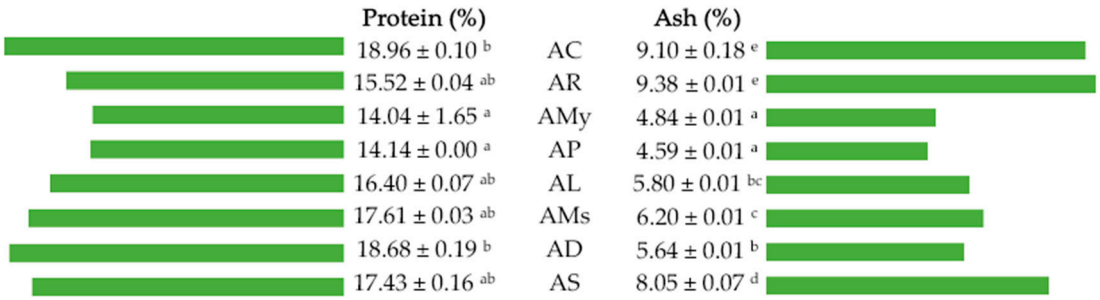

Figure 1. Nutritional value of acacia leaf species, on a dry matter basis (% in DM) (mean ± standard). AMy—*A. melanoxylon*, AD—*A. dealbata*, AC—*A. cyclops*, AR—*A. retinodes*, AMs—*A. mearnsii*, AP—*A. pycnantha*, AL—*A. longifolia*, AS—*A. saligna*. Different letters in the same column indicate statistically significant differences by the L.S.D. test ($p < 0.05$) for each analytical determination.

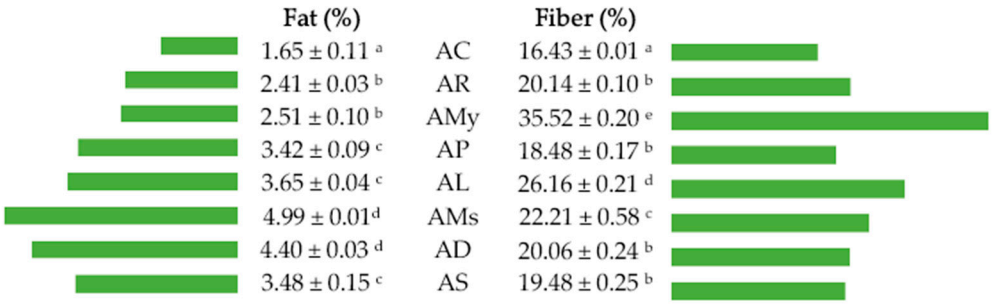

Figure 2. Nutritional value of acacia leaf species, based on dry matter (mean ± standard). AMy—*A. melanoxylon*, AD—*A. dealbata*, AC—*A. cyclops*, AR—*A. retinodes*, AMs—*A. mearnsii*, AP—*A. pycnantha*, AL—*A. longifolia*, AS—*A. saligna*. Different letters in the same column indicate statistically significant differences by the L.S.D. test ($p < 0.05$) for each analytical determination.

Research has indicated that tree species have abundant natural polyphenolic compounds (like tannins) and typically have higher protein levels. The ash content in leaves ranged from 4.59 (for AP) to 9.38% (for AR), with significant differences observed among the various species. Kolobe et al. [21] reported values ranging from 2.34 to 9.81% for different species, with *A. saligna* demonstrating higher values (9.81%) in our study. Additionally, Gebeyew et al. [20] reported ash content values of 9.1% and 7.7%.

Figure 2 illustrates the variations in fat content among different *Acacia* species, ranging from 1.65% to 4.99%. AP exhibited the lowest ash content, while AR demonstrated the highest. Notably, there is a lack of reported fat values for acacia leaves, although comparatively higher values have been observed in contrast to pods [25]. Significant differences were observed among the analyzed samples regarding fiber composition. AMy exhibited the highest fiber content, at 35.52%, whereas AC exhibited the lowest, at 16.43%. To the best of our knowledge, there are no scientific studies providing specific fiber values for the species studied in our manuscript, so it is not possible for us to make comparisons. The only existing study is that by Kolobe et al. [21], which reported the fiber content of *A. hockii*, *A. karroo*, and *A. saligna*, with values ranging from 5.71 to 25.9%, respectively, for *A. hockii* and *A. karroo*. Despite involving different species from those studied in our article, the values found in our work are similar to those published by these authors.

