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Elemental Quantification in Different Conventional Medicinal Plants and Nutritional Therapeutic Assessment

*Tarun Saikia

D.C.B. Girls' College, Department of Chemistry, Jorhat, 785001, Assam, India.

*Corresponding author: E-mail: saikia.tarun55@gmail.com ORCID ID: <https://orcid.org/0000-0001-8375-6871>

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Abstract:

Plants are tremendous sources of different macro and micronutrients that are involved in diverse physiological functions in human body. Quantification of different macro- and micronutrients in traditionally famous medicinal plants is very essential from nutritional and therapeutic perspective. In the present study, micro- and macronutrient contents were determined in 10 medicinal plants collected locally in upper Assam, India to score the nutrients efficacy of the plants. Following standard estimation protocols, moisture content, ash percentage, micronutrients (Fe and Cu) and macronutrients (Na, K, Ca, Mg and P) were determined in the leaves of selected plants in dry weight basis. The results of the current study have documented the maximum Cu concentration (0.142 mg g^{-1}) in the leaves of *Sessile Joyweed* and the available P content (1.62 mg g^{-1}) in *Fenugreek* leaves. However, Cu (0.007 mg g^{-1}) and P (0.107 mg g^{-1}) concentrations were found to be lowest in *Mint* and *White Goosefoot*. Fe concentration was maximum (0.331 mg g^{-1}) in *Skunkvine*, while the lowest but nearly similar results of this element were found in *Pungent Leaf* (0.109 mg g^{-1}), *Sessile Joyweed* (0.109 mg g^{-1}) and *Green Amaranth* (0.112 mg g^{-1}) respectively. In this study, the highest moisture content (9.89 %) was recorded in the leaves of *Amaranth*, but ash percentage (7.84 %) and Ca (3.22 mg g^{-1}) content were found maximum in *Green Amaranth*. Similarly, K (9.29 mg g^{-1}) and Na (5.02 mg g^{-1}) were found highest in *Spinach* and *Sessile Joyweed*. The results of K (5.54 mg g^{-1}) and Na (2.31 mg g^{-1}) were lowest in *Pungent Leaf*. In contrast, the lowest Ca and Mg were recorded in *Mint* (1.57 mg g^{-1}) and *White Goosefoot* (0.51 mg g^{-1}). However, Mg content was highest (2.09 mg g^{-1}) in the leaves of *Spinach*. The findings of the current study have revealed that elemental quantification in different medicinal plants is very relevant in terms of therapeutic use and nutritional intake.

Keywords: Medicinal plants, elemental quantification, protocols, physiological, nutritional.

Introduction

Medicinal plants are potential sources of different biologically active compounds that have strong therapeutic significance. Moreover, the edible parts of such medicinal plants also have high mineral malnutrition values for human health. A large number of wild and native plants which are the good source of medicine play an imperative role in the survival of ethnic and tribal communities. They are essential for daily life requirements of human beings, such as food, fiber, and therapeutics. The therapeutic use of indigenous plant products for ethnomedicinal and nutritional purposes has attracted the interest of researchers^{1,2}. Medicinal plants possess essential food resources such as carbohydrates, proteins, and fat. These components are important for human health and are used in various physiological, metabolic, and morphological activities³. Numerous plant-based healthcare products are used in medications and nutritional supplements, and many medicines are derived directly or indirectly from plant resources⁴.

In the current years, nutritional therapy gains a commanding position over allopathic medications. The conceptual papers, reviews are found in the literature^{5,6,7}, many of them focus on specific health care applications, such as the pharmaceutical services, plant based nutritional medications. Nutrition therapy is the treatment of a medical condition through changes of diet, by adjusting quantities, qualities and methods of nutrients intake. Proper nutrition plays a vital role in preventing and managing various health conditions such as diabetes, cardiovascular disease, obesity, and renal diseases and it can help regulate blood sugar levels, improve lipid profiles, manage weight, reduce inflammation, and support organ functions. In this context, rigorous study of nutritional status and proper assessment of macro- and micronutrient contents in medicinally popular plants is becoming most promising research. People in rural areas prefer herbal medicines to health care. Moreover, in rural regions where conventional medicines are widely available, peoples have growing interest in plant-based medications. Medicinal plants include a variety of physiologically active components, including minerals and phytochemicals, which have numerous physiological effects on human health.

Plants are static organisms that must cope permanently with diverse environments. The growth and level of different macro-and micronutrient contents in plant bodies depend on many abiotic

factors such as light, temperature, water, and soil nutrient availability. Plants through their evolution have developed multiple mechanisms that allow their survival and growth to cope with these capricious environments. The uptake of soil minerals is achieved via many processes to minimize the mineral deficiency⁹ including the stimulation of root transporter activities, competitive migration of soil minerals toward transporter proteins, vertical or horizontal root growth, and/or root exudation. Moreover, soil macro-and micronutrient levels also determine the concentrations of these elements in the plant body. In some soil environments, plants may also increase soil mineral availability and improve their nutrient uptake through interactions with rhizospheric microorganisms by applying sorption-desorption mechanism.

