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ESTIMATION OF MACRO AND MICRO NUTRIENTS CONTENT IN THE LEAVES OF *MURRAYA KOENIGII* (L.) SPRENGE

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ABSTRACT: The macro and micro nutrients content estimation was carried out for leaves of indigenous medicinal plant *Murraya koenigii* (L.) Spreng collected from different places of North Karnataka region viz. Bidar, Kalaburagi, Shahapur, Sandur and Kappathuguda. About few kg of leaves of *Murraya koenigii* (L.) Spreng plant material was collected and then they were washed in deionized water to eliminate contamination due to dust and environmental pollution. The washed leaves were air-dried under shade for more than a month and then grinded to get fine powder. The powdered sample was heated at higher temperature to get ash. The obtained ash was digested with conc. HCl, deionized water and ash (25:25:1:950). The contents of macro, micro nutrients and harmful heavy metals such as K, Ca, Mg, Fe, Mo, Cu, Mn, Zn, Al, V, Cd and Ti were estimated by an analytical atomic absorption spectrometry technique. The experimental results confirmed the presence of mineral nutrients which are beneficial to the human body and are within the limit. The heavy metal which are harmful to human body i.e., Cd, Al, within the limit but concentration of Al is absent for the leaves of Shahapur and Kappathuguda.

INTRODUCTION: Medicinal plants are the richest bio-resource of drugs for traditional systems of medicine and play an important role in meeting the global health care needs. Medicinal plants supply minerals, vitamins and certain hormone precursors in addition to protein and energy to the body^{1, 2}. According to the survey reported by World Health Organization (WHO), about 80 % of the world's population consumes traditional medicinal plants in direct and indirect ways to treat their diseases. In traditional medicine system indigenous medicinal plants are widely consumed as home remedies to improve their health, life-style and also for curing and preventing various diseases.

The property of curing and prevention of the indigenous medicinal plants depends on their mineral nutrient contents. The concentration level of the mineral nutrients in the plants play an important role in chemical, biological, biochemical, metabolic, catabolic and enzymatic reactions in the living organism which will lead to the formation of active organic constituents³ and these minerals varies by the geochemical characteristics of the soil and environmental conditions⁴.

During the past decade, it has seen a significant increase in the use of traditional medicine due to their minimal side effect, availability and acceptability⁵. Numerous indigenous medicinal plants and their formulations are used for treating diseases in ethno-medical practices as well as in traditional systems of medicine. Essential macro and micro nutrients in indigenous medicinal plants have been investigated by many researchers to strengthen the importance of mineral nutrients content analysis with respect to human health⁶.

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Macro nutrients include carbohydrates, fats and proteins and are the structural and energy-giving caloric components. Whereas the micro nutrients are the vitamins, minerals, trace elements, phytochemicals, and antioxidants that are essential for good health. The human body requires a number of mineral nutrients to maintain a good health⁷. In this context several attempts have been made to determine the mineral nutrient contents of indigenous medicinal plants using different elemental analysis techniques from many countries all over the world⁸.

In the present study, *Murraya koenigii* (L.) Spreng commonly available garden plant leaves were collected from different place of North Karnataka region viz. Bidar, Kalaburagi, Shahapur, Sandur & Kappathgudda. They are used in traditional medicinal system for the treatment of variety of diseases due to its antidiabetic, cholesterol reducing property, anti-diarrheal, antioxidant, antiulcer, antimicrobial, antibacterial and many more useful medicinal properties. The collected leaves of *Murraya koenigii* (L.) Spreng were investigated for their mineral nutrients content using analytical AAS technique.

This technique measures the concentrations of elements. Atomic absorption is so sensitive that it can measure down to ppb (parts per billion) or ppm (parts per million) of a gram ($\mu\text{g dm}^{-3}$ or 10^{-6}) in a sample. The technique makes use of the wavelengths of light specifically absorbed by an element present in the sample. They correspond to the energies needed to promote electrons from one

energy level to another, i.e., higher energy level. Atomic absorption spectroscopy has many uses in different areas such as clinical analysis, environmental analysis, pharmaceuticals, industry, mining and agriculture^{9,10}.

