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Research Article

Comparative Evaluation of the Proximate, Mineral, and Phytochemical Profiles of *Megaphrynium macrostachyum* Seeds and Leaves

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Abstract

A comparative study was carried out to evaluate the proximate, mineral, and quantitative phytochemical compositions of *Megaphrynium macrostachyum* seeds and leaves to determine their nutritional and therapeutic potential for human and livestock nutrition. The laboratory trial was conducted in triplicate (n=3) at the Department of Biochemistry, Gandhi College of Agriculture, Rajasthan, India. The results demonstrated that the seeds possessed significantly ($p<0.05$) higher concentrations of almost all evaluated parameters compared to the leaves, with the sole exception of crude fibre. Proximate analysis revealed that the seeds contained 14.45% moisture, 10.21% crude protein, 7.67% ether extract, 13.81 % ash, and a high gross energy value of 1339.8 kcal/100g, whereas the leaves contained 12.90 % moisture, 7.33% crude protein, 5.50% ether extract, 11.66 % ash, and 1220.1 kcal/100g of energy. Conversely, crude fibre was significantly ($p<0.05$) higher in the leaves (9.07%) than in the seeds (7.67%), reflecting the structural demands of vegetative foliage. Mineral profiling using Atomic Absorption Spectrophotometry (AAS) showed a significantly higher accumulation of elements in the seeds than in the leaves. The macro- and micro-mineral profiles for the seeds and leaves were: Calcium (296.4 vs. 192.5 mg/100g), Phosphorus (105.1 vs. 88.9 mg/100g), Potassium (831.9 vs. 551.6 mg/100g), Magnesium (106.5 vs. 92.7 mg/100g), Iron (11.6 vs. 8.3 mg/100g), Zinc (5.85 vs. 2.65 mg/100g), Manganese (0.81 vs. 0.50 mg/100g), and Copper (2.08 vs. 1.02 mg/100g), respectively. Quantitative screening also indicated a higher concentration of bioactive phytochemicals (alkaloids, flavonoids, tannins, and saponins) in the leaves than in the seeds. In conclusion, while *M. macrostachyum* leaves serve as a viable source of structural fibre and phytochemicals, the seeds represent a far superior, nutrient-dense source of protein, lipids, essential macrominerals, trace elements, and dietary energy. These findings suggest that *M. macrostachyum* seeds have strong potential for development into functional seed meals or alternative feed ingredients, provided target processing is applied to manage anti-nutritional thresholds.

Keywords: *Megaphrynium macrostachyum*, Seed meal, Proximate composition, Mineral profiling, Phytochemicals, Alternative feedstuff.

Introduction

The global livestock and aquaculture industries are under intense pressure to identify alternative, non-conventional feed resources to meet the soaring demand for animal protein [1]. Traditional feed ingredients, particularly soybean meal, maize, and fishmeal, face extreme price volatility, en-

vironmental sustainability challenges, and direct competition with human food security (food-feed competition) [2-4]. This economic strain has forced animal nutritionists and biochemists to explore underutilized tropical plants and wild forest biomass as potential low-cost, nutrient-dense alternatives.

Among the diverse flora of tropical ecosystems, the family Marantaceae comprises several resilient species that generate substantial biomass but remain heavily underexploited [5].

Megaphrynium macrostachyum (Benth.) Milne-Redh., commonly found across tropical rainforests and humid zones, is a large, rhizomatous perennial herb characterized by broad, robust leaves and dense seed-bearing structures [6]. Historically, its large, durable leaves have been utilized locally for food packaging, preservation, and temporary roofing, while various parts of the plant find applications in ethnomedicine for treating ailments ranging from simple wounds to complex metabolic imbalances [6,7]. Despite this local utility, the plant continues to grow wild with very little structured cultivation or systematic harvesting for animal or human nutrition. Biochemically, plants allocate nutrients differently across their anatomical structures depending on vegetative and reproductive demands [8,9]. Leaves operate primarily as metabolic factories driven by photosynthesis, whereas seeds act as physiological sinks designed to store macromolecular compounds, essential lipids, and mineral reserves to support embryonic growth during germination [10,11]. Understanding these precise differences in nutrient allocation is essential for determining how best to utilize the plant [12].