Mineral malnutrition is considered to be the most serious global challenge for mankind. Plants are the source of nearly all food chains, the production of biofortified seeds, fruits or edible vegetative organs with increased concentrations of micronutrients could reduce the multiple micronutrient deficiencies what is considered “hidden hunger”⁹. Although micronutrients and minerals are required in minor quantities and are essential for the good health of humans, deficiencies of such micro or macro nutrients in the diet may have long-term negative effects on human health and lead to micronutrient deficiency diseases. Our body requires calcium for muscles to move and for nerves to carry messages between the brain and every part of the body. Similarly, Mg is a key element in regulating muscle and nerve function, blood sugar levels, blood pressure, and the production of proteins, bones, and DNA. Copper is an essential trace element for human health. Copper-containing proteins act as catalysts to assist a number of body functions¹⁰. Similarly, iron is essential as it forms a part of the protein hemoglobin, an oxygen-carrying pigment. It forms part of many enzymes involved in an array of cell functions. In addition to micronutrients, high amounts of major elements are required for good health. Phosphorus is a major element in many biochemical reactions taking place in the body, such as the conversion of foods to energy, helps with muscle contractions, nerve conduction, and normal kidney functions, and helps to build strong bones¹¹. Potassium is required for the maintenance of the total body, fluid volume, electrolyte balance, normal cell functions and acidity of the blood. It functions as an electrolyte and regulates the amount of water in the body. Muscle cells require potassium for growth, and the heart depends on potassium to maintain normal electrical activity¹¹.

In different parts of India, there has been growing interest in the study of medicinal plants and their traditional uses over the last few decades. Folk medicines, which have tremendous importance in curing many ailments, have lost value to the younger generation in recent years. Educational extension of plant-based medications among young people and industrial development of herbal medicines may reduce the negative impact of many allopathic drugs and may also increase employment opportunities. Deforestation, environmental degradation, and modernization are currently occurring in the region, putting the knowledge of plant-based remedies for different diseases at risk. To prevent medicinal plant resources from becoming extinct, it is crucial time in scholarly level for developing various management strategies in collaboration with local communities, such as village administrative councils. The most important aspect is to provide multidirectional awareness and training to the local communities in the study region for sustainable plant exploitation.

Many authors have reported the importance of elemental constituents of herbal drugs in their studies and have enhanced the awareness of trace elements in plants^{12, 13, 14, 15}. Most of these studies have concluded that essential metals can also produce toxic effects or pseudo-toxic effects when intake is high, whereas non-essential metals are toxic even at very low concentrations for human health. It has been well documented that the presence of macro- and micronutrients in medicinal plant species is important in alleviating different diseases, but very little research has been conducted to determine the effectiveness of medicinal plants with respect to their macronutrient and micronutrient content in regional level. The current study has been undertaken to determine the micronutrient and macronutrient contents of ten important medicinal plants used to alleviate different diseases in Assam, Northeast India. The selected plant species, which have high nutritional values, are well known cooking recipes among the people of this region. Besides, inhabitants of this region frequently use the fresh leaves of these plants as oral supplements or as external medication for curing different diseases. To confirm the nutrient index and limit the ingestion doses, knowledge of elemental status in these medicinal plants is utmost essential from a nutritional as well as therapeutic point of view. Such elemental quantification in medicinal plants could contribute to the precise intake level of essential elements in treating different diseases. Moreover, there is an urgent need to document the knowledge of traditional medicines and plant-based nutritional therapy among the new generation from the study site before it is lost to society. Indeed, revolutionary steps are essential

in scientific documentation on necessary minerals and proximate composition in different parts of plant species to promote and scientifically confirm their nutritional and ethnomedicinal advantages.

Materials and Methods:

Sample collection:

In this study, 10 unlike plant species were collected from subsistence farmers' fields of different places (Table 1) of Upper Assam, India. Leaves of these plants are edible as vegetable and have tremendous nutritional and therapeutic importance (Table 2). The fresh leaves of the selected medicinal plants were cleaned with double distilled water repeatedly and shade dried until all the water molecules evaporated. The shade dried leaves were finally oven dried carefully at 60 °C -- 70°C. The dried leaves were grinded with mechanical grinder and transferred into airtight poly packets with proper labeling for future use.

Preparation of leaf extract:

Exactly 2 g of powered leaf materials of each of the plant species were digested in tri-acid mixture (Nitric acid, Perchloric acid and Sulphuric acid in 10:4:1 ratio) carefully and finally extracted with few ml dilute HNO₃. The extracts were filtered through Whatman - 42 filter paper and transferred to properly labeled 100 ml volumetric flasks which were finally volumized to 100 ml mineral solution with double distilled water. These mineral solutions were used later on for micro and macro-nutrients analysis.

Methods of analysis:

In this study, the analysis of soil samples was carried out according to standard methods using analytical-grade reagents. Moisture and ash contents were analyzed with AOAC standardized method¹⁶. The Na, K content was determined using flame photometer from mineral solution obtained after tri-acid digestion¹⁷. P was estimated from mineral solution converting phosphate to phosphomolybdic acid and finally reducing with hydroquinone. The blue colour developed was measured in a spectrophotometer (Systronics UV-VIS Spectrophotometer 118) at 660nm¹⁷. Ca, Mg, Fe & Cu were determined by Atomic Absorption Spectrophotometer (Agilent 200FS Series AA System) from mineral solution¹⁶.

All quality control and assurance measures were taken including replicate analysis of the samples. The obtained data from chemical analysis was subjected to simple descriptive statistics (mean and standard deviation) using SPSS software. Concentrations of studied parameters are expressed as the mean value ($\text{mg.g}^{-1} \pm \text{SD}$) in dry weight basis (DW).