MATERIALS AND METHODS:

Plant Material: Fig. 1 show the Karnataka and North Karnataka region map and Fig. 2 Show the leaves of the *Murraya koenigii* (L.) Spreng, an indigenous medicinal plant.

Sample Collection: North Karnataka locally known as Uttara Karnataka is a geographical region consisting of mostly semi-arid plateau from 300 to 730 meters (980 to 2,400 ft.) elevation that constitutes the northern part of the South Indian state of Karnataka. Districts of North Karnataka are Bidar, Belgaum, Gulbarga, Yadagiri, Raichur, Koppal, Bellary, Bijapur, Bagalkot, Gadag, Dharwad, Haveri, and Uttara Kannada Districts.

Murraya koenigii (L.) Spreng indigenous medicinal plant leaves are picked from different places of North Karnataka region viz. Bidar, Kalaburagi, Shahapur, Sandur and Kappathgudda. About a few kg of leaves of plant material was collected and then they were washed in deionized water to eliminate contamination due to dust and environmental pollution. The washed leaves of *Murraya koenigii* (L.) Spreng indigenous medicinal plant were air-dried under shade for more than 30-45 days and then grinded to get fine powder which was further used for the mineral nutrients content analysis.

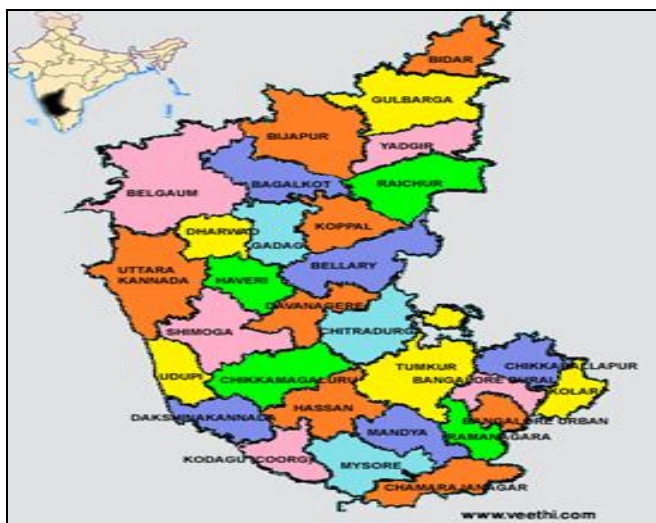


FIG. 1: KARNATAKA WITH NORTH KARNATAKA REGION MAP



FIG. 2: COLLECTED LEAVES OF *MURRAYA KOENIGII* (L.) SPRENGE

Sample Preparation for Mineral Nutrients Content Analysis: A 10 gm of powder *Murraya koenigii* (L.) Sprengel plant leaves was taken in a silica crucible and then kept in an oven for 2 - 3 h



at 250 - 350 °C to get ash. The obtained ash was used for preparation of solution. The solution was prepared by mixing of concentrated HCl, double distilled water and 1 g of ash in the ratio 25: 25:1. The mixed solution was then stirred for few minutes; it was then filtered using walt man filter paper 41. A 950 ml of double distilled water was added to the filtered solution to make it 1000 ml solution. The same procedure was repeated for all other plant material samples¹¹. The prepared solutions are as shown in Fig. 3 and the same are for the measurement of mineral nutrients content analysis using AAS technique.



FIG. 3: SAMPLES FOR MINERAL NUTRIENTS CONTENT ANALYSIS

Determination of Elements: The mineral nutrients such as K, Ca, Mg, Fe, Mo, Cu, Mn, Zn, Al, V, Cd and Ti in the leaves of *Murraya koenigii* (L.) Sprengel plant samples were analyzed using analytical atomic absorption spectrophotometer. It is manufactured by Thermo ScientificTM with a

model no. iCETM-3000 series and it is equipped with dedicated flame, furnace or combined flame and furnace option. Air -C₂H₂ and N₂O- C₂H₂ flame was used for determination mineral nutrients content. The instrument was operated with the conditions shown in Table 1.