Wild flora like *Megaphrynium macrostachyum* produce vast amounts of unharvested biomass annually, there is a profound lack of empirical data regarding their precise nutritional and biochemical compositions. Wild plants naturally synthesize secondary metabolites (such as alkaloids, tannins, and saponins) as an evolutionary defense mechanism against herbivores [13,14]. Without quantitative profiling of these phytochemicals across different plant tissues, it is impossible to predict potential anti-nutritional effects, such as reduced protein bioavailability, poor palatability, or localized systemic toxicity in target animals [15,16].

This comparative investigation provides critical empirical evidence required to transition *Megaphrynium macrostachyum* from an underutilized wild plant into a structured, valuable asset for animal nutrition and biochemical processing. First, by establishing the precise proximate and energy values of both the seeds and leaves. The structural profiling of macro- and micro-minerals (such as Calcium, Phosphorus, Zinc, and Iron) through high-precision Atomic Absorption Spectrophotometry (AAS) clarifies the plant's potential to combat trace mineral deficiencies in livestock without relying on expensive synthetic premixes. Identifying the specific tissues that accumulate these vital minerals can optimize targeted processing.

Therefore, this study was designed to establish a comprehensive comparative baseline of the proximate, mineral, and quantitative phytochemical profiles of *M. macrostachyum* seeds and leaves, determining their suitability for systematic incorporation into livestock feed formulation and functional food matrices.

Materials and Methods

Experimental Site and Duration

The laboratory evaluations were conducted at the Department of Biochemistry, Gandhi College of Agriculture, Rajasthan, India. The state of Rajasthan is geographically situated in the northwestern region of India, bounded within latitudes 23°03' to 30°12' North and longitudes 69°30' to 78°17' East.

Collection, identification, and authentication of plant materials

Fresh leaves and mature seeds of *Megaphrynium macrostachyum* were harvested from their natural habitat. To ensure botanical consistency and structural integrity, samples were placed in sterile, labeled polyethylene bags and immediately transferred to the herbarium for processing. Taxonomic identification and formal authentication were performed by a plant taxonomist at the institutional herbarium. Voucher specimens were subsequently assigned identification codes (ASD/2023/HN; ASE/2023/HM for seed and leaves respectively) and deposited in the repository for future reference. The cleaned plant parts were shade-dried at room temperature until a constant weight was achieved, pulverized into a fine powder using a localized laboratory mill, and stored in airtight glass containers at 4°C until analysis.

Proximate analysis

Proximate profiling was carried out in triplicate following standard laboratory protocols according to AOAC [17]. Moisture Content: Determined gravimetrically by drying 2.0 g of sample in a forced-air drying oven at 105°C for 3 hours until a constant weight was attained.

Crude Protein: Evaluated via the macro-Kjeldahl digestion method. Total nitrogen was determined and converted to crude protein using the traditional empirical factor of 6.25.

Ether Extract (Crude Fat): Quantified by continuous Soxhlet extraction using analytical-grade petroleum ether (boiling point 40–60°C) as the solvent Matrix.

Crude Fibre: Determined through consecutive boiling of the fat-free residue with 1.25% H₂SO₄ and 1.25% NaOH solutions, followed by filtration, drying, and subsequent ignition in a muffle furnace.

Total Ash: Estimated by incinerating the pre-charred samples in a digital muffle furnace at 550°C for 5 hours to burn off all organic matter.

Gross Energy (kcal/100g) = (4× % Carbohydrate) + (4×% Crude Protein) + (9×% Ether Extract)

Mineral Profiling

For mineral quantification, 1.0 g of each sample was subjected to wet digestion using a high-purity triple-acid mixture (HNO₃: H₂SO₄: HClO₄ in a 5:1:1 ratio) on a digital hotplate inside a fume hood.