Scientific Names	Leaf Photo	Collection sites	Geographical Co-ordinates	
			Latitude	Longitude
<i>Trigonella Foenum Graecum</i>		Nazira, Sivsagar	26.902314°	94.718971°
<i>Amaranthus Viridis</i>		Mariani, Jorhat	26.651955°	94.325659°
<i>Mentha Piperita</i>		Dhekiajuli, Jorhat	26.6634°	94.192921°
<i>Spinacea Oleracea</i>		Kakoti Gaon, Jorhat	26.748563°	94.197879°
<i>Chenopodium Album</i>		Mariani, Jorhat	26.650457°	94.310646°
<i>Cinnamomum Tamala</i>		Mariani, Jorhat	26.7029509°	94.2492069°
<i>Tropical Almond</i>		Mariani, Jorhat	26.7129721°	94.1904515°
<i>Paederia Foetida</i>		Bongaon, Majuli	26.8198698°	94.1627041°
<i>Alternanthera Sessilis</i>		Bongaon, Majuli	26.6350965°	94.0945145°
<i>Amaranthus Spinousus</i>		Mariani, Jorhat	26.6010754°	94.02340656°

Table 1: Leaf photo of the selected medicinal plants and geographical coordinates of the collection sites.

Scientific Name	Local Name	English Name	Therapeutic values	Leaf Morphology
<i>Trigonella Foenum Graecum</i>	Methi	Fenugreek	Loss of appetite, inflammation of stomach, constipation,. Used for diabetes, painful menstruation, polycystic ovary syndrome.	It is an erect, smooth, herbaceous plant. The leaves are alternate, compound, trifoliolate, 7-12 cm long.
<i>Amaranthus Viridis</i>	Morisha	Amaranth	Stomach ulcers, atherosclerosis, tuberculosis, as well as antiseptic, antifungal, and anti-inflammatory preparations	Leaves are normally elliptical, with an acute tip and a cuneate base, leaf size varies significantly within species.
<i>Mentha Piperita</i>	Pudina	Mint	Use in indigestion, irritable bowel syndrome, respiratory complaints, improve brain power, boosts immunity, help in breastfeeding Pain	Mint species are wide-spreading stolons that grow both under and above ground. Leaves are oblong to lanceolate in shape.
<i>Spinacea Oleracea</i>	Paleng	Spinach	Prevents cancer, reduces blood sugar, aids in good bone health, weight loss, reduces hypertension	Leaf size around 5 to 8 cm, may be curly leaves, broad flat leaves, and smooth leaves
<i>Chenopodium Album</i>	Jhilmil	White Goosefoot	Rich in vitamins A, C and B, full of essential minerals and antioxidants	Leaves are greenish-blue to gray, 4 inches to 6 feet tall and 4 to 12 inches wide depending on soil nutrients.
<i>Cinnamomum Tamala</i>	Tejpatt	Pungent leaf	Useful in diabetes, prevents the damage of pancreatic beta cells, enhances insulin secretion, lowers blood sugar levels.	The bark is brittle, brown in colour with good odour. Leaves are thick, 4 to 7 inch in length with deep vein like structure.
<i>Tropical Almond</i>	Arjun	Arjun	Use as cardiotoxic in heart failure, ischemic, cardiomyopathy, anaemia. atherosclerosis, myocardium necrosis, blood diseases,	Leaves are simple, alternate thick-coriaceous, 15-25 cm in length and 6-7.5 cm in width and petiole is 0.6-0.9 cm long.
<i>Paederia Foetida</i>	Vedailata	Skunkvine	Leaves are used in rheumatism pain, infertility, paralysis, abdominal pain, abscesses, arthritis, overeating.	Climbing, woody plant with slender stems, twining, up to 10m long and short hairs scattered on young parts.
<i>Alternanther Aessilis</i>	Mati-kanduri	Sessile Joyweed	It reduces eye diseases, wound healing, increase heart function, enhances blood circulation and induces brain.	Flowers are white with a paper-like texture and rounded clover-like heads in the leaf axils.
<i>Amaranthus Spinousus</i>	Khutura Saag	Green amaranth	Used in atherosclerosis, stomach ulcers, tuberculosis, as well as antiseptic, anti fungal and anti-inflammatory.	Annual herb, light green stem of about 60–80 cm in height and leaves are ovate, 3–6 cm long, 2–4 cm wide, with long petioles.

Table 2. The leaf morphology and some curative properties of the selected medicinal plants.

Results and Discussions:

In this study, we determined % moisture contents, % ash contents, macronutrients (Na, K, Ca, Mg, P) and micronutrients (Cu, Fe) from the leaves of 10 medicinal plants which have high nutritional values and have remarkable therapeutic importance (Table 2). The results of different macro- and micronutrients of the selected plants are compared with the nutrients' results recorded in USDA database and other earlier resources (Table – 3). These results help to choose plants with high quantity of the nutrients and also validate the efficacy of the medicinal activity of the plants. The findings of current study showed a large variation in macro and micronutrient levels among the plant species. The variability in the contents of nutrients offers opportunity to the scholars working on food and nutritional branches as well as pharmacological science. On the basis of these results, the selected medicinal plants may be categorized in terms of maximum content of macro and micro nutrients.

Plants Names	Mineral contents in mg.g ⁻¹							Data sources
	Ca	Mg	K	Na	P	Fe	Cu	
Fenugreek	1.76	1.91	7.7	0.67	2.96	0.335	0.011	<i>USDA Database, updated 1977</i>
Amaranth	2.15	0.55	6.11	0.2	0.5	0.023	0.0016	<i>USDA Database, updated on 1984</i>
Mint	2.43	0.8	5.69	0.31	0.73	0.051	0.0033	<i>USDA Database, updated on 1984</i>
Spinach	0.99	0.79	5.58	0.79	0.49	0.027	0.0013	<i>USDA Database, updated on 1984</i>
White Goosefoot	3.12 9	3.92 9	13.2 7	1.38	0.789	0.057 9	0.0033	<i>Guerrero & Isasa et al.,1998</i>
Pungent leaf	10	0.6	4.31	0.1	0.64	0.083	0.0034	<i>USDA Database, updated on 2007</i>
Arjun	49.6 5	1.86 8	9.36 4	0.02 7	0.051	0.179	0.015	<i>Chakradhari et al,2019²³</i>
Skunk vine	0.1	1.4	8.0	----	1.3	0.347	0.081	<i>Upadhyaya et al., 2010²²</i>
Sessile Joyweed	5.1	0.8	44.5	2.86	0.487	0.098	0.016	<i>Lalitha & Vijayalakshmi, 2018²⁹</i>
Green Amaranth	2.09	0.55	6.4	0.21	0.72	0.022	0.0016	<i>Nutrition Advance, 2024</i>

Table 3: Concentrations of different inorganic elements recorded in *USDA database* and other published reports for the selected plants.