The moisture content values ranged from 41.66 to 70.87%. The leaves of the AC species exhibited the most significant variation compared to other species and possessed

the highest water content. Substantial differences were also observed among the other species, with the AMs species displaying the lowest values. Considering that the main components of the plant cell wall are cellulose, hemicelluloses, pectin, and lignin, and recognizing the potential use of leaves as animal feed, we also assessed the content of NDF, ADF and ADL in this study. These components are critical for understanding the digestibility and nutritional value of plant material for ruminants, as they directly influence how efficiently animals can utilize the feed. However, their quantities vary depending on the species and the plant's stage of development [34].

The concentration of cellulose, hemicelluloses, and lignin (the primary constituents of the plant cell wall) are included in the NDF measurement. The NDF contents varied significantly for the AP and AL species and ranged from 36.61% to 49.98%, respectively (Table 2). The average NDF among the different species was observed to be relatively higher compared to the NDF contents (24.6%) reported by Zapata-Campos et al. [35] and values reported by Kolobe et al. [21] (18.6-62.1%) and Rubanza et al. [24] (25.0-50.5%). The hemicellulose content ranged from 13.05% for AR to 21.90% for AC (Table 2), higher than that reported by Abdulrazak et al. [19] (40–98 g/kg DM) for *A. nubica* and *A. brevispica* from Kenya. ADF represents the cellulose and lignin fractions of the plant cell wall [27]. For the different varieties of *Acacia* species, ADF ranged from 21.34% (AC) and 46.55% for AMy, respectively. The average cellulose content of the different *Acacia* species ranged from 7.65% for AD to 16.03% for AMy. As far as we know, no studies have presented values for this parameter in this species. The digestibility of cell walls is highly variable and depends on the degree of lignification, which is expressed as ADL [34]. The highest ADL content was recorded for Amy, at 30.52%, while the lowest was observed for AC, at 9.31%. Table 2 shows that the NFC content ranged from 18.42 to 41.23%. The highest values were found in the AP species, while AMy was the species with the lowest values.

Table 2. Nutritional value of acacia leaf species on a dry matter basis (% in DM) (mean ± standard deviation).

Species	NDF (% DM)	Hem (% DM)	ADF (% DM)	Cel (% DM)	ADL (% DM)	NFC (% DM)
AMy	60.18 ± 0.07 ^{f}	13.63 ± 0.22 ^{a}	46.55 ± 0.28 ^{f}	16.03 ± 0.12 ^{f}	30.52 ± 0.41 ^{g}	18.42 ± 1.47 ^{b}
AD	41.32 ± 0.27 ^{c}	18.68 ± 0.45 ^{d}	22.64 ± 0.18 ^{b}	7.65 ± 0.37 ^{a}	14.99 ± 0.19 ^{c}	29.95 ± 0.49 ^{d}
AC	43.24 ± 0.06 ^{d}	21.90 ± 0.48 ^{e}	21.34 ± 0.42 ^{a}	12.03 ± 0.26 ^{e}	9.31 ± 0.16 ^{a}	27.05 ± 0.14 ^{c}
AR	38.81 ± 0.79 ^{b}	13.05 ± 1.00 ^{a}	25.76 ± 0.21 ^{c}	10.68 ± 0.10 ^{d}	15.08 ± 0.11 ^{c}	33.89 ± 0.88 ^{e}
AMs	41.45 ± 0.26 ^{c}	15.19 ± 0.23 ^{bc}	26.26 ± 0.03 ^{c}	9.78 ± 0.05 ^{bc}	16.47 ± 0.02 ^{d}	29.75 ± 0.30 ^{d}
AP	36.61 ± 0.39 ^{a}	14.21 ± 0.30 ^{ab}	22.39 ± 0.09 ^{b}	9.14 ± 0.19 ^{b}	13.26 ± 0.10 ^{b}	41.23 ± 0.29 ^{f}
AL	49.98 ± 0.10 ^{e}	16.26 ± 0.15 ^{c}	33.71 ± 0.05 ^{e}	10.29 ± 0.08 ^{cd}	23.42 ± 0.13 ^{f}	24.17 ± 0.14 ^{b}
AS	41.97 ± 0.05 ^{c}	13.91 ± 0.26 ^{ab}	28.07 ± 0.31 ^{d}	10.74 ± 0.23 ^{d}	17.32 ± 0.08 ^{e}	29.07± 0.29 ^{cd}

AMy—*A. melanoxylon*, AD—*A. dealbata*, AC—*A. cyclops*, AR—*A. retinodes*, AMs—*A. mearnsii*, AP—*A. pycnantha*, AL—*A. longifolia*, AS—*A. saligna*, NDF—neutral detergent fiber, Hem—hemicelluloses, ADF—acid detergent fiber, Cel—cellulose, ADL—acid detergent lignin. Different letters in the same column indicate statistically significant differences by the L.S.D. test ($p < 0.05$) for each analytical determination.

