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Research Article

Qualitative and Quantitative Screening of Grasses for Macro and Micro Nutrients

R. Hari babu* and N. savithramma

Dept of Botany, S. V. University, Tirupati – 517 502, Andhra Pradesh, India

Correspondence should be addressed to R. Hari babu; harisan82@gmail.com

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Abstract

The present study is aimed to screen the selected grasses of South India for qualitative and quantitative mineral content. The results revealed that the grass species accumulate the major and minor elements at various concentrations. The concentration of macroelements were found to be 9503, 51360, 4650 and 3473 (ppm) of Ca, K, Mg and P respectively; and microelements were 40, 219, 2698, 261, 68 and 108 for B, Cu, Fe, Mn, Mo and Zn (ppm) respectively. 17% of ash content and 3826 cal/g of calorific value were found as the selected species. The levels of macro and micro elements of the grasses were determined by using ICP –OES. Elemental analysis studies of the grass species revealed that large amounts of major and minor elements were found rich in *Fimbristylis cymosa* and *Scleria lithosperma*, which may be suggested for livestock feeding.

Key words: ICP-OES, Grass, Ash, Calorific value and Elemental analysis

Introduction

A major constraint to livestock production in developing countries is the scarcity and fluctuating quantity and quality of the year-round feed supply. Grasslands provide the primary forage for ruminants particularly cattle, throught much of the year. Many minerals make up the body composition of animals, they play many fundamental roles and many of them are essential to the normal vital function of animals. In the animal body, major minerals constitute more than 100 mg/g (Ca, P, Mg, Na, K, Cl and S), while micro minerals or trace elements (Fe, Zn Cu, Mb, Se, I, Mn, Pb, Cd, Co, Cr, Al, As, Si Ni and Sn) are present in lower amounts [1]. In general fewer mineral deficiencies or toxicities occur in animals when a major portion of the diet consists of concentration levels of production as well as the genotype of the animal influences mineral requirements and tolerance of animal for minerals [2]. Elements are essential for normal growth of plants, their protection against viruses and completion of their life cycle [3].

There is an increasing awareness of the need to pay greater attention to the role of elements in plant and animal nutrition and welfare. Some elements are essential nutrients for plant growth and often also for food and feed quality because the primary route for their intake by humen and animals is plants. Animals having developed a dependency on these trace elements, which are an important physiological effects when present at concentrations other than those associated with classical toxicity and with extreme deficiency.

The essential elements are involved in many metabolic processes of physiology, especially as enzymes activators, e.g., Fe, Zn and Mn [4]. They can also interact with some organic compounds such as flavonoids,influencing their biological activity [5]. This present study has conducted with the objective of assessing the major and trace elements in ten grass specie belonging to cyperaceae family, which are used to feed livestock. Major trace elements play a very important role and often toxic in higher doses. The assessment of trace elements is essential which could potentially be useful to animals. The nutritive values of grasses had been studied by [6, 7, 8] but studies on elemental analysis are scanty. Hence the present work includes qualitative and quantitative determination of various elements in ten selected grass species were carried out by using ICP-OES technique. Due to the lack of knowledge on nutritive value of feed and feed ingredients farmers are unable to formulate the balance diet for their animals. As a result quality status of these feed ingredients and their effect on animal performance is not known properly. Scientific study on the evaluation of the quality of such feeds and feed ingredients thus becomes necessary to satisfy the farmers, scientists and the feed manufacturers for various purposes. So determining the quality of these feed ingredients will help to address this problem.

Material and methods

Material

Ten grass species of cyperaceae Bulbostylis barbata (Rottb.) C.B. Clarke, Cyperus difformis L., Cyperus rotundus L., Fimbristylis cymosa R. Brown., Fimbristylis eragrostis (Nees.) Hanse, Paspalidium flavidum (Retz.) A.Campus., Kyllinga monoceps (J.R. Forst) Dandy., Kyllinga triceps (rottb.) Descr., Scleria lithosperma (L.) Sw.and Fimbristylis monostachya (L.) Hassk., were selected and collected from different places of South India, in 2010, authenticated by BSI Coimbatore (Tamilnadu) and were dried at 1050C for 24h. Dried samples were grinded and homogenized using an agate homogenizer and stored in poly-ethylene bottles until analysis.

