

## Essential and Non-Essential Elemental Composition of Five Different Major Parts of *Butea monosperma* (Palash) by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES)

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### Abstract

*Butea monosperma* (BM) (lim.) Kuntz also known as flame of forest is being used in traditional medicines to treat so many disease conditions. Preliminary phytochemical screening of this plant revealed the following-Alkaloids, cynogenic glycosides, phenolic compounds, flavonoids, terpenoids, tannins and saponins. It contains butrin, isobutrin, butin, palasitricin, and butein. The present study aimed at investigating the metal composition of five major parts of BM using a highly sensitive Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES). Eighteen essential mineral elements viz Ag, Ba, Be, Bi, B, Ca, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Se, Sr, V and Zn and three non-essential elements (As, Cd, Pb) were determined for the first time in *B. monosperma*. Wet digestion method using a mixture of 2 ml of concentrated HNO<sub>3</sub>:HClO<sub>4</sub> (4:1 v/v), 1 ml H<sub>2</sub>O<sub>2</sub> and 1ml milliQ water was used for digestion of the samples using microwave in a Microwave Digestion System (MDS) and analyzed by ICP-OES. The concentration of toxic elements viz As and Pb detected were found below the FAO/WHO maximum permissible limit; hence they are safe for human consumption. This study provides information on the nutritive value of *B. monosperma* which indicates that it is good for health and also serves as a good source of essential nutrients and has low levels of toxic elements.

**Keywords:** *B. monosperma*; ICP-OES; Essential elements; Nutritional value; Wet digestion

### Introduction

Medicinal plants are the richest bio-resource of drugs for traditional medicines, modern medicines, nutraceuticals as well as chemical entities for synthetic drugs [1]. Pollution of foods by heavy metal in living tissues through food chain is a worldwide phenomenon. Studies have revealed that fruits and leafy vegetables are vulnerable to heavy metal contamination

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from soil, wastewater and air pollution [2,3] and as such serve as the pathways by which heavy metals enter the human tissues leading to deterioration of health [4]. *Butea monosperma* (family Fabaceae) commonly called Palash and "Flame of the forest" is reported in various ancient literatures to have medicinal potential. The plant has proven efficacy for antioxidant [5] anti-obesity [6] anticancer, and chemoprotective [7] potential. It is also useful against neuropathic [8] and peripheral pain. The plant is also known for hepatoprotective and antidiabetic potential [9-11]. However, all parts of BM have been reported for its biological functions' accounting for its extensively used in Ayurveda, Unani and Homeopathic medicine. It has become a cynosure of modern medicine. The usefulness of various parts of BM has been reported viz roots are useful in filariasis, night blindness, impotence, in snake bites, helminthiasis, piles, ulcer and tumours. It is reported to possess anti-fertility, aphrodisiac and analgesic activities.

Flowers are useful in diarrhoea, astringent, diuretic, depurative, tonic, leprosy, skin diseases, gout, thirst, burning sensation. The stem bark is useful in indigenous medicine as treatment for dyspepsia, diarrhoea, dysentery, ulcer, sore throat and snake bite. Besides medicinal uses it is also having the economic use such as leaves are used for making platters, cups and bowls. Bark fibres are used for making cordage. Seeds of BM are used in inflammation, skin and eye diseases, bleeding piles, urinary stones, abdominal troubles, intestinal worms and tumour. When seeds are pounded with lemon juice and applied to the skin, they act as a rubefacient [12-14].

Many analytical methods are available for the determination of trace elements in plant material such as AAS (Atomic Absorption Spectroscopy) but these methods require the decomposition of the sample which is time consuming and at the same time exposes the sample to contaminations by microbes [15,16]. Sample preparation is a critical step for the analysis of metals due to presence of different type of matrices but with the advancement of technology such as MDS, digestion is now rapid and is an efficient method for sample digestion prior to the determination of trace metals [17,18]. Moreover, ICP-OES is one of the most accurate and latest analytical techniques for the determination of trace elements in numerous sample types. However, most studies on *B. monosperma* centered more on their medicinal values with little or no attention on their metal composition which invariably contributes to their known pharmacological actions. Before the commencement of this work, there are no literature reports on the metal composition of BM in spite of its extensive use. Hence, the main objective of this study was to comparatively evaluate the levels of essential (Ag, B, Ba, Be, Bi, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Se, Sr, V and Zn) and non-essential elements (As, Cd, Pb) of five major parts of *B. monosperma* using ICP-OES with the help of MDS.