3.3. Mineral Analysis

Tables 3 and 4 present the mineral concentration of *Acacia* species leaves. The high mineral concentrations in the leaves of different species suggest their possible beneficial role as mineral supplements in ruminant diets.

The results indicate that K, Ca, and Mg were the predominant minerals in the analyzed samples, while Cd was the least abundant. Maintaining low Cd concentrations in animal feed and soil ensures regulatory compliance, food safety, and environmental protection. It helps prevent bioaccumulation, safeguards soil and ecosystem health, and avoids economic losses and potential human health issues [36]. Pb was not detected in any of the samples analyzed. The nitrogen total (NT), obtained using the Kjeldahl method, varied significantly among the species, with values ranging from 2.25% to 3.03% for AMy and AC, respectively. The NT in the leaves can be attributed to the ability of leguminous species to fix nitrogen [20].

Table 3. Concentration of total nitrogen, phosphorus, calcium, potassium, sodium, and magnesium for studied *Acacia* species.

Species	NT (%)	P (mg/kg)	Ca (mg/kg)	K (mg/kg)	Na (mg/kg)	Mg (mg/kg)
AMy	2.25 ± 0.26 ^{a}	1293.82 ± 33.18 ^{d}	8917.50 ± 483.05 ^{a}	9269.58 ± 30.57 ^{ef}	1466.83 ± 31.14 ^{c}	1936.45 ± 8.53 ^{a}
AD	2.99 ± 0.03 ^{d}	684.65 ± 28.37 ^{c}	9428.17 ± 5.19 ^{a}	3512.75 ± 239.96 ^{a}	149.85 ± 2.43 ^{a}	2841.44 ± 153.82 ^{bcd}
AC	3.03 ± 0.02 ^{d}	1211.19 ± 1.21 ^{d}	28,465.31 ± 1362.98 ^{b}	11,475.85 ± 272.50 ^{g}	2756.19 ± 85.59 ^{d}	2348.43 ± 59.22 ^{abc}
AR	2.48 ± 0.01 ^{ab}	1285.58 ± 78.13 ^{d}	27,071.86 ± 3038.61 ^{b}	8376.89 ± 340.66 ^{de}	2478.51 ± 18.79 ^{d}	2933.77 ± 115.52 ^{bcd}
AMs	2.82 ± 0.00 ^{d}	783.04 ± 22.24 ^{c}	11,526.46 ± 18.54 ^{a}	10,753.46 ± 697.09 ^{fg}	2802.78 ± 116.86 ^{d}	2256.18 ± 171.41 ^{ab}
AP	2.26 ± 0.00 ^{a}	309.61 ± 17.54 ^{a}	14,786.66 ± 1060.03 ^{a}	5557.24 ± 41.19 ^{b}	933.10 ± 5.17 ^{b}	3271.26 ± 27.66 ^{d}
AL	2.62 ± 0.01 ^{bc}	515.57 ± 19.57 ^{b}	8452.90 ± 152.96 ^{a}	7313.79 ± 45.19 ^{cd}	972.88 ± 54.88 ^{b}	1889.80 ± 65.46 ^{a}
AS	2.79 ± 0.03 ^{bcd}	1135.24 ± 10.46 ^{d}	27,974.32 ± 1387.97 ^{b}	5844.57 ± 444.85 ^{bc}	1173.00 ± 134.24 ^{bc}	2999.95 ± 305.64 ^{cd}

AMy—*A. melanoxylon*, AD—*A. dealbata*, AC—*A. cyclops*, AR—*A. retinodes*, AMs—*A. mearnsii*, AP—*A. pycnantha*, AL—*A. longifolia*, AS—*A. saligna*, NT—total nitrogen, P—phosphorus, Ca—calcium, K—potassium, Na—sodium, Mg—magnesium. Different letters in the same column indicate statistically significant differences by the L.S.D. test ($p < 0.05$) for each analytical determination.