TABLE 1: OPERATING PARAMETER FOR WORKING ELEMENTS

Elements Flow	Wavelength (nm)	Slit width (nm)	Lamp Current	Flame Type	Fuel Flow (L/min)	Characteristic Conc. mg/L	Burner height (mm)
Mg	285.2	0.5	75%	Air-C ₂ H ₂	1.2	0.0170	7
Al	309.3	0.5	100%	N ₂ O- C ₂ H ₂	4.3	12.0442	11
K	766.5	0.5	100%	Air-C ₂ H ₂	1.2	0.0567	7
Mn	279.5	0.2	75%	Air-C ₂ H ₂	1.0	0.0860	7
Fe	248.3	0.5	75%	Air-C ₂ H ₂	0.9	0.2344	7
Cr	357.9	0.5	100%	N ₂ O- C ₂ H ₂	4.2	0.6196	8
Ca	422.7	0.5	100%	N ₂ O- C ₂ H ₂	4.2	0.2340	11
Cu	324.8	0.5	75%	Air-C ₂ H ₂	1.1	0.1119	7
Zn	213.9	0.2	75%	Air-C ₂ H ₂	1.2	0.0333	7
Cd	228.8	0.5	50%	Air-C ₂ H ₂	1.2	0.0344	7
Si	251.6	0.5	75%	N ₂ O- C ₂ H ₂	4.9	2.698	11
Mo	313.3	0.5	75%	N ₂ O- C ₂ H ₂	4.7	3.6551	11
V	318.5	0.5	75%	N ₂ O- C ₂ H ₂	4.7	4.2067	11
Ti	365.4	0.5	75%	N ₂ O- C ₂ H ₂	4.7	45.6638	11

The calibration has been carried out using different hollow-cathode lamps for Al, Cu, Mg, Zn and Cd were employed as radiation source and calibrated

using 100 ml standard solutions in equal ratio. A detector measures the wavelengths of light transmitted by the sample, and compares them to

the wavelengths which originally passed through the sample. Atoms of each element will emit a characteristic spectral line. Every atom has its own distinct pattern of wavelengths at which it will absorb energy, due to the unique configuration of electrons in its outer shell. This enables the qualitative analysis of a sample.

The absorption wavelength for the determination of each element with its linear working range and correlation coefficient were calibrated for the analysis. A monochromator is used to select the specific wavelength of light that is absorbed by the sample and to exclude other wavelengths.

The selection of the specific wavelength of light allows for the determination of the specific element of interest when it is in the presence of other elements. **Fig. 4** shows an instrument process of an atomic absorption spectrometer.

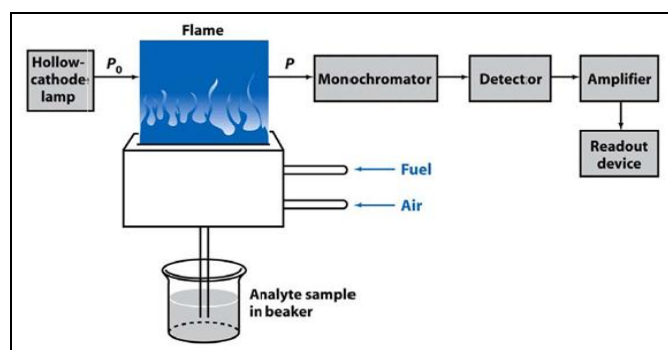


FIG. 4: INSTRUMENT PROCESSES OF AN ATOMIC ABSORPTION SPECTROMETER

RESULTS AND DISCUSSION: **Fig. 1** Show the images of *Murraya koenigii* (L.) Sprenge leaves of the indigenous medicinal plants. **Table 2**, **Table 3** and **Table 4** show concentrations of essential macro nutrients, essential micro nutrients and harmful heavy metals measured in the leaves of *Murraya koenigii* (L.) Sprenge an indigenous medicinal plant collected from different places of North Karnataka region.

Essential Macro Nutrients:

Calcium (Ca): The concentration of Ca is found in all the collected leaves of *Murraya koenigii* (L.) Sprenge medicinal plants and the concentration of calcium are highest compared to all other macro nutrients. The presence of high amount of the Calcium concentration in the leaves of *Murraya koenigii* (L.) Sprenge medicinal plant could be due to the fact that the soil of North Karnataka region

contains maximum amount of Calcium and the same one is reflected in the medicinal plants. The level of calcium is varied from 68.1831 - 75.9759 mg/L in all samples.