Macro-minerals (Calcium, Phosphorus, Potassium, Magnesium) and micro-minerals (Iron, Zinc, Manganese, Copper)

were analyzed using Agilent Atomic Absorption Spectrophotometer (Model: Agilent 240FS AA Dual-Beam System). The system was calibrated utilizing certified reference material (CRM) standard solutions for each specific element to ensure accuracy. It is equipped with an echelle monochromator with a narrow-range photomultiplier tube (PMT) detector (optical system) and adjusted to a wavelength range of 185 nm to 900 nm.

Phytochemical Screening and Quantification

Quantitative determination of major secondary metabolites (alkaloids, flavonoids, tannins, and saponins) was completed via colorimetric methods measured on a high-throughput UV-Vis spectrophotometer (Shimadzu UV-1900i Double-Beam Spectrophotometer) adjusted at a wavelength Range of 190 nm to 1100 nm, photometric range (-4.0 to 4.0 Abs) and spectral bandwidth of 1.0 nm across the entire operational spectrum to ensure precision in results.

Statistical analysis

The data analysis was performed using the SPSS version 25 statistical software. To assess significant variations in mean values, a one-way analysis of variance (ANOVA) was adopted.

Results

Table 1 reveals the proximate composition of *Megaphrynium macrostachyum* seed and leaf moisture, crude protein, ash, and ether extract concentrations were significantly higher ($p < 0.05$) in *Megaphrynium macrostachyum* seed (MMS), compared to *Megaphrynium macrostachyum* leaf (MML). Conversely, MMS contained lower ($p < 0.05$) concentration of crude fibre than MML. Table 2 shows the mineral composition of *Megaphrynium macrostachyum* seed and leaf. Calcium, phosphorus, potassium, magnesium, iron, zinc, manganese and copper concentrations was higher in MMS compared to MML.

Table 3 reveals the composition of phytochemicals in *Megaphrynium macrostachyum* seed and leaf. MML had higher concentrations of tannins (20.44 mg/g), alkaloids (45.93 mg/g), saponins (14.91 mg/g), flavonoids (87.93 mg/g) and phenols (102.4 mg/g) compared to tannins (12.89 mg/g), alkaloids (30.82 mg/g), saponins (9.77 mg/g), flavonoids (49.21 mg/g) and phenolic compound (50.82 mg/g) ($p < 0.05$).

Parameters (%)	Seed	Leaf	SEM	P –value
Moisture	14.45 ^a	12.90 ^b	0.19	0.01
Crude protein	10.21 ^a	7.33 ^b	0.01	0.02
Crude fibre	7.92 ^b	9.07 ^a	0.01	0.02
Ether extract	7.67 ^a	5.50 ^b	0.01	0.01
Ash	13.81 ^a	11.66 ^b	0.22	0.03

Energy (Kcal/100g)	1339.8 ^a	1220.1 ^b	131.3	0.04
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Table1: Proximate composition of *Megaphrynium macrostachyum* seed and leaf

Parameters (mg/100g)	Seed	Leaf	SEM	P –value
Calcium	296.4 ^a	192.5 ^b	1.98	0.02
Phosphorus	105.1 ^a	88.90 ^b	0.69	0.02
Potassium	831.9 ^a	551.6 ^b	3.23	0.03
Magnesium	106.5 ^a	92.70 ^b	0.08	0.02
Iron	11.60 ^a	8.30 ^b	0.02	0.01
Zinc	5.85 ^a	2.65 ^b	0.01	0.001
Manganese	0.81 ^a	0.50 ^b	0.01	0.001
Copper	2.08 ^a	1.02 ^b	0.01	0.001

Table2: Mineral composition of *Megaphrynium macrostachyum* seed and leaf

Parameters (mg/g)	Seed	Leaf	SEM	P –value
Tannins	12.89 ^b	20.44 ^a	0.03	0.01
Alkaloids	30.82 ^b	45.93 ^a	0.15	0.02
Saponins	9.77 ^b	14.91 ^a	0.09	0.001
Flavonoids	49.21 ^b	87.93 ^a	0.10	0.03
Phenols	50.82 ^b	102.4 ^a	0.16	0.03