Table 4. Concentration of iron, manganese, copper, zinc, nickel, cadmium, and chromium for studied *Acacia* species.

Species	Fe (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Ni (mg/kg)	Cd (mg/kg)	Cr (mg/kg)
AMy	82.81 ± 2.57 ^{c}	60.66 ± 2.40 ^{bcd}	11.33 ± 0.09 ^{abc}	13.39 ± 0.56 ^{ab}	6.76 ± 0.00 ^{b}	2.21 ± 0.20 ^{b}	10.89 ± 0.48 ^{a}
AD	94.09 ± 4.30 ^{de}	284.64 ± 27.45 ^{f}	12.36 ± 0.78 ^{c}	15.11 ± 0.13 ^{abc}	10.41 ± 0.01 ^{fg}	2.45 ± 0.10 ^{b}	10.40 ± 0.04 ^{a}
AC	65.16 ± 4.86 ^{b}	32.88 ± 0.22 ^{abc}	12.47 ± 0.27 ^{c}	39.05 ± 0.20 ^{de}	9.62 ± 0.11 ^{ef}	2.45 ± 0.03 ^{b}	10.10 ± 0.81 ^{a}
AR	73.66 ± 1.37 ^{b}	71.46 ± 1.62 ^{cd}	12.71 ± 0.10 ^{c}	35.45 ± 2.89 ^{d}	5.13 ± 0.32 ^{a}	2.24 ± 0.31 ^{b}	10.46 ± 0.64 ^{a}
AMs	127.26 ± 0.28 ^{f}	11.38 ± 1.39 ^{ab}	10.16 ± 0.43 ^{ab}	19.19 ± 2.27 ^{bc}	7.92 ± 0.34 ^{c}	0.23 ± 0.02 ^{a}	11.31 ± 0.92 ^{a}
AP	102.02 ± 6.51 ^{e}	159.89 ± 5.12 ^{e}	10.16 ± 0.32 ^{ab}	9.15 ± 1.11 ^{a}	8.89 ± 0.02 ^{de}	0.39 ± 0.03 ^{a}	10.78 ± 0.64 ^{a}
AL	55.22 ± 4.14 ^{a}	10.16 ± 0.81 ^{a}	11.58 ± 0.57 ^{bc}	22.57 ± 2.04 ^{c}	8.69 ± 0.17 ^{cd}	0.37 ± 0.00 ^{a}	10.09 ± 0.25 ^{a}
AS	85.95 ± 3.02 ^{cd}	84.39 ± 4.01 ^{d}	9.51 ± 0.10 ^{a}	44.77 ± 1.67 ^{e}	10.65 ± 0.03 ^{g}	2.53 ± 0.01 ^{b}	10.01 ± 0.08 ^{a}

AMy—*A. melanoxylon*, AD—*A. dealbata*, AC—*A. cyclops*, AR—*A. retinodes*, AMs—*A. mearnsii*, AP—*A. pycnantha*, AL—*A. longifolia*, AS—*A. saligna*, Fe—iron, Mn—manganese, Cu—copper, Zn—zinc, Ni—nickel, Cd—cadmium, Cr—chromium. Different letters in the same column indicate statistically significant differences by the L.S.D. test ($p < 0.05$) for each analytical determination.

P concentration varied greatly, ranging from 310 mg/kg in AP to 1290 mg/kg in AMy. This last result suggests that the leaves with a higher phosphorus content than 1000 mg/kg (AMy, AC, AR, and AS) [37] are adequate for specialized fodder meeting the requirements of sheep [38]. AP, AL, and AD had lower P content than other fodder species [37]. Higher values were recorded by Brown et al. [17] for the species *A. reficiencs* (156,000 mg/kg) and *A. senegal* (238,000 mg/kg). The phosphorus concentrations in acacia leaves found in this study were higher than those observed for pods for the same species [39]. Concerning Ca concentration, the results showed significant differences ($p < 0.05$), ranging from 8452 mg/kg for AL to 28,465 mg/kg for AC. Our results are within the global range of Ca content (14,600 to 31,500 mg/kg) reported by Rubanza et al. [24] for six species of *Acacia* (*A. angustissima*, *A. drepanolobium*, *A. nilotica*, *A. polyacantha*, *A. senegal*, and *A. tortilis* in the region of Tanzania, although Brown et al. [17] recorded higher values for *A. reficiencs* (879,000 mg/kg) and *A. senegal* (878,000 mg/kg). Significant differences ($p < 0.05$) were observed for K among the species, ranging from 3510 to 11,470 mg/kg, for AD and AC, respectively. Mapiye et al. [30] obtained K content values of 1400 mg/kg for *A. karro* in Africa, and Kolobe et al. [21] recorded values between 8300 and 18,000 mg/kg in *A. tortilis*, *A.nilotica*, *A. agustissima*, *A. rubusta*, *A. xanthophloea*, and *A. nigrescens*. Regarding sodium content, which ranged from 150 to 2802 mg/kg for AD and AMs, respectively, significant differences were found among the species. To the best of our knowledge, no research or study regarding Na content specifically in acacia plants is available. Determining sodium (Na) content in leaves is essential for assessing their suitability for animal feed and soil amendments. This study is the first to measure Na levels in acacia leaves, establishing a valuable baseline for evaluating their potential effects. For animal feed, an adequate concentration of Na is crucial for regulating blood pressure and volume, ensuring proper cell function, and maintaining the correct pH in the gastrointestinal tract, which aids in diges-