Experimental Apparatus

Perkin Elmer 7000DV model ICP-OES was used for the determination of elements. Its appearance alone sends out a definite signal: The Perkin Elmer optical emission spectrometer (Perkin Elmer 7000DV, USA) is different from conventional ICP-OES. The Perkin Elmer can be out fitted with an interface for either axial or radial plasma observation. The proprietary ICAL system logic automatically monitors operation of the Perkin Elmer guaranteeing continuous optimum operating conditions. The operating parameters of ICP-OES were set as recommended by the manufacturer. The ICP-OES operating conditions and analytical characteristics of elements are listed in Tables1 and 2, respectively. Milestone start D microwave (Sorisole-BgItaly) closed sys- tem (maximum pressure 1500psi, maximum temperature 300C was used.

Reagents and solutions

All chemicals used throughout the experiments were of analytical reagent grade (Merck, Darmstadt, Germany). HNO_3 (65%), H_2O_2 (30%) and HCI (37%) were of suprapur quality (E. Merck, Darmstadt, Germany). All glassware and polyethylene bottles were kept overnight by soaking in 10% HNO_3, and cleaned by rinsing five times with distilled de-ionized Ultra High Quality (UHQ, chemical resistivity: 18MXcm1) water (Millipore, Bedford, MA, USA) prior to use. Aliquots of an ICP multi element standard solution (10 mg/L) containing the analyzed elements (B, Ca, Cu, Fe, K, Mg, Mn, Mo, P and Zn) was used in the preparation of calibration solutions. These solutions were prepared by serial dilution With 0.2% (v/v) HNO_3 to the required concentrations with UHQ water prior to use. For calibration, commercially available standard solutions were used. The ranges of the calibration curves (6 points) were selected to match the expected concentrations (10–500 mg/L) for all the elements of the sample studied by ICP-OES

Mineral analysis

Grass samples were digested and made up the final solution to volume with UHQ water, the concentrations of each of Ca, K, Mg, P, B, Cu, Fe, Mn, Mo, and Zn were determined by ICP-OES equipped with an auto-sampler. Prior to analysis, the instrument was calibrated according to manufacturer's recommendation. Blank digestion was also carried out by completion of full analytical procedure without plant sample. All determinations were made in triplicate. We used standard addition method for possible matrix effect.

Table.1 The operating parameters of determination of elements by ICP-OES.

INSTRUMENT	PERKIN ELMER
Viewing height (mm)	12
Wavelength	nm
Replicates	3
RF Power (W)	1300
Spray chamber	cross flow nebulizer
Nebulizerflow (L/min)	0.8
Plasma Torch Quartz, fixed, 3.0 mm injector tube	
Replicate read time	30 s per replicate
Plasma Gas Flow (L/min)	15
Auxiliary Gas Flow (L/min)	0.2
Sample aspiration rate (mL/min)	1.0

Table. 2
Analytical characteristics of analyte ions by ICP-OES.

Elements	Analyte Wavelength(nm)	Slopes of the calibration curves
Boron	249.677	0.99998
Calcium	317.933	0.99989
Copper	327.393	0.99989
Iron	238.204	0.99987
Potassium	766.490	0.99998
Magnesium	285.213	0.99991
Manganese	257.610	0.99994
Molybdenum	202.031	0.99952
Phosphorus	213.617	0.99954
Zinc	206.200	0.99975

Calorific value

An Digital Bomb Calorimeter (Model – RSTB- 3, Rico, INDIA) was used to determined the calorific value of the dry powders of the by using the following formula sample.

$$\text{Formula: } CVs = \frac{T \times W - (CVT + CVW)}{M}$$

T= Final rise in temperature in Degree Celsius.

M= Mass of sample in grams.

H= Known Calorific Value of Benzoic Acid in cal/gram.

W=Water Equivalent in calories per degrees centigrade.

CV T= Calorific Value of thread.

CV W= Calorific Value of Ignition wire.

CV S=Calorific Value of sample.

Ash content

Method recommended in pharmacopoeia of India (Anonymous, 1966), and British Pharmacopoeia. [9] were followed for determining Ash value and percentage method.

Preparation of Ash - 3g of dried powder of plant sample was incinerated in a Silica crucible over the burner. The charred material was heated in muffle furnace for six hours at 600-650^{o}C.The ash formed was white and free from carbon. It was cooled and weighed on the ash less filter paper.