The results (Table 3) collected from *USDA Database* suggest high Mg, K, P and Fe contents in Fenugreek leaves, while for White Goosefoot and Spinach the Ca and Na contents are high. However, the results of current study documented large variations in studied parameters (Table 4). The moisture percentage varied from 9.78 to 7.18 % and ash percentage varied from 1.87 to 7.84. With respect to macronutrients, Ca varied from 1.57 – 3.22 (mg. g⁻¹), Mg from 0.51– 2.09 (mg.g⁻¹), K contents varied from 5.54 – 9.29 (mg g⁻¹). The variations in Na and available P ranged from 2.31 to 5.01 and 0.033 to 0.91 0.18–5.79 mg g⁻¹ respectively. Likewise, variations in micronutrients Fe and Cu were found within the range 0.109 – 0.331 and 0.007 – 0.142 mg. g⁻¹ respectively in dry weight basis.

Leaf water content is also an important indicator in the sense plants’ biochemical synthesis and accumulation of macro- and micronutrient contents. Percentage leaf moisture content is a vital parameter that needs to be considered during storage of raw herbal material. High relative water content (RWC) in leaves gives suitable conditions for the proliferation of living organisms. Thus, the increase in leaf moisture content of medicinal plants may affect their herbal properties through the increase of microbial damage to leaf phyto-chemical properties and valuable active agents. In this study, range of moisture content recorded highest in Green Amaranth (9.48%) to lowest in Spinach (7.18 %) on dry weight basis (Table 4, figure 1). Most of the plant species displayed comparable moisture percentage measured on dry weight basis. Similarly highest ash percentage was observed in Green Amaranth (7.84%) followed by Spinach (7.46%), Fenugreek (7.11%), Skunkvine (6.78%) and Sessile Joyweed (6.35%). Ash percentage was found lowest in Arjun and Amaranth.

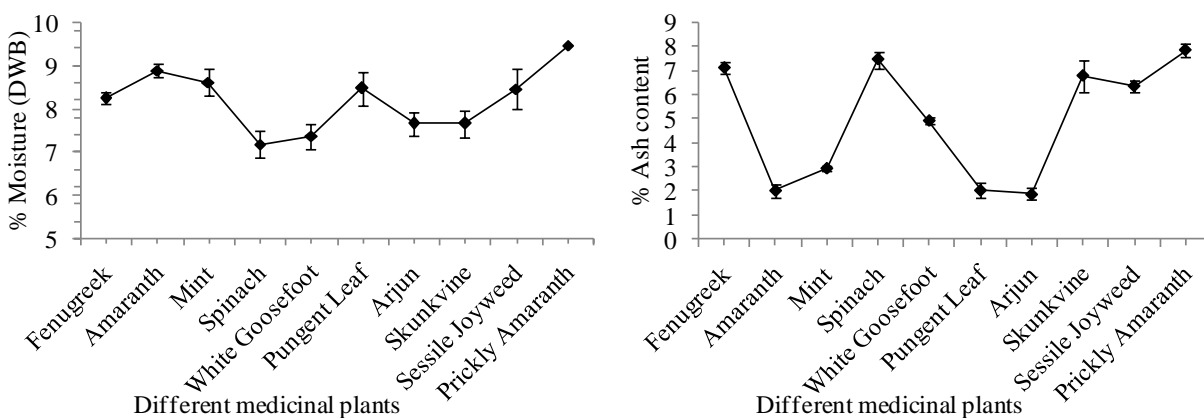


Figure -1: Leaf moisture contents and Ash contents (in %) in different medicinal plants. The vertical bars represent the ±SEM (standard mean errors) of the results.

Wide variations in copper (Cu) concentrations (0.007 – 0.142 mg g⁻¹) were observed in the current investigation (Table 4). Copper is an essential element for human body that regulates oxidation–reduction reactions, energy production, neurotransmitter synthesis, and involved in iron metabolism¹⁸. However, a high ingestion of copper can cause other physiological disorders like vomiting, dermatitis, abdominal pain, diarrhea, and liver damage¹⁸.

In this investigation, lowest Cu content was recorded in Mint (0.007 mg g⁻¹) where Sessile Joyweed showed maximum value (0.142 mg g⁻¹). Cu concentration in the leave of Skunkvine (0.126 mg g⁻¹) and White Goosefoot (0.112mg g⁻¹) were found higher than the other plant species (Figure 2). Some earlier reports^{19, 20} have documented 0.13 mg g⁻¹ Cu contents in the leaves of White Goosefoot while Dghaim et al (2015)²¹ recorded maximum 12.32 mg k⁻¹ Cu in Mint which was higher than the value found in the current study. However, Upadhyaya et al (2010)²² reported a lower Cu content (Table 3) in the shoot of *P. Foetida* (0.018 mg g⁻¹) compared to the result recorded in this study. In contrast, Reddy and Reddy (1997)¹³ reported that the range of Cu contents in the 50 medicinally important leafy material growing in India were 17.6 to 57.3 ppm. In that study, Cu concentration in the leaves of Fenugreek, Amaranth, Spinach, Pungent leaf, Arjun, and Prickly Amaranth were 0.074, 0.027, 0.051, 0.079, 0.067 and 0.073 mg g⁻¹ respectively. Although the permissible limit for Cu in medicinal plants has not yet been set by the WHO, the limits of the oral reference dose for medicinal plants as set by China and Singapore are 0.020 and 0.150 mg per g, respectively¹⁸. Based on these results, we can conclude that the Cu concentrations in the leaves of these ten medicinal plants were not more than the standard concentration reported for similar plants. However, regular intake of Sessile Joyweed, Skunkvine, and White Goosefoot may cause detrimental effects on human health.