tion [40]. When considering acacia leaves for soil fertilization, it is important to recognize that Na, in small amounts, can serve as a secondary nutrient, particularly in plants adapted to utilize it efficiently. Nevertheless, careful management is required to prevent excessive salinity issues when incorporating these plants into the soil [41]. Significant differences were also observed in Mg concentration, with AMy recording the lowest (1940 mg/kg) and AS the highest (2990 mg/kg) contents. Previous studies reported higher Mg values for *A. reficiencs* and *A. senegal*. AC showed the highest NT, K, and Ca concentrations, while P and Mg had lower values than in other samples. Observing Table 4, significant differences ($p < 0.05$) among the species can be noted, except for Cr. AMs showed notably high Fe and Cr concentrations. Iron levels were lower than those found in other studies on acacia leaves. Mn concentration varied widely, ranging from 10.16 mg/kg in AL to 284.64 mg/kg in AD, consistent with data recorded by Gebeyew et al. [20] in *A. reficiencs* (111.00 mg/kg) and *A. senegal* (141.00 mg/kg). In our study, Cu levels were higher than those reported for AMs leaves but lower than those obtained by other researchers. Zn concentration was significantly higher for AS and AC than for other species. Regarding Cd values, the AMs, AP, and AL species presented the lowest concentrations; there are no previous studies on this mineral.

As a final remark, and considering the results obtained and previously discussed regarding mineral composition, the leaves of acacia contain notable concentrations of essential minerals that are highly beneficial for plant growth.

When these leaves are incorporated into the soil, they undergo decomposition, releasing vital nutrients that promote nutrient cycling and enhance soil fertility. This process offers a significant environmental advantage by reducing the dependency on chemical fertilizers, as the nutrients absorbed by the plants are returned to the soil for uptake in subsequent cultivation cycles [42].

Additionally, when acacia leaves are spread across the soil surface, they decompose gradually, providing a slow and sustained release of minerals and nutrients. Simultaneously, they act as a natural mulch, forming a protective layer that reduces water evaporation, contributing to better soil moisture retention and aiding in soil conservation. Another key benefit is the potential for incorporating acacia leaves into compost. When added to compost piles, the leaves support the formation of humus, enriching the organic matter content. The resulting compost can then be applied to the soil, not only supplying essential nutrients but also improving soil structure and stability through enhanced organic matter content [43]. This creates a more sustainable method of soil enhancement, as the slow release of nutrients and increased humus content improve soil properties over the long term, fostering healthier and more productive agricultural ecosystems.

For better understanding and ease of interpretation of the results, we created a heat map with the nutritional results and a specific examination of the results relating to the content of mineral elements obtained for different species. A heat map is a graphical representation of data, where values within a matrix are depicted using distinct colors [39]. Green colors represent a positive correlation between nutritional levels and species, while yellow colors represent a negative correlation. The heat map (Figure 3) was generated using each species' nutritional and mineral content. The heat maps grouped acacia leaf species into different groups according to their nutritional composition. Regarding the results obtained, it was possible to verify that Fib, ADF, ADL, Cel, and NDF are the most abundant in AMy, followed by AL, although there are some differences between them. These species present the highest quantities for these analyses, as previously confirmed. The remaining species present a modest negative correlation for these analyses. AC stands out with abundant nutritional parameters, namely H, NT, protein, Hem, and ash, while all others show a weak negative correlation with all species. NFC demonstrates a weak negative correlation with all species except AP and AR. On the other hand, AL demonstrates the highest weak positive correlation compared to the other species. NT, protein, Hem, and fat are the only study parameters that show a weak positive result for the AD species; all other investigated compounds demonstrate a weak negative connection. The same trend