Results and Discussion

There are various sources contributing to the metal composition of the grass species. The sample pre-treatment procedure must take in to account the analyte interest, the matrix characteristics and the minimal required time period of the analytical technique considered. In this work. The major elements like Ca, K, Mg, P, and minor elements like B, Cu, Fe, Mn, Mo, and Zn were analysed by using ICP – OES. The results of the analyses are summarized in **table.3**

The calcium concentrations varied from 4331 to 9053 ppm. *Kyllinga monoceps* had the lowest concentration and *Paspalidium flavidum* had highest. The concentrations of calcium were comparable in *Fimbristylis eragrostis*, *Bulbostylis barbata* and *Kyllinga triceps, Fimbristylis cymosa* with a range of 5267 to 5311 and 6551 to 6564 ppm respectively. Concentration sufficient for growth of plant is 5000 mg/kg Calcium is required by meristematic and differentiating tissues. During cell division it is used in the synthesis of cell wall, particularly as calcium pectate in the middle lamella. It is also needed during the formation of mitotic spindle. It accumulates in older leaves. It is involved in the normal functioning of the cell membranes. Calcium is the most abundant element in the animal body and it is fundamental for the activity of many enzyme systems, coagulation of blood, transmission of nerve impulses, contraction of muscles, flocculation of casein in the stomach and many other [10]. About 26 to 30% of total ash content of most animals is Ca, NRC lists the maximum tolerable levels of Ca as 2% of diet dry matter. Calcium is used in the development and maintenance of bone structure. It plays functional role in the clotting process, nerve transmission, hormone function and metabolism of vitamin D etc.

The potassium concentrations of selected species varied from 21190 to 51360 ppm. Highest potassium content observed in *Fimbristylis cymosa* followed by *Fimbristylis monostachya*, *Kyllinga monoceps* and *Bulbostylis barbata*. While lowest content observed in *Cyperus rotundus* 21190 ppm. The potassium concentrations were comparable in *Scleria lithosperma* and *Cyperus difformis* ranges from 32240 to 32460 ppm. 10,000 mg/kg concentration is sufficient for growth of plant. Potassium together with sodium helps to regulate the water balance within the body and transfer of nutrients to the cell, transmits electrochemical impulses and is necessary for normal growth enzymatic reactions [11]. Potassium is the main intracellular cation and plays a role of primary importance in nerve and muscle excitability [12].

Magnesium concentrations was found 1988 to4650 ppm. Most of samples having the content between 3000 to 4600 ppm. *Fimbristylis cymosa* had the highest concentration and *Kyllinga monoceps* had the lowest concentration. The magnesium concentrations comparable in *Fimbristylis monostachya*, *Kyllinga triceps and Paspalidium flavidum, Fimbristylis cymosa* ranges from 3238 to 3278 and 4617 to 4650 ppm. 2,000 mg/kg concentration is sufficient for growth of plant. Magnesium is a key element in cellular metabolisms. For high metabolic rate, cells require high magnesium. In presence of higher percentage of potassium and phosphorous, absorption of magnesium increases. Loss of magnesium leads to hyper irritability. Adults may suffer muscles tremors, memory loss, inability to concentrate, apathy and depression [11]. About 60-70% of the total magnesium of the organism is localized in the skeleton, where it is closely associated with calcium and phosphorus the remainder is found in the soft tissues and body fluids [1].

The phosphorus concentrations of selected grass species varied from 1563 to 3473 ppm. *Kyllinga triceps* had the highest concentration and *Bulbostylis barbata* had the lowest concentration. The concentration of phosphorus were comparable in *Paspalidium flavidum,Cyperus rotundus* and *Fimbristylis monostachya*, *Fimbristylis eragrostis* ranges from 1685 to 1703 and 2263 to 2284 ppm. 2,000 mg/kg is necessary for growth of plant. Phosphorous is tied to calcium in bone structure and plays a significant role in CNS function. Phosphate is the primary iron in extracellular and intracellular fluid; it aids absorption of dietary constituents, helps to maintain the blood at a slightly alkaline level, regulatory enzyme activity and is involved in the transmission of nerve impulses [11.