Iron is another biologically vital element found in soils. Plants uptake iron from the soil through the emergence of different phyto-chelating proteins, and this bio-available iron involve in the synthesis of numerous iron-containing bioactive compounds. In plant health, Fe is engaged in chlorophyll biosynthesis, photosynthesis, electron transport and respiration, nitrogen fixation, root growth and development, DNA and RNA synthesis, and plant defense mechanisms. Iron also has several key functions in the human body, including oxygen supply, energy production, and immunity. Although, iron is a vital element and has a considerable role in different metabolic processes, the toxic iron level has a detrimental effect on diverse metabolic functions

and cardiovascular systems, and causes gastrointestinal disorders. Its overdose may also cause several physiological disorders like symptoms of dizziness, nausea and vomiting, diarrhea, joint pain, shock, and liver damage.

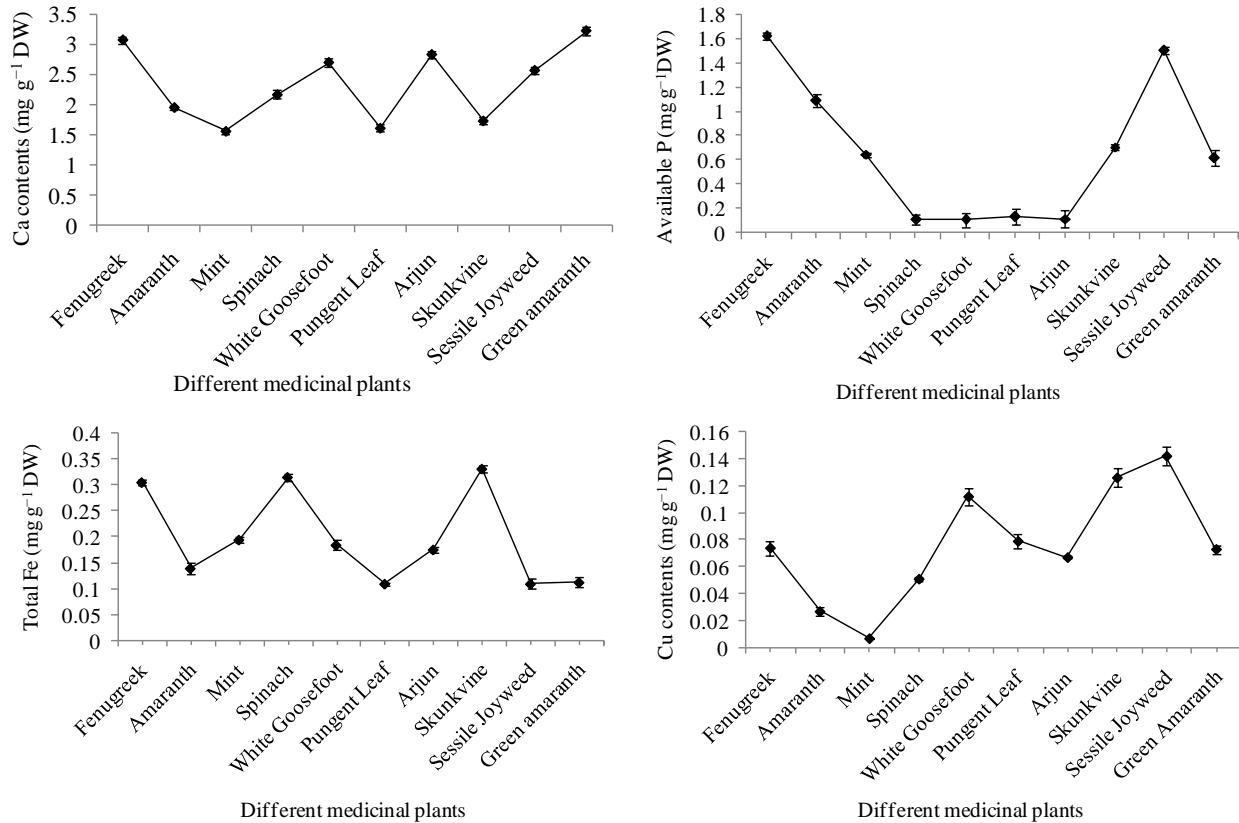


Figure -2: Concentration ($\text{mg g}^{-1} \text{DW}$) of leaf Ca, available P, total Fe and Cu in different medicinal plants. The vertical bars represent the $\pm \text{SEM}$ of the results.