is observed in AMs species, although with some differences. In the case of AS species, it presents a weak negative correlation for Hem, Fib, and NDF, while, conversely, it presents a weak positive correlation for H, protein, ash, and fat. AR stands out as the species with the highest amount of ash, followed by AC and AS, and all other parameters, except NFC, show a negative correlation. The results of the mineral content analysis reveal significant disparities among the species under investigation. Mn emerged as the mineral exhibiting a notable positive correlation and stood out as the most prevalent mineral in the AD species. This species demonstrated positive correlations with Cd, Mg, Fe, Cu, and Ni and a weak positive correlation for Cr. Conversely, the AC, AR, and AS species confirmed their status as the species where most of the analyzed minerals were detected (Ca, Zn, Cd, P, K, Na, and Cu), thus exhibiting positive correlations for these minerals, albeit with significant differences observed between the species. Conversely, a negative correlation between the AD and AP species was found. As previously stated, AMs displayed a higher Fe content compared to other species, thus manifesting a strong positive correlation. Notably, AMs was the sole species demonstrating a negative correlation for all analyzed minerals except Cu.

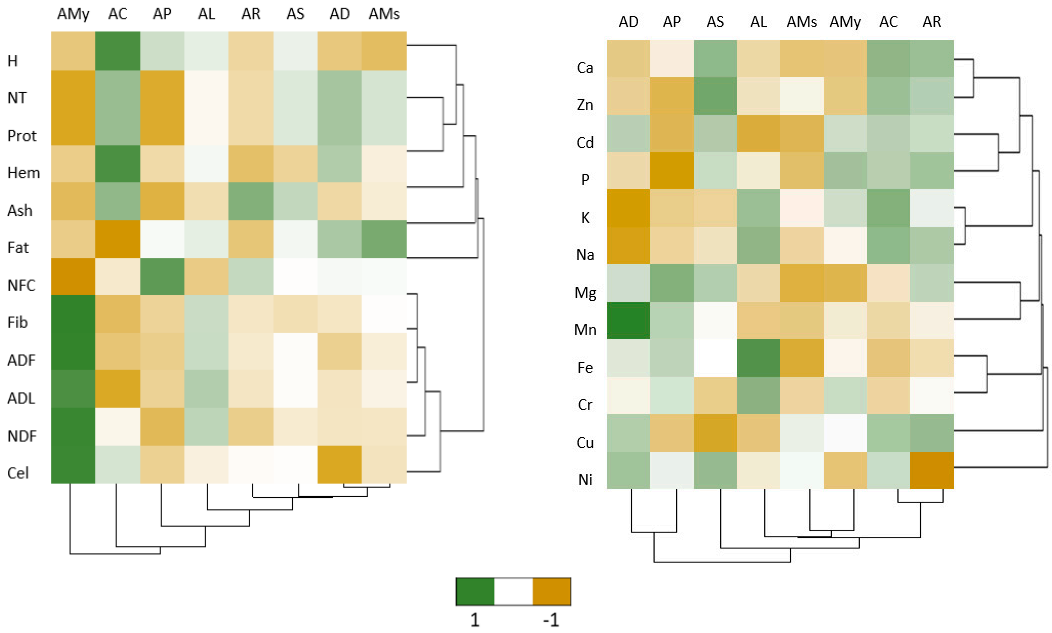

Figure 3. Heat maps plotting clusters of nutritional composition groupings of AMy—*A. melanoxylon*, AD—*A. dealbata*, AC—*A. cyclops*, AR—*A. retinodes*, AMs—*A. mearnsii*, AP—*A. pycnantha*, AL—*A. longifolia*, AS—*A. saligna*. Legend: H—humidity; NT—nitrogen total; Prot—protein; Hem—hemicelluloses; Ash—ash; Fib—fiber; ADF—acid detergent fiber; ADL—acid detergent lignin; NDF—neutral detergent fiber; Cel—cellulose; Fat—fat; NFC—non-fibrous carbohydrates; Ca—calcium; Zn—zinc; Cd—cadmium; P—phosphorus; K—potassium; Na—sodium; Mg—magnesium; Mn—manganese; Fe—iron; Cr—chromium; Cu—copper; Ni—nickel.