Fig. 5 shows, the calcium concentration in the leaves of *Murraya koenigii* (L.) Sprenge is least for Bidar and is highest for Shahapur. Calcium is essential for all organisms, used in cell walls, bones. It helps in transporting of long chain fatty acids which helps in preventing high blood pressure, heart diseases, cardiovascular diseases, repair worn out cells, strong teethe in humans, building of RBCs and body mechanism. Therefore, Calcium has been extensively used for treatment of various diseases.

Potassium (K): The concentration of potassium (K) is found in all collected leaves of *Murraya koenigii* (L.) Sprenge medicinal plants and it is the second dominant essential macro nutrients. The presence of high amount of the K concentration in the leaves of *Murraya koenigii* (L.) Sprenge medicinal plants could be due to botanical structure as well as the mineral composition of the soil and also other factors like use of fertilizers, water irrigation and geological conditions of the region. The level of K is varied from 16.2793 - 17.5753 mg/L in all the samples collected from different places of North Karnataka region. The mineral concentration level is same as the Ca. *i.e.* mineral concentration is least for Bidar and highest for Shahapur can be seen in **Fig. 5**. K is essential to all organisms with the possible exception of blue green algae. It is a major cation and is important in nerve action. Potassium reduces blood pressure and it is moderately toxic to mammals when injected intravenously.

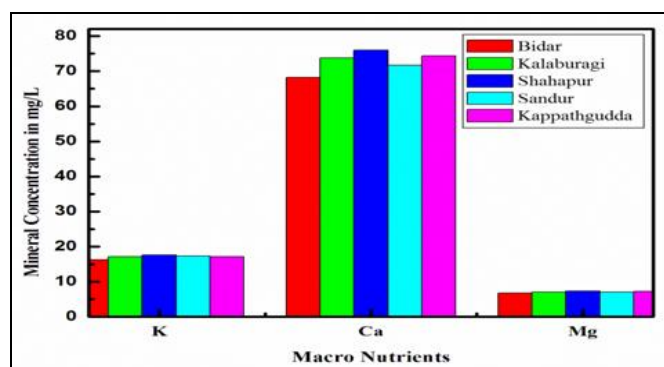


FIG. 5: COMPARATIVE STUDY OF ESSENTIAL MACRO NUTRIENTS IN THE LEAVES OF MURRAYA KOENIGII (L.)

Magnesium (Mg): The concentration of magnesium (Mg) is also found in all collected and it is the third dominant mineral. The level of Mg is varied from 6.8058 - 7.3278 mg/L in all samples collected from different places of North Karnataka region. Like Ca and K the mineral concentration level of Mg is almost same for the leaves collected from different places which can be seen in **Fig 5**. Magnesium works with calcium to help transmitting nerve

impulse in the brain. Magnesium has calming effect and works on the nervous system of those peoples, suffering from depression. In blood its quantity is 2 - 4 mg/100 ml. Magnesium has an important role in the phosphorylation reactions of glucose and its metabolism. Its deficiency has been implicated in insulin resistance, carbohydrate intolerance, dyslipidemia and complications of diabetes.

TABLE 2: ESSENTIAL MACRO NUTRIENTS CONCENTRATION (mg/L) OF *MURRAYA KOENIGII* (L.) SPRENGE LEAVES

Mineral	Bidar	Kalaburagi	Shahapur	Sandur	Kappathgudda
K	16.2793	17.1518	17.5753	17.3008	17.0827
Ca	68.1831	73.779	75.9759	71.6995	74.3512
Mg	6.8058	7.0280	7.3278	7.0660	7.2192

Essential Micro Nutrients: Zinc (Zn): The concentration of zinc is found in all the collected leaves of *Murraya koenigii* (L.) Spreng medicinal plants and their level is the range of 0.0744 - 0.2086 mg/L. The mineral nutrient concentration of Zinc is in very small amount. Several biological roles of zinc have been reported and over 200 proteins and enzymes contain zinc and produce important role in DNA synthesis, brain development, steroidogenesis, bone formation, wound healing.