Fe concentrations in selected medicinal plants were found within the range from 0.109 mg g^{-1} in Sessile Joyweed to 0.331 mg g^{-1} in Skunkvine. The leaves of Fenugreek recorded lower Fe (0.305 mg g^{-1}) content as recorded in USDA data base (0.335 mg g^{-1}) updated 1977 (Table 3) while Spinach, Amaranth, Mint, White Goosefoot and Pungent leaf documented higher Fe content (Table – 4) compared to this database. In contrast, earlier reports^{19, 20} have documented 0.255 mg g^{-1} Fe in the leaves of White Goosefoot on dry weight basis, which was higher than the

PlantsNames	% Moisture	Ash %	Mineral contents in mg g ⁻¹ DW								
			Mg	Ca	P	Ca : P	K	Na	Na:K	Fe	Cu
Fenugreek	8.26±0.15	7.11±0.26	1.82±0.08	3.07±0.06	1.62±0.03	1.89	8.29±0.17	4.24±0.24	0.51	0.305±0.005	0.074±0.005
Amaranth	8.89±0.15	2.03±0.28	0.91±0.05	1.96±0.04	1.09±0.05	1.79	8.07±0.18	3.81±0.31	0.47	0.139±0.008	0.027±0.003
Mint	8.62±0.3	2.95±0.1	0.85±0.04	1.57±0.05	0.646±0.02	2.43	7.41±0.10	3.40±0.19	0.46	0.194±0.005	0.007±0.001
Spinach	7.18±0.3	7.46±0.35	2.09±0.07	2.17±0.07	0.11±0.045	19.72	9.29±0.24	5.02±0.34	0.54	0.315±0.007	0.051±0.002
White Goosefoot	7.37±0.3	4.92±0.14	0.51±0.06	2.70±0.07	0.107±0.06	25.23	7.58±0.23	4.93±0.08	0.65	0.184±0.009	0.112±0.006
Pungent leaf	8.48±0.38	2.04±0.3	0.89±0.06	1.62±0.06	0.135±0.05	12	5.54±0.19	2.31±0.29	0.41	0.109±0.003	0.079±0.005
Arjun	7.66±0.26	1.87±0.24	1.71±0.07	2.83±0.06	0.115±0.07	24.6	7.31±0.11	4.41±0.17	0.6	0.175±0.005	0.067±0.001
Skunkvine	7.66±0.3	6.78±0.65	1.44±0.03	1.74±0.05	0.706±0.03	2.46	6.26±0.17	2.74±0.19	0.44	0.331±0.006	0.126±0.007
Sessile Joyweed	8.47±0.25	6.35±0.27	1.39±0.05	2.57±0.06	1.504±0.03	1.7	8.76±0.24	5.02±0.33	0.57	0.109±0.008	0.142±0.007
Green amaranth	9.48±0.45	7.84±0.28	1.42±0.02	3.22±0.07	0.62±0.06	5.19	7.81±0.35	4.81±0.25	0.61	0.112±0.007	0.073±0.003

Table 4: Results of mineral contents in the leaves of different medicinal plants in mg g⁻¹ ± SEM. Results are expressed in Dry Weight basis.

value documented in this study ($0.1840.335 \text{ mg g}^{-1}$). Likewise, Dghaim et al (2015)²¹ recorded maximum of $821.02 \text{ mg k}^{-1}\text{Fe}$ in Mint which was much higher than the value found in the current study (0.194 mg g^{-1}). In the current study, Fe concentration in Arjun was $0.175 \text{ } 0.305 \text{ mg g}^{-1}$ leaves which was analogous to the result reported by Chakradhari et al (2019)²³. In fact, the Fe concentrations observed in this study were above the permissible limit (0.015 mg g^{-1})^{24, 25}, in all the plant samples. The higher level of Fe in the current study might be due to higher soil iron where the plants were cultivated and potential rhizospheric iron sorption mechanism through the expression of novel iron shipping genes at the root periphery²⁶.

Phosphorous is one of the crucial macro-elements needed for the growth, maintenance, and repair of all tissues and cells. Phosphorous is an essential constituent of nucleic acids, cellular membranes, and many other enzymes. It is vital for the production of the genetic building blocks, DNA and RNA. It is also required to help balance and use other vitamins and minerals, including vitamin D, iodine, magnesium, and zinc. In plants, phosphorous acts as an activator of different enzymes, regulates moisture content, and reduces iron and salt stress effects. However, an elevated intake of phosphorus may pull calcium out of bones, making them weaker. Higher phosphorus and calcium levels also lead to dangerous calcium deposits in blood vessels, lungs, eyes, and hearts. Long-term intake of high-dose P can lead to an increased risk of heart attack, stroke, or death. The average daily phosphorus requirement varies with age, but an adult's recommended amount is 700 mg ²⁷.

In this study, P content was highest (Figure 2) in Fenugreek (1.62 mg g^{-1}) and lowest in White Goosefoot (0.107 mg g^{-1}). However, in the line of USDA database, the P content is too high (2.96 mg g^{-1}) in Fenugreek than the result of the current study. But the observed result of phosphorous in the leaves of Amaranth (1.09 mg g^{-1}) was relatively higher than the USDA database. The leaf P contents in Mint, Spinach, Pungent leaf, and Green Amaranth were $0.646, 0.11, 0.135$ and $0.62 \text{ mg g}^{-1}\text{DW}$ respectively (Table 4), which were lower than the established data (Table -3). In contrast, Bahadur et al. (2011)²⁸ has reported much lower P content (0.0155 mg/g) in dry leaves of White Goosefoot. However, the P content in Arjun (0.115 mg g^{-1}) was superior to the result of an earlier report²³ while Sessile Joyweed showed higher P content ($1.504 \text{ mg g}^{-1}\text{DW}$) than the earlier report of Lalitha and Vijayalakshmi, (2018)²⁹. The lower P contents detected in this study

might be due to poor soil P level where the plants were grown. There is a close relationship between soil P levels and the root iron uptake mechanism. Lower soil P content increases the biologically available ferrous form of iron in the root periphery. Hence, this Fe-P antagonistic relationship in soil accelerates the root Fe uptake process, which might be another reason for the higher iron concentration observed in the current study. The lower P levels in the selected plants indicate that normal dietary intake or using the leaves of these plants as herbal medicine will not be harmful in P malnutrition.