The highest amount of Boron recorded in *Paspalidium flavidum* 40.20 ppm followed by *Fimbristylis ergrostis*, *Fimbristylis monostachya* and *Fimbristylis cymosa*. While least amount was recorded in *Cyperus difformis* 17.23 ppm. The concentrations of Boron comparable in *Cyperus rotundus* and *Kyllinga triceps* ranges from 28.05 to 28.24 ppm. Concentration sufficient for growth of plant is 20 mg/kg. Boron is required for uptake and utilisation of Ca^{2+}, membrane functioning, pollen germination, cell elongation, cell differentiation and carbohydrate translocation. Agricultural practice have well established that adequate B supply is imperative for obtaining high yield and good quality. Knowledge about metabolic functions of B in plants remains incomplete. Recent research findings have greatly improved our understanding for B uptake and transport processes [13 and roles of B in cell-wall formation [14], cellular membrane functions and anti-axidative defense systems have been suggested [15]. A beneficial or even essential role of B in animal metabolism is supported by the findings that low B concentrations induce the MAPK pathway in cultured animal cells with a knockout of the B transporter Na B C1, the mammalian homolog of At B or 1, stop to develop and proliferate [16].

The copper concentrations varied from 23.12 ppm to 219.2 ppm in ten species. *Paspalidium flavidum* had the lowest concentration and *Kyllinga monoceps* had the highest. The concentrations of copper content were comparable in species *Fimbristylis eragrostis* and *Scleria lithosperma* ranges from 53.76 to 54.56. 6 mg/kg concentration sufficient for growth of plant. Copper is an important mineral in dopamine synthesis. Low level of dopamine results in decrease in activity of central nervous system. A deficiency of copper may cause hypertension, antibiotic sensitivity, hyperactivity, hyperglycemia, manic disorders insomnia, allergies and osteoporosis [11]. Cu is universally important cofactor and activator of numerous enzymes which are involved in development and maintenance of the cardiovascular system. A Cu deficiency can result in a decrease in the tinsel strength of arterial walls, leading to aneurysm formation and skeletal maldevelopment [17]. Copper is essential for the synthesis of haemoglobin it is involved in the synthesis of coetaneous pigments and crimp tensile strength, elasticity and affinity for dyes of wool. Cu is the main constituent of the bone, connective tissue, brain, heart, and many other body organs [18].

Table: 3 Elemental detection of ten grass species on a dry weight basis expressed as ppm sample

S.NO	Scientific name of the Grass	Ca	K	Mg	P	B	Cu	Fe	Mn	Mo	Zn
1	*Bulbostylis barbata* (Rottb.) C.B. Clarke	5311 ±221	33720± 191	2957± 21	1563± 82	22.39±2.11	102.4±0.98	1121±3.21	211.1±1.36	30.44±2.10	62.01±3.23
2	*Cyperus difformis* L.	8306 ± 163	32460 ± 253	4226± 28	3201±27	17.23±1.11	176.4±2.21	1272±3.32	167.3±2.25	34.92±1.25	58.92±2.89
3	*Cyperus rotundus* L.	5526 ± 192	21190 ± 120	2396± 41	1703±36	28.05±2.12	125.5±3.11	471.2±2.87	59.66±1.20	15.57±1.50	80.99±2.45
4	*Fimbristylis cymosa* R. Brown.	6564 ± 142	51360 ± 410	4650±54	2150±21	34.48±2.35	118.8±1.41	1336±4.36	159.9±2.98	39.86±4.13	59.38±5.21
5	*Fimbristylis eragrostis* (Nees.) Hanse	5267 ± 216	27970± 201	2582± 63	2284±95	37.21±1.28	53.76±2.65	2698±6.32	192.5±1.90	68.00±1.83	53.99±4.29
6	*Fimbristylis monostachya* (L.) Hassk.	5794 ± 169	41980 ± 326	3238± 49	2263±36	36.75±4.35	112.7±3.25	813.3±3.28	105.2±3.58	20.45±3.21	59.84±2.51
7	*Kyllinga monoceps* (J.R. Forst) Dandy.	4331± 135	37250± 451	1988± 52	2343±87	18.32±1.84	219.2±1.63	524.2±5.21	182.0±2.54	19.44±1.25	108.3±2.38
8	*Kyllinga triceps* (rottb.) Descr.	6551 ± 192	28880± 362	3278± 56	3473±29	28.24±1.90	93.07±2.03	724.3±5.32	112.9±5.25	22.63±2.59	65.66±2.89
9	*Paspalidium flavidum* (Retz.) A.Campus.	9503±125	26670 ± 812	4617± 87	1685±26	40.20±1.28	23.12±2.52	356.9±1.20	140.4±2.47	13.98±1.20	60.21±2.56
10	*Scleria lithosperma* (L.) Sw.	5875± 127	32240 ± 635	4121± 47	2633±33	23.53±3.39	54.56±1.69	706.6±9.23	261.0±2.36	25.59±2.98	75.66±8.25

