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Determination of some elemental content in Fabaceae family Cancer medicinal plants from North- East Karnataka region using SEM- EDX and FAAS spectrometry

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KEYWORDS

Medicinal plants**,** Militia pinnata, Cassia tora, Elements, Energy dispersive X-ray and Flame atomic absorption, WHO permissible limit.

ABSTRACT

The study aimed to determine the elemental content of Militia pinnata and Cassia tora Fabaceae family medicinal plants, traditionally used in cancer management and their medicinal properties. The analysis used Scanning microscope-Energy dispersive X-ray (SEM-EDX) and Flame atomic absorption (FAAS) techniques. The total 16 elemental content like the C, O, Mg Al, Si, Mo, K Sb, Ca Ti, Cr, Mn, Fe, Co, Ni and Zn in the plants sample. The results showed trace elements concentration in plants, with major are Mg, K and Ca and minor are Mn, Fe, Cu, Zn and Mo elements, the concentration of Mg, K and Ca is highest, which is compared to Mn, Fe, Cu, Zn and Mo were determined in plants sample. These major and minor elements are within WHO ((World Health Organization) permissible limits.

1. Introduction

Plant and herbs used in the folk and traditional medicine have been accepted currently as one of the main source of chemoprevention drug discovery and development [1]. Around 60% of currently used anticancer agents are derived in one way or another from natural sources, including plants, marine source and micro organisms. Herbal medicines are plant derived materials or preparations, which contain raw or processed ingredient from one or more plants with therapeutic value and used as dietary supplements to fight or prevent common diseases in various systems of medicine such as Ayurveda, Unani and Siddha. Plant products have been used throughout human history for various purposes including medicine[2]. Herbs can be viewed as biosynthetic chemical laboratories, producing a number of chemical compounds. Herbal drugs ranges from parts of plants to isolated purified active constituents. They may come from mostly leaves, roots, barks, seeds and flower. They are eaten, swallowed, drunk, inhaled or applied to the skin. Several secondary metabolites are produced by the higher plants as natural defence against disease and infections. The Indian system of medicine known as Ayurveda uses mainly plant based drug or formulation to treat various diseases including cancer: and around. Approximately 877 small molecule drugs are introduced worldwide between 1981- 2002, 61% can be traced back to their origin in natural products. Recent surveys suggest that one in three persons use medicinal natural products daily and possibly one in two cancer patients use them well[3].Pongamia pinnata (Linn) Pierre is medium sized glabraous tree popularly known as Karanja in Hindi, Indian beech in English and Pongam in Tamil1. Most of the Tamil Nadu physicians of Indian system of traditional medicine Ayurveda and siddha use Pongamia pinnata treat various kinds of diseases including diabetes mellitus [6]. Historically pongamia has been used folk medicinal plant, particularly in Ayurveda and siddha system tumours, piles, skin diseases, itches, painful rheumatic joints wounds, ulcers, diarrhoea etc. It is well of Indian medicine [7].

Cassia tora (Leguminosae) is a wild crop and grow in most parts of India as a weed. According to Ayurveda, the leaves and seeds are acrid, laxative, anthelmintic, ophthalmic, liver tonic, cardio tonic and expectorant[8]. The leaves and seeds are also useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, and cardiac disorders [9 10].

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The Thermo Scientific™ iCE™ 3000 Series Flame atomic absorption FAAS enables simple measurement of elements in a wide range of samples. The flame atomizer is ideal for the measurement of high concentration samples, whilst the furnace atomizer achieves sub ppm detection. The dual atomizer system with Stockdale optics incorporates advanced background correction with a unique quad line deuterium source and optional Zeeman correction to ensure accurate analysis[11]. Energy dispersive X-ray (EDX) analysis is a micro analytical technique for the quantitative analysis of elements that uses a characteristic spectrum of X-rays. In principle, when an electron beam impinges on a sample it ejects the secondary electrons from an inner shell and the vacancy generated gets filled up by the higher shell electrons. The excess energy is released in the form of X-rays which are not only characteristic of the element but also depend on the shell from which electrons move into the holes [12].