Iron (Fe): Iron (Fe) is an essential mineral to prevent anemia and cough associated with angiotensin-converting enzyme (ACE) inhibitors. The mineral concentration of Iron (Fe) was highest in samples of Bidar followed by Kappathgudda, Sandur, and Kalaburagi and Shahapur in the range of 1.1948 - 8.8503 mg/L. For the formation of hemoglobin iron is necessary. For the transfer of oxygen, Fe is required in human body¹². Iron deficiency is the most prevalent nutritional deficiency in humans¹³.

Copper (Cu): The mineral concentration of copper is 0.0298 mg/L in leaves of *Murraya koenigii* (L.) Spreng medicinal plant sample of Sandur and for Kappathgudda it is 0.0527 mg/L. Copper (Cu) plays an important role in the treatment of chest wounds, to prevent inflammation in arthritis and similar diseases. It is required for some essential enzymes such as super oxide dismutase, cytochrome oxidase, lysyl oxidase, etc.

Excess consumption of copper results in dermatitis, metallic taste in the mouth, hair and skin discoloration etc. Copper play role in some

neurological conditions like Alzheimer's disease, Wilson's disease¹⁴.

Manganese (Mn): The mineral concentration of Mn is 0.1145 mg/L for leaves of *Murraya koenigii* (L.) Spreng medicinal plants sample of Sandur and 0.3240 mg/L in leaf sample of Bidar. Manganese (Mn) can help to assist the body in metabolizing protein and carbohydrates.

Molybdenum (Mo): The concentration of Molybdenum (Mo) is found in all the collected leaves of *Murraya koenigii* (L.) Spreng medicinal plants and it varied from 0.0049 - 0.3454 mg/L. Molybdenum is a rare mineral, but it is essential for human body for various metabolic processes.

The amount of Mo in the plant depends on the soil content in the growing area. Molybdenum is stored in the body, particularly in the liver, kidneys, glands, and bones. It is also found in the lungs, spleen, skin, and muscles. About 90% of the molybdenum eaten in foods is eliminated by the body through the urine.

Vanadium (V): The concentration of Vanadium (V) is found in all the collected leaves of *Murraya koenigii* (L.) Spreng medicinal plants and it varied from 0.4903 - 0.7333 mg/L. Vanadium affects carbohydrate metabolism including glucose transport, glycolysis, glucose oxidation, and glycogen synthesis¹⁴. At a dose of 100 mg/day vanadyl sulfate improves insulin sensitivity¹⁵. It's possible mechanism of action in glycemic control is thought to be primarily insulin mimetic with up regulation of insulin receptors.

Titanium (Ti): The concentration of Titanium (Ti) is found in all the collected leaves of *Murraya koenigii* (L.) Spreng medicinal plant and it varied

from 0.7563 - 2.5326 mg/L. Titanium is a physically promotive trace mineral. The function of Titanium is not known yet. It is harmless to our body.

TABLE 3: ESSENTIAL MICRO- NUTRIENTS CONCENTRATION (mg/L) OF MURRAYA KOENIGII (L.) SPRENGE LEAVES

Mineral	Bidar	Kalaburagi	Shahapur	Sandur	Kappathgudda
Ti	0.7984	1.7325	2.5326	1.5826	0.7563
V	0.5803	0.7094	0.5831	0.4903	0.7333
Mn	0.324	0.2419	0.284	0.1145	0.3217
Fe	8.8503	3.1464	1.1948	3.7004	6.111
Cu	0.0364	0.046	0.0919	0.0298	0.0527
Zn	0.1071	0.0744	0.2086	0.1192	0.1198
Mo	0.0049	0.1639	0.2702	0.3454	0.2918

Harmful Heavy Metals:

Cadmium (Cd): The concentration of cadmium is found in all the collected leaves of *Murraya koenigii* (L.) Spreng medicinal plants and their level is the range of 0.0040 - 0.0188mg/L. The mineral concentrations of leaves collected from different place of North Karnataka are very low and

are within the permissible limit set by World Health Organization. Cadmium is a non- essential harmful heavy metal which biochemically replaces zinc and causes high blood pressure. It also damages kidney and liver¹⁶ and causes a disease known as Itai-Itai.