Results are mean of triplicate estimation on dry weight basis ± standard error

Table: 4 Ash and Calorific values of ten grass species on a dry weight basis expressed as ppm sample

S.No	Grass name	Calorific value cal/g	Ash %
1	*Bulbostylis barbata* (Rottb.) C.B. Clarke	3298 ± 421	17 ± 0.13
2	*Cyperus difformis* L.	3093 ± 289	13± 0.25
3	*Cyperus rotundus* L.	3826 ± 361	16± 0.39
4	*Fimbristylis cymosa* R. Brown.	2996 ± 273	13± 0.11
5	*Fimbristylis eragrostis* (Nees.) Hanse	3313 ± 236	10± 0.15
6	*Fimbristylis monostachya* (L.) Hassk.	3777 ± 203	06± 0.12
7	*Kyllinga monoceps* (J.R. Forst) Dandy.	3069 ± 270	14± 0.44
8	*Kyllinga triceps* (rottb.) Descr.	3435 ± 230	14± 0.19
9	*Paspalidium flavidum* (Retz.) A.Campus.	2971 ± 250	13± 0.20
10	*Scleria lithosperma* (L.) Sw.	2874 ± 236	10± 0.25

Results are Mean of triplicate estimation on dry weight basis ± standard error

The average Iron content of 882.3 ppm. the highest amount of Fe content found in *Fimbristylis eragrostis* 2698 ppm followed by *Fimbristylis cymosa, Cyperus difformis* and *Bulbostylis barbata,* while least amount of Fe content found in *Paspalidium flavidum* 356.9 ppm. 100 mg/kg concentration is sufficient for growth of plant. Iron plays a significant role in oxygen transport in the body. A deficiency of Iron can impair neuronal development, sweating, rapid pulse, prolonged sleep, cessation of the menses, aversion to eating and heavy feeling of body [19]. Iron is an essential mineral and an important component of proteins involves in oxygen transport and metabolism [20]. Most iron is combined with proteins, it participates in the composition of haemoglobin, myoglobin and cytochromes.

The highest amount of Manganese recorded in *Scleria lithosperma* 258 and the least amount was recorded in *Cyperus rotundus* 59.66 ppm. 50 mg/kg concentration is sufficient for growth of plant. Mn is a component of several enzymes including manganese-specific glycosyltransferase and phosphoenolpyruvate carboxykinase and essential for normal bone structure. Mn deficiency can manifest as transient dermatitis, hypocholesterolemia, increased ALP level, skeletal abnormalities, retarded bone growth, change in hair colour to growth, abnormalities in pancreas, and disturbances in lipid and carbohydrate metabolisms [19]. Mn is an important electrolyte also responsible for proper bones, liver, kidneys, pancreas and pituitary gland function. It also works as co-factor for more than 300 metabolic reactions [21]. Manganese was shown to prevent ataxia in lambs. The present results show that the level of 'Mn' is well acceptable. Manganese participates to the non-toxic function of the iron, vitamin C and the potentiation of the hypoglycaemic effect of adrenaline [22].

The molybdenium concertrations varied from 13.98 to 68 ppm. Highest molybdenum concentrations observed in *Fimbristylis eragrostis* and least concentration in *Paspalidium flavidum*. The molybdenium concentrations were comparable in *Kyllinga monoceps* and *Fimbristylis monostachya* ranges from 19.44 to 20.45 ppm. 0.1 mg/kg is required for growth of plant. Molybdenium is a component of coenzyme that is essential for the activity of xanthine oxidase, sulphite oxidase and aldehyde oxidase [23]. It acts as a detoxification agent in the liver as a part of the sulfite oxidase enzyme and it possibly retards degenerative diseases, cancer and ageing. Molybdenum is important essential trace element involved in mentbolism through metalloenzymes [24].