2. Material and methods

Sample collection

Samples of Militia pinnata and Cassia tora their parts like (seed and leaves) were collected from different three districts including Bidar, Raichur and Bijayapura of North- East Karnataka region. The sample collected map as shown in figure 1 below and details and images of medicinal plants as shown in table 1 and figure 2. Plant samples, were first washed several times with distil water in order to eliminate soil particles and dust, and after ground.

Table.1 Details of cancer cure medicinal plants

SL.NO	Botanical Name	Local Name	Common Name	Sample code	Family	Parts used	Medicinal uses
1	Militia pinnata	Honge mara	karanja	HOA1	Fabaceae	Seed	Tumors,
2	Cassia tora	Chagache	Sickle senna	CHE2	Fabaceae	leaves	Human cervical cancer cells

Study area

The North-East Karnataka region covers about 30% of the Karnataka and 50% of the North-Karnataka region a large number of different kinds of ores or mining's factories are running in this region which falls under Manganese, Iron, Gold ores and Uranium ores/mining's, along with cement factories and thermal power plant are ongoing in this region. The study includes latitudes range from 17'. 27 º -13'.83 º North and longitudes range from 77º 44'– 77 º 25' East respectively, which spans a geographical area of 44138 Sq. Km which covers three districts. In the present study only three distracts likes Bidar, Raichur and Bijayapura covered. Here Bidar fall in the Deccan plateau region, but the Raichur district doesn't fall in this region than the soil type of Bidar is red and Bijayapura black, soil type is black and medium black, similarly, in Raichur district Introduction 11 soil type is pink, red and black soils, such a type of soils are naturally observed in three regions.

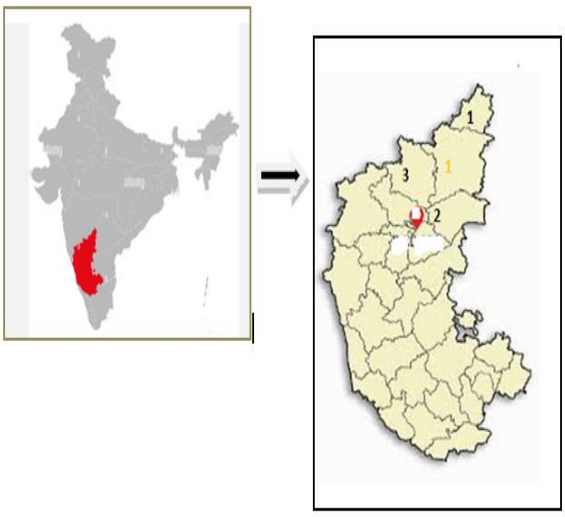

Figure. 1 Sample collected map: 1. Bidar; 2.Raichur; 3. Bijapur.

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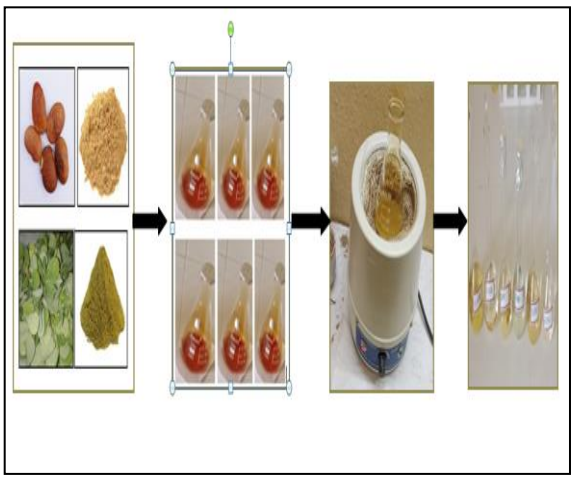
Figure 2. Images of Fabaceae family medicinal plants: 1. Militia pinnata seed; 2. Cassia tora leaves.

Sample preparation and Elemental analysis

Flame atomic absorption (FAAS) technique

The collected leaves and seeds were washed in tap water and rinsed thoroughly with double distilled water in order to remove contamination and then dried in shade laboratory at room temperature about 35 days and subsequently powdered by using Electrical Grinder. A quantity of 250 grams of each powder sample was weighed.

0.20g of each of the powdered plant seeds and leaves samples digested in 7 ml of acid solution (HNO_3, H_2SO_4, and HClO4). The corresponding solution was heated until white fumes appeared. The clear solution was diluted upto 100 ml with double distilled water and filtered with Whatman filter paper no.42, for the elemental analysis using FAAS Technique.