3.4. Spectroscopic Analysis

Figure 4 exhibitions the PCA graph, which is centered on the mean and generated from the spectra obtained through FT-NIR analysis. The algorithm applied combines the Savitzky–Golay filter with singular value decomposition and utilizes the cross-validation method with 20 segments for analyzing all acacia leaf samples.

This unsupervised multivariate statistical analysis shows that this promising technique can distinguish leaf raw materials by their distinct characteristics, including mineral elements, phenolic profiles, and flavonoid content. The results demonstrate a clear separation between species observed by the different leaves analyzed. Therefore, FT-NIR spectroscopy has shown potential as a valuable technique for monitoring the composition

of acacia leaves. Nevertheless, further research is required to have a more accurate result. The application of the PCA method is a helpful tool to distinguish between the composition of acacia leaves. These results align with similar ones obtained with FT-NIR analysis in previous studies [25], as well as with those obtained using FT-RAMAN [6,44] and FTIR-ATR [45]. However, previous studies used plant extracts, whereas in this study, we aimed to determine whether we can assess species differentiation and quality using unprocessed, directly harvested leaves. Measuring solid samples with a rotating sample cup to obtaining more representative spectral information is only feasible with FT-NIR.

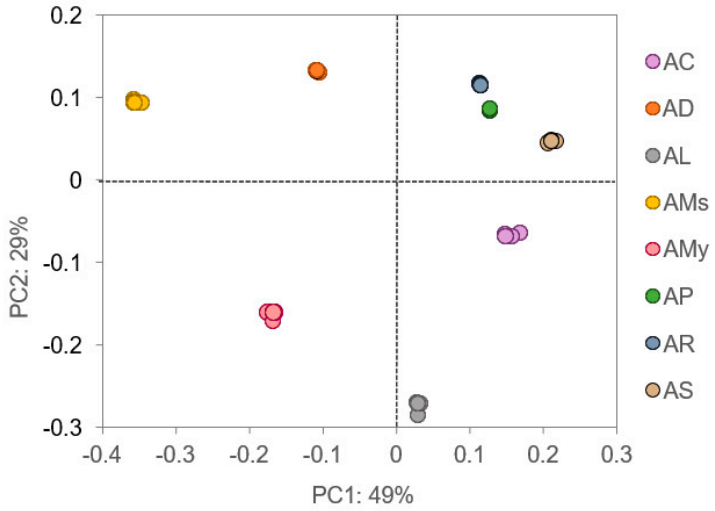

Figure 4. Score plot of the first two principal components of the PCA performed with FT-NIR spectra of acacia leaf samples.

The first two components can account for 78% of the variation, thus allowing us to distinguish between species.

4. Conclusions

The findings of this study underline the potential of acacia tree leaves as valuable resources with multifaceted applications. A comprehensive assessment of their nutritional composition and mineral content revealed promising attributes to use as feed resources for ruminants and mineral sources in soils. The incorporation of acacia leaves into the soil was shown to enhance nutrient availability, reducing the need for chemical fertilizers. The leaves break down and release essential minerals, contributing to the soil's nutrient profile while affecting carbon (C) and nitrogen (N) cycling. The non-cytotoxicity of the extracts after cell incubation further supports their suitability as an alternative feed for animals, indicating an option for supplementation; however, more studies are needed. Furthermore, the different protein and fiber contents between species highlight the diversity in nutritional profiles, offering opportunities for personalized dietary strategies. All species' moderate to high levels of essential minerals such as calcium, phosphorus, and potassium emphasize their potential contributions to meeting dietary needs while improving soil properties. The distinct profiles observed between species highlight the importance of species-specific considerations in utilization strategies. The effectiveness of vibrational spectroscopy in distinguishing between species provides a valuable tool for future research and applications in optimizing resource utilization. Overall, these findings contribute to expanding our understanding of the potential of acacia tree leaves as versatile and valuable resources, both as animal feeds and as natural soil fertilizers.

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preparation, S.I.P., J.G. (Joana Gonçalves), O.A. and E.G.; writing—review and editing, S.I.P., C.H. and J.G. (Jorge Gominho); supervision, O.A. and E.G.; funding acquisition, O.A., E.G. and J.G. (Jorge Gominho). All authors have read and agreed to the published version of the manuscript.

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