TABLE 4: HARMFUL HEAVY METALS CONCENTRATION (mg/L) OF MURRAYA KOENIGII (L.) SPRENGE LEAVES

Mineral	Bidar	Kalaburagi	Shahapur	Sandur	Kappathgudda
Cd	0.0095	0.0188	0.0097	0.0044	0.0040
Al	0.5249	0	0	0.3495	1.7302

Aluminium (Al): The concentration of Aluminium is not found in all the leaves of *Murraya koenigii* (L.) Spreng medicinal plant. It presents in *Murraya koenigii* (L.) Spreng leaves collected from Bidar, Sandur and Kappathgudda in the range of 0.3495 - 1.7303mg/L. The concentration of aluminum is high for leaves collected from Kappathgudda and is totally absent in Kalaburagi and Shahapur. Aluminum is usually not harmful. Some studies show that aluminum may develop

Alzheimer's disease, but other studies have not found this to be true. In the present study 12 mineral nutrients viz. macro nutrients, micro nutrients and harmful heavy metal concentration were determined and found to be varies from place to place shown in **Fig. 5** and **Fig. 6**. These mineral nutrients help us to prevent and cure various diseases and are very essential for the human health.

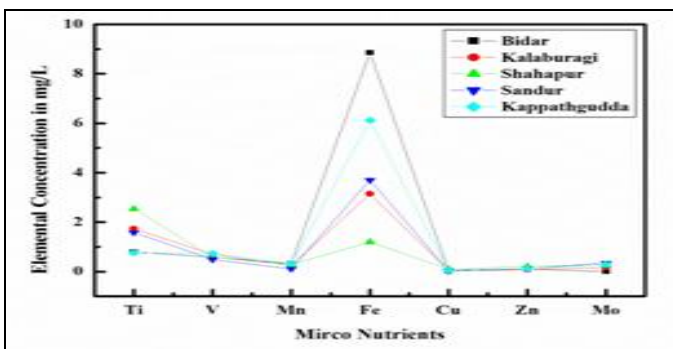
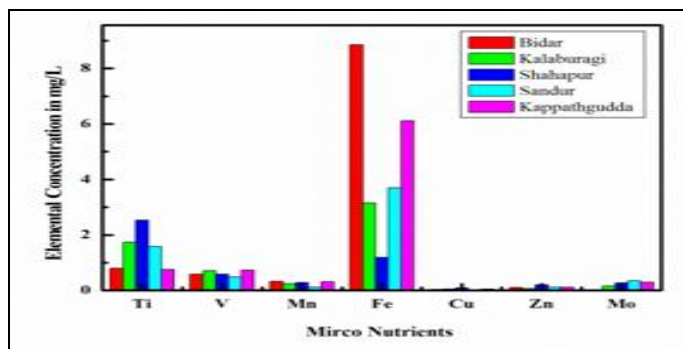


FIG. 6: COMPARATIVE STUDY OF ESSENTIAL MICRO NUTRIENTS IN THE LEAVES OF MURRAYA KOENIGII (L.)

CONCLUSION: The present study on estimation of macro and micro nutrients content in the leaves of *Murraya koenigii* (L.) Spreng reveals the presence of various mineral nutrients, but the concentration of the minerals was found to be

varies from place to place. This is attributed to the presence of the minerals of the soil, the different botanical structure of the medicinal plant or soil, environmental factors including atmosphere and pollution, season of collection sample, age of

indigenous medicinal plant and soil conditions in which plant grows.

From this study it is also verified that the leaves of *Murraya koenigii* (L.) Spreng medicinal plants contains concentration of micro nutrients viz. copper and zinc along with macro and other micro nutrients, which are required for the metabolism as per the recommendations of WHO^{16, 17, 18}. The data obtained in present study will be helpful in the synthesis of new modern drugs with various combinations of plants which can be used to cure many diseases. However, more detailed study of chemical composition of the indigenous medicinal plants is required and work is progressive in this direction.

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