Calcium is the most abundant element in the body. Almost all calcium in the body is stored in bones and teeth, giving them structure and hardness. It is involved in regulating almost all biological functions of the body, such as the heart and muscle contractions, neuro-information transmission, learning and memory, embryo formation and development, cell proliferation, cell division and differentiation, cell energy metabolism, protein phosphorylation and dephosphorylation modification, and gene expression and regulation. Therefore, calcium has been extensively used for the treatment of various diseases. The levels of calcium were varied from 1.57 – 3.22 mg g⁻¹ in all samples (Table 4). Highest Ca was found in the leaves of Green Amaranth and lowest in the leaves of Mint plant (Figure 2). The Ca contents in Spinach, White Goosefoot, Arjun and Sessile Joyweed were 2.17, 2.70, 2.83 and 2.57 mg g⁻¹ respectively. Ca content in Fenugreek leaves was 3.07 mg g⁻¹ which was much higher than the value recorded as per USDA database. However, Gharneh and Davodahosseini (2015)³⁰ reported 2.00 to 4.55 mg g⁻¹ of Ca in 7 fenugreek genotypes native to Iran on a fresh weight basis. Conversely, Ca concentration in the leaves of Amaranth, Mint, Pungent leaf and White Goosefoot were found lower than the results established in USDA database (Table -3 and Table-4). Similarly, Chakradhari et al, (2019)²³ recorded much higher Ca (49.65 mg g⁻¹) in Arjun than the result of current study (2.83 mg g⁻¹). As per the report of Nutrition Advance, 2024, Ca content in Green Amaranth is 2.09 mg g⁻¹, but in this study 3.22 mg g⁻¹ of Ca was found in the leaves.

Although Ca has an enormous biological role in human health, a high blood Ca level may cause hypercalcemia. Too much calcium in the blood can weaken bones and create kidney stones. It can also affect the heart and brain. Calcium and phosphorus are associated with each other for the development and proper functioning of bones, teeth, and muscles³¹. However, within the body, both are inversely related: as blood calcium levels rise, phosphate levels fall. For adequate intake of calcium and phosphorus, the appropriate ratio is 1 to 2:1. In this study, except Spinach,

White Goosefoot, Arjun and Pungent leaf, other plant species had a suitable Ca and P ratio (Table 4). Regular intake of plants with a higher Ca/P ratio than the ratio 1:1, may cause vomiting, poor appetite, or constipation³². Therefore, phosphorus-rich food materials should be used together with the leafy vegetables. Indeed, in terms of Ca, controlled consumption of the leaves of those plants with high Ca concentrations would be more effective from an ethnomedicinal and nutritional perspective.

Mg²⁺ ion is essential for healthy muscles, nerves, bones, and blood sugar levels. It acts as a physiological Ca²⁺ antagonist within cells since it can compete with Ca²⁺ for binding sites in proteins and Ca²⁺ transporters³³. These abilities are involved in the observed effect of magnesium on the cardiovascular system, muscles, and brain. Magnesium blocks the calcium channel in the N-methyl-D-aspartate (NMDA) receptor and must be removed for glutamatergic excitatory signaling. Low serum Mg²⁺ levels increase NMDA receptor activity, thus enhancing Ca²⁺ and Na⁺ influx and neuronal excitability. For these reasons, a deficiency of Mg²⁺ has been considered in many neurological disorders, such as migraine, chronic pain, epilepsy, Alzheimer's, Parkinson's, stroke, anxiety, and depression³⁴. Conversely, if one doesn't get enough magnesium in their diet over a long period of time, they may be at a higher risk of health problems such as heart attack, stroke, diabetes, or osteoporosis. Normally, blood Mg content is 2–4 mg/100 ml. In this study, the level of Mg varied from 2.09 to 0.51 mg g⁻¹ in the leaf samples of different plant species (Table 4). The leaves of Fenugreek, Mint, and White Goosefoot recorded 1.82, 0.85, and 0.51 mg g⁻¹ respectively, comparable Mg levels as reported in the USDA database (Table 3). However, earlier reports^{30, 35} have documented much higher Mg content (1.30-3.70 mg g⁻¹) in the fresh leaves of different Iranian Fenugreek genotypes compared to the current result. Conversely, Mg concentrations (Figure 3) in Amaranth (0.91 mg g⁻¹), Spinach (2.09 mg g⁻¹) and Pungent leaf (0.89 mg g⁻¹) were higher than the records in the USDA database. In this study, the Mg content found in Arjun was 1.71 mg g⁻¹ dry leaves, an analogous result to the report of Chakradhari et al., (2019)²³. However, Mg content in the dry leaves of Green Amaranth was higher (1.42 mg g⁻¹) than the established report of Nutrition Advance, 2024 but much lower than the value 403.13 mg/100g, reported by Umar et al. (2011)³¹. The high magnesium concentration expected in this study might be due to the higher chlorophyll contents in the green leaves of different plant species.