Sample preparation procedure for FAAS

Scanning microscope- energy dispersive X-ray (SEM-EDX)

For EDX Technique the dried 10mg leaves fine powder sample was taken and prepared a pallet of 1 cm^2 disks, and the powder sample was coated with 15 nm thick gold layers for contact purposes and these samples were kept for about 30 min one at a time and subjected for elemental analysis.

3. Result and Discussion

Flame Atomic Absorption Spectroscopy (FAAS) and energy dispersive X-ray (**EDX**) techniques are a very common technique for detecting elements present in solution samples. The concentrations of various elements present in the Fabaceae family medicinal plants at different places of Bidar, Raichur and Bijayapura districts. The medicinal plant leaves and seed samples were subjected to the analysis of different elemental concentrations and its differential level analyzed by using table form. The first rows gives place of plant collected, second rows gives botanical code of medicinal plant and the rest of the rows gives the elemental concentrations expressed in ppm and weight % is equal to ppm are shown in table 2 table 3.The elements like **C**, **O,** Mg Al, Si, Mo, K Sb, Ca Ti, Cr, Mn, Fe, Co, Ni and Zn.

Table. **2** The concentrations of Elements (ppm) in Fabaceae medicinal plants using FAAS.

Elements	B1HOA	B1`CHE	RHOA	RCHE	B2HOA	B2CHE
Mg	8.57	5.96	4.03	13.95	7.7	7.11
Al	0.35	0.05	0.17	0.17	0.60	0.34
Si	3.23	4.41	6.58	5.44	4.93	3.63
Mo	0.47	0.57	0.51	0.52	0.51	0.51
K	3.12	1.86	1.98	1.78	3.12	2.09
Sb	0.06	0.05	0.18	0.05	0.40	0.02
Ca	5.270	6.054	5.337	2.037	6.424	8.927
Ti	0.01	0.01	0.01	0.01	0.01	0.05
Cr	0.01	0.01	0.03	0.02	0.11	0.09
Mn	2.10	0.81	0.13	0.26	0.94	0.99
Fe	0.18	0.21	0.22	0.22	0.21	0.20
Co	0.02	0.06	0.08	0.01	0.13	0.00
Ni	0.03	0.07	0.15	0.03	0.05	0.03
Zn	0.28	0.34	0.21	0.26	0.22	0.22

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Table3. The concentrations of Elements (wt %) in Fabaceae medicinal plants using EDX.

Elements	B1HOA	B1`CHE	RHOA	RCHE	B2HOA	B2CHE
C	75.94	57.46	75.72	51.00	75.13	63.22
O	22.44	35.98	20.29	41.91	21.80	35.35
Mg	0.13	0.18	0.32	0.19	0.04	0.08
Al	0.04	0.04	0.04	0.03	0.08	0.09
Si	0.10	0.01	0.03	0.01	0.01	0.01
Mo	0.18	0.68	0.42	0.29	0.24	0.36
K	0.14	0.50	0.70	0.90	0.24	0.34
Sb	0.06	0.05	0.18	0.05	0.40	0.02
Ca	0.59	4.63	0.29	4.86	0.21	0.69
Ti	0.00	0.00	0.08	0.06	0.28	0.00
Cr	0.08	0.03	0.02	0.11	0.05	0.01
Mn	0.02	0.02	0.20	0.23	0.13	0.25
Fe	0.10	0.31	0.33	0.23	0.13	0.32
Co	0.02	0.06	0.08	0.01	0.13	0.00
Ni	0.02	0.06	0.08	0.01	0.13	0.00
Zn	0.02	0.06	0.08	0.01	0.13	0.00

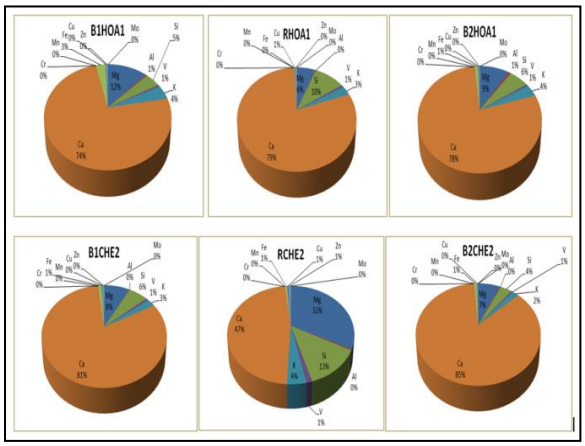

Figure. 3 Average elemental concentrations in Fabaceae family plants in three districts plant Samples.