The highest amount of Zinc concentration observed in *Kyllinga monoceps* 108.3 ppm followed by *Cyperus rotundus, Scleria lithosperma* and *Kyllinga triceps*, while the lowest amount observed in *Fimbristylis eragrostis*. *Fimbristylis cymosa, Fimbristylis monostachya* and *Paspalidium flavidum* were comparable concentration ranges from 59.38, 59.84 and 60.21 ppm. Required content for growth of plant is 20 mg/kg. Zinc deficiency are associated with mental impairments, lethargy, emotional disorder and irritability [25]. Zinc has the tendency to accumulate in bone tissue rather than in the liver, but it is found in every tissue and reaches rather high levels in skin, hair and wool. It is an activator of several enzyme systems and enteres in the composition of insulin. Zinc is necessary for the functioning of over 300 different enzymes and plays a vital role in an enormous number of biological process [26]. The physiological activities of the plant influence the Zn absorption and the interaction with many elements like Fe and Mn. Cu affects Zn uptake [27] and its deficiency causes many physiological disorders. Besides, it is responsible for stimulating growth of epidermal and epithelial cells [28].

Most of the species had the ash content between 10 to 17%. *Bulbostylis barbata* had the highest content followed by *Cyperus rotundus, Kyllinga monoceps, Kyllina triceps* and *Cyperus difformis*. While sample *Kyllinga monoceps*, *Kyllina triceps Cyperus difformis*, *Fimbristylis cymosa* and *Paspalidium flavidum, Scleria lithosperma* had the equal content 14, 13 and 10%. The results of the analyses are summarized in **table.4.** The amount and composition of ash remaining after combustion of plant material varies considerably according to the part of the plant, age, treatment etc. The constituents of the ash also vary with time and from organ to organ. Ash usually represents the inorganic part of the plant.

The calorific value varied from 2874 to 3826 cal/g. *Cyperus rotundus* had the highest calorific value and *Scleria lithosperma* had the lowest concentration. The calorific value were comparable in *Paspalidium flavidum*, *Fimbristylis cymosa* and *Kyllinga monoceps, Cyperus difformis* ranges from 2971 to 2996 and 3069 to 3093 cal/g. Highest calorific values in partly attributed to the slight difference in the moisture content of the material at the time of analysis. The results of the analyses are summarized in **table.4.** The starch as a binder has been reported to have ability of increasing the calorific values [29]. Calorific concentrations in plants increase with decreasing total available isolation over the span of a growing season.

The differences in concentration of various elements may be due to the differences in botanical structures and genetic variability's of the plants and also due to the mineral composition of the soil and absorbing capacity and accumulation efficiency of species. Moreover the difference may be due the ability of plants to accumulate the elements from the surrounding aerial or aquatic environment either for their physiological requirement or as a precautionary measure. This in turn enables some plants to be used as bio monitors for environmental pollution [30]. The level of essential trace elements in the plants varies by the geochemical characteristics of the soil and also by the ability of the plants to select and absorb some of these elements. Further, the bioavailability of the elements depends on the nature of their association with the constituents of the soil. Plants are readily assimilate elements through their roots. The additional sources of these elements for plants are rainfall, atmospheric dusts, plant protection agents and fertilizers that can be absorbed though the leaf blendes [31](Lozak *et al.*, 2002). Generally it is concluded that the studied products are rich source of essential elements Mg, Ca, Zn, and Cu, hence might play an important role in the maintenance of the nutritional requirements. The results presented in this paper enlarge the knowledge of the elemental composition of these grass species, will be useful and of interest in the toxicological and nutritional fields.

Conclusion

The above results indicate that the grass samples are a good source of essential nutrients required for the well being of livestock feed. The presence of potassium, phosphorus, calcium, iron, magnesium, copper etc. in high concentrations in the grass samples suggests its use in therapeutic purposes. Thus the presence of the nutraceutically valued minerals in the plants points toward the possibility of their use to restore the different imbalances caused in the body. It is therefore concluded that the selected grass species of the study are rich in elements may also help in biodiversity function etc. From the results it indicated that variations in elemental composition and concentration among the species and different ecological areas. There reflecting differences in physiological functioning of the specific plants depending upon the elemental interaction within it. Our preliminary study having baseline information about mineral constituents of grass species of South India, it will be helpful to develop an approach towards direct link between elemental content and its curative probability having coherence with traditional use and livestock feed. The present results may provide an useful information to formers for cultivation of grasses which are consisting high mineral and calorific values.

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