Potassium, the most vital intracellular cation, is an essential nutrient that is naturally present in many foods and is available as a dietary supplement. It is an essential mineral for the normal functioning of all cells. To maintain a balanced potassium level in the body, one should consume not less than 400–800 mg of potassium per day. Potassium regulates the heartbeat, ensures

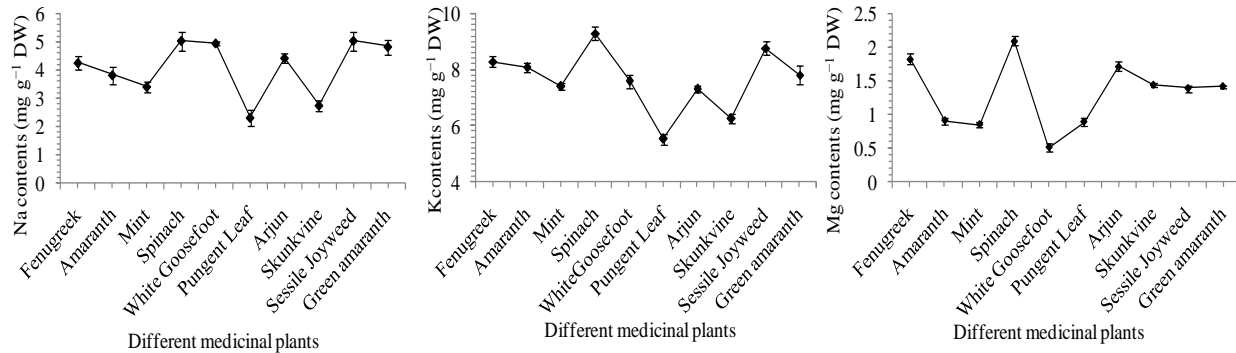


Figure -3: Concentration (mg g⁻¹DW) of leaf Na, K and Mg in different medicinal plants. The vertical bars represent the \pm SEM of the results.

proper function of the muscles and nerves, and is vital for synthesizing protein and metabolizing carbohydrates. However, too much high intake of potassium can be dangerous. Potassium affects the heart's muscle work; the heart may beat irregularly, which, in the worst cases, can cause a heart attack. In the current study, K contents were found to be maximum in Spinach (9.29 mg g⁻¹ dry leaves) and minimum in Pungent leaf (5.54 mg g⁻¹). Among the other plant species, Fenugreek, Amaranth, Sessile Joyweed, and Green Amaranth were also documented to have higher potassium contents, viz., 8.29, 8.07, 8.76, and 7.81 mg g⁻¹ respectively on dry weight basis (Table 4). Conversely, in earlier reports^{31, 36}, quite higher K contents, 2,391.67 mg/100g and 34.6 mg/g were detected in the leaves of *A. Viridis* compared to the current results in Amaranth (*Amaranthus Viridis*) and Green Amaranth (*Amaranthus Spinousus*). Similarly, Lalitha and Vijayalakshmi, (2018)²⁹ reported remarkably higher K (4450 mg /100gm) in the leaves of *Alternanthera sessilis* than the current study. However, in this study, the K contents in different plant species were found higher than the reputable USDA database. The plants species which are rich in K contents have high therapeutic values in regulating the heartbeat and ensuring proper function of the body muscles and nerves.

Na is another important electrolyte that the body needs in relatively large amounts. Sodium helps the body keep fluids in a normal balance. It also plays a key role in normal nerve and muscle function. However, too low Na level (hyponatremia symptoms) or much higher Na level

in body (hypernatremia symptoms) may result brain dysfunction. Severe hyponatremia or hypernatremia symptoms can lead to confusion, muscle twitching, seizures, coma and death. Sodium to potassium intake ratio (Na:K) has been considered to be an important predictor of hypertension. The Na:K ratio in the body is of great concern for prevention of high blood pressure. A diet low in sodium and high in potassium (Na:K < 1) is widely recommended as a strategy to lower blood pressure and to reduce the risk of cardiovascular diseases^{19, 20}. Hence, the knowledge leaf Na level is very essential in nutritional intake of different medicinal plants. In this study, the different plant species recorded lower Na to K ratios (Na:K < 1) which signify their potential nutritional and therapeutic efficiency in reducing high blood pressure diseases (Table 4). In the current observation, Na concentration was found lowest 2.31 mg g⁻¹ in Pungent leaf and highest 5.02 mg g⁻¹ in Spinach and Sessile Joyweed. Leaves of Fenugreek, White Goosefoot, Green Amaranth and Arjun recorded 4.24, 4.93, 4.81 and 4.41 mg g⁻¹ Na in dry weight basis (Table 4). All the plant species showed higher Na concentrations than the results reported in USDA database (Table-3). Besides, sodium contents in the samples were found much lower to potassium contents, which agreed with the earlier reports for leafy vegetables^{31, 37, 38}. The plant leaves with low sodium content may be suggested the possibility of incorporating into diets of obese patients.

CONCLUSION:

Nutrition therapy is the treatment of a medical condition without going through deeper allopathic medications. In the current century, a number of chronic diseases are identified that can be cured through changes in diet by adjusting quantities, qualities, and methods of nutrient intake. In the USA, medical nutrition therapy has a legal definition and is provided by a registered dietitian. So, rigorous study of nutritional status and proper assessment of macro- and micronutrient contents in medicinally renowned plants is becoming most promising research. The findings of the current study have documented that some of the studied parameters were found to be higher than the safety standards of the WHO. Beside, in this study diverse macro- and micronutrients contents were detected among the plant species. Results of this study have demonstrated that oral supplements of vegetables like fenugreek, spinach, and sessile joyweed, either in cooking form or as fresh leaves, have good nutritional benefits with high macro- and micronutrient contents. However, concentration variability in the results of different macro- and

micro-elements demonstrate the need for precise study of medicinal plants or their products used as bio-medicines; otherwise, they may be harmful to health. Moreover, the variation in the results of different biogenic elements reflects that the elemental concentration is a function of plant's genotypic character and soil physicochemical properties.

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