The pie chart figure 3 and (Table 2) is measured average elemental concentrations in Fabaceae family plants. Therefore the Bidar , Raichur and Bijayapura plant leaf and seed contain total average of elements at 100 appreciable range and the B2CHE plant contain Calcium (Ca) is high compare to the Potassium (K) Magnesium (Mg) and all other elements. The Bidar samples like B1HOA1 and B1CHE2 are regulating the variation trend is Ca>K>Mg range. The net percentage variations of major and minor elements are found in differential form of Raichur (RCHE2) medicinal plant and similarity in Bidar and Bijayapura region medicinal plants. In Table 2 and table 3 are shows the concentrations of Elements (ppm and weight %) different organs of medicinal plants of species determined by FAAS and EDX methods. The analysis of various elemental concentrations in the two same families sampled Ayurvedic medicinal plants indicated that the Mg Al, Si, Mo, K Sb, Ca Ti, Cr, Mn, Fe, Co, Ni and Zn were present in all samples which are responsible for curing cancer diseases.

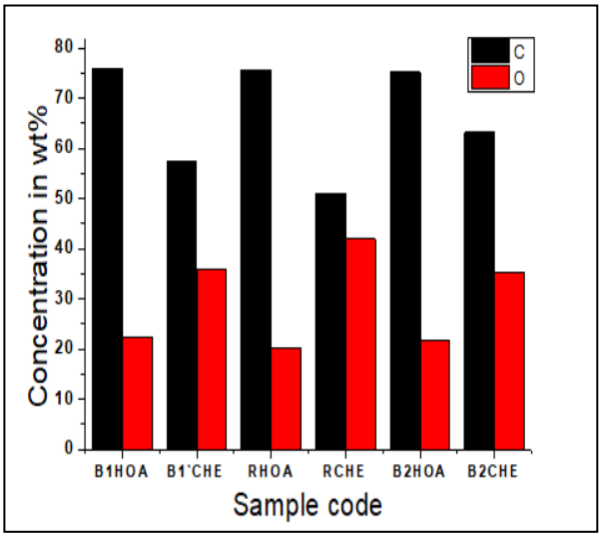

Figure.4 variations of Cancer Fabaceae family Medicinal plants.

The figure 4 shows that the average elemental variations of both Carbon (51.00 wt% 75.94 wt %) and Oxygen (20.29 wt% to 35.98wt %) in two plant samples Millettia pinnata (HOA), Cassia tora (CHE), Fabaceae family medicinal plants. Further, the majority of Carbon (75.94 and to75.13 wt %) element is found to be in Bidar and Bijayapura Millettia pinnata (HOA), seed sample as compare to the other leaf samples. The reaming elemental content was found in the average range of 2 Fabaceae family plants. The figure 4 it is noticed that the maximum abundance of Carbon elements in Bidar (B1HOA), Raichur (RHOA) and Bijayapura (B2HOA) medicinal plants as compare to Bidar (B1CHE), Raichur(RCHE) and Bijayapura (B2CHE) medicinal plants but the Oxygen elements observed comparatively high in Bidar and Raichur as Bijayapura medicinal plant leaves and seed samples.