Table3: Phytochemical composition of *Megaphrynium macrostachyum* seed and leaf

Discussion

The comparative evaluation of *Megaphrynium macrostachyum* seeds and leaves reveals distinct nutritional profiles that reflect the distinct biological roles of these anatomical parts. While leaves function primarily as transient metabolic engines driven by photosynthesis, seeds serve as terminal physiological sinks designed to store complex nutrient reserves [18,19]. The proximate profiles in this experiment shows that *M. macrostachyum* seeds possess a significantly superior protein and gross energy yield compared to the leaves. Seeds exhibited a higher protein concentration (10.21%) and fat content (7.67%) than the leaves (7.33% CP and 5.50% EE). This distribution aligns with the ecological role of seeds, which accumulate storage proteins and lipophilic compounds to sustain embryonic growth during early germination phases [20]. The higher concentration of ether extracts in the seeds directly accounts for their higher gross energy value (1339.8 kcal/100g) compared to the leaves

(1220.1 kcal/100g). Conversely, crude fibre and minerals was the only proximate index where the leaves outperformed the seeds. This structural variance is expected due to the cellular requirements of foliage, which possesses dense matrices rich in cellulose, hemicellulose, and structural lignin to maintain physical posture and optimize photosynthesis. The crude protein content in *Megaphrynium macrostachyum* seeds and leaves recorded in this study corresponds with the reports of [21,22]. The moisture content reported for MMS and MML was within 12.00 – 16.50 % recorded by [23] for *Delonix regia* leaf and seed respectively. This outcome discourage the activities of microbes which may affect their shelf life [23].

Despite the seeds yielding a higher total ash percentage (13.81%) compared to the leaves (11.76%), individual elemental quantification showed that *M. macrostachyum* seeds contained significantly higher structural concentrations of both macro- and micro-minerals. Potassium emerged as the most abundant element in both tissues, with seeds accumulating a substantial 831.9 mg/100g compared to 551.6 mg/100g in the foliage, highlighting its role in active cellular osmoregulation and enzyme system stabilization. Calcium (296.4 mg/100g) and phosphorus (105.1 mg/100g) were also markedly elevated in the seeds relative to the leaves. These two elements act synergistically in animal nutrition, where a balanced ratio is essential for structural bone mineralization, cellular signaling, and energy transduction via nucleotide pathways. The higher trace element concentration in the seeds specifically iron (11.6 mg/100g) and zinc (5.85 mg/100g)—further emphasizes their value. Iron is vital for hemoglobin formation and cellular respiration [24], while zinc operates as a catalytic cofactor for over three hundred structural transcription factors and metabolic enzymes [25]. The elevated concentration of secondary metabolites in the leaves relative to the seeds points to an evolutionary chemical defense strategy [26]. This accumulation provides strong practical benefits when applied to animal nutrition or pharmacology [27]. These secondary metabolites provide strong therapeutic, antioxidant, and antimicrobial properties that can help stabilize intestinal microbiota and lower systemic oxidative stress in livestock [28-30]. However, their inclusion levels in animal rations must be carefully managed, as high concentrations can reduce palatability or interfere with protein bioavailability [30]. Ultimately, the robust nutritional, mineral, and phytochemical profile of *Megaphrynium macrostachyum* seeds demonstrates their potential to be processed into high-energy, nutrient-dense seed meals for livestock feed or human dietary supplementation, provided any associated anti-nutritional factors are managed through target processing techniques [31].

Conclusion

This study demonstrates that *Megaphrynium macro-*

stachyum seeds serve as a far more potent source of dietary energy, crude protein, ether extract, and essential minerals than its leaves. Conversely, the leaves offer value as a dietary source of crude fibre and phytochemicals. The rich nutritional profile of the seeds highlights their potential to be processed into high-energy, nutrient-dense seed meals for livestock feed or human dietary supplementation, provided any associated anti-nutritional factors are managed during processing.

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