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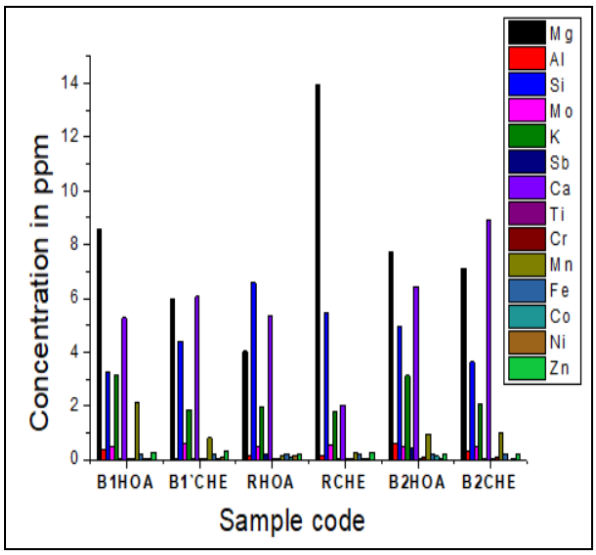

Figure.5 FAAS: variations of trace elemental content in Medicinal plants

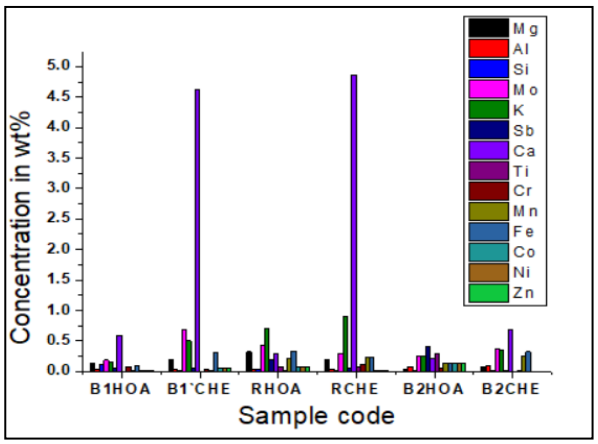

Figure.6 EDX**:** variations of trace elemental content in Medicinal plants

The data indicates that the major elements such as Magnesium (Mg), Potassium (K) and Calcium (Ca). Similarly minor or trace elements such as Aluminium (Al), Silicon (Si), Chromium (Cr), Manganese (Mn), Iron (Fe), Copper (Cu) and Zinc (Zn) and Molybdenum (Mo) contents. The major element concentration is found to be more than the minor or trace elemental concentration. The regional variations of elemental concentration in two Fabaceae family medicinal plants are shown in figure 6 in the form of column. The first abundance of elements like Potassium (K) is1.78 to 3.12 ppm and Calcium (Ca) is 2.037 to 8.927 ppm and second abundance of elements are Magnesium (Mg) and Aluminium (Al) content high in Bidar plants and in Raichur first set of high elements like Potassium (K) 1.78 to 3.12ppm and Calcium 2.037 to 6.054ppm and other elements are Magnesium (Mg) and Aluminium (Al) but in Bidar Calcium (Ca) is 6.054ppm fund to be high content as compare to Raichur and Bijayapura samples and Magnesium (Mg), Aluminium (A) Silicon (Si) and Potassium (K) elements are shows less variations in studied medicinal plants and the reaming elements are detected in appreciable limits in BHOA,BCHE, RHOA1, RCHE, B2HOA and B2CHE samples. Figure 6 in the form of column the first highest of elements like Potassium (K) is 0.14 weight % to 0.90 weight % in RCHE and Calcium (Ca) is 0.29 weight % to 4.63 weight % in B1CHE.Other elements are shows less variations in studied**.**

4. Conclusion

The present investigation provides the information on the elemental concentration of traditionally used ayurvedic medicinal plants in North East-Karnataka regions of Bidar, Raichur and Bijayapura districts. Total two Fabaceae families of medicinal plants collected and examined by using EDX and FAAS. And experimentally compared with FAAS and EDX techniques are measured in ppm and weight %. The all major elemental concentrations comparably high in two Fabaceae family medicinal plant seed and leaf samples as such that the minor and trace elements. Moreover, the present analysis represents that all detected elements are shown within the permissible limits of WHO/FAO and some other standard permissible limits of medicinal plants. Fabaceae family medicinal plants results concluded that the Militia pinnata seed Cassia tora leaves are showing a low toxicity level with approximate weight percent of 16 elementary namely **C**, **O,** Mg Al, Si, Mo, K Sb, Ca Ti, Cr, Mn, Fe, Co, Ni and Zn respectively. According to the Ayurveda, these are highly anticancer; antioxidant and antibiotic ayurvedic medicinal plants. In present analysis Carbon and Oxygen concentration were sufficiently high in above cited four medicinal plants.

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