## **Materials and Methods**

### **Reagents**

The standards for ICP-OES were prepared from stock solutions of studied elements at 1000 mg/L concentration obtained from Perkin Elmer (USA). All other reagents and solvents used in this study were of analytical grade obtained from Fischer Scientific (USA). Double distilled water was used throughout the experiments. To minimize the risk of contamination, 6.0 M HNO<sub>3</sub> was used to clean plastic bottles and glassware and rinsed with double distilled water prior to use. Milli-Q water was used in preparation of sample and standard solutions.

### **Collection of plant material, identification, processing and extraction**

The leaves, roots, seeds, flowers and stem bark of *B. monosperma* were collected from a small bush near Era Lucknow Medical College and Hospital (ELMCH) India and following prior identification. Afterwards, the stem bark, leaves, flowers, seeds and roots were cleaned, dried in a hot air oven (50°C), powdered using a food processor (Kenwoo True Compac Blender), passed through 60 mesh sieve (BS) and stored in a labeled airtight container at 4°C till further use.

### **Determination of total ash value**

Pre-cleaned silica crucible was heated at 600°C until the weight of the crucible became constant. Five-gram powdered plant material was taken in the silica crucible and heated in a muffle furnace at 400°C till there was no evolution of smoke. The crucible was cooled at room temperature in a desiccators and ash was moistened with concentrated H<sub>2</sub>SO<sub>4</sub> (0.5 ml). Crucible was placed on hot plate and heated until fumes of H<sub>2</sub>SO<sub>4</sub> ceased to evolve. The crucible with sulphated ash was then heated in a muffle furnace at 600°C till the weight of the content became constant [19,20].

### **Physico-chemical analysis**

The physico-chemical analysis includes number of parameters such as physical state, colour, taste, percentage loss on drying as per standard methods adopted by Gupta [19,20] and Indian Pharmacopoeia [21], ash content as per methods adopted by Indrayan et al. [22] and Gupta [19,20], ash value as per method followed by Ahmed and Sharma [23], pH value were measured by pH meter [24,25]. Proximate analysis: Chemical compositions of the plants parts were determined using the AOAC methods [26]. Moisture (method 14:004), total ash (method 14:006), crude fiber (method 14.020), total fat (method 7.056) and protein (method 2.057) were assayed and carbohydrate was obtained by difference.

### **Elemental analysis (Microwave digestion)**

The microwave-assisted closed vessel digestion technique was used for digestion of the samples due to its superior digestion capability and sample throughput. Digestions were performed using MDS (Multiwave 3000, Anton Paar, Perkin Elmer) with the Rotor 16HF100 (100 ml PFA vessels, 40 bar) and Pressure, Temperature (P/T) sensor. To ensure improved precision, three sub-samples (fruit, flower, leave, bark and seed) were digested. 0.1 g samples were weighed into ceramic easy prep vessels, to which, 2.0 mL HNO<sub>3</sub>:HClO<sub>4</sub>, 1.0 ml of H<sub>2</sub>O<sub>2</sub> and 1.0 ml of H<sub>2</sub>O was added and allowed to pre-digest for 1 hr before sealing. For all the samples, the microwave power was ramped to 500 W for the first 15 min, where it remained for the next 15 min, then ramped to 650 W for 15 min during which complete digestion occurred. The microwave power was reduced and the bombs cooled by forced ventilation for 15 min. sample digest were poured into 20 ml polyethylene bottles for elemental analysis made up to the mark with double distilled water. All samples were analyzed for As, Ag, Ba, Be, Bi, B, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Se, Sr, V and Zn by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). The samples were analyzed in three replicates of selected plant part (TABLE 1).

**ICP-OES analysis:** The calibration standards for ICP-OES were prepared by diluting the stock standard solution (1000 mg/L) in 0.2% (v/v) nitric acid. Working solutions were prepared from the stock as necessary. The calibration curves for all the studied elements were prepared by different concentrations of standards in the range 0.005 mg/L to 1.0 mg/L from working solution (FIG. 1). The above clear solution obtained after microwave digestion was analyzed by ICP-OES (Optima 8000, Perkin Elmer) for elemental analysis with following operational conditions. The analytical wavelengths were selected based on minimum spectral interferences and maximum analytical performance. TABLE 2 shows the wavelengths chosen for

each of the elements analyzed by ICP in this study. Initially the 3 most sensitive lines were chosen. From these lines, the lines with no interfering elements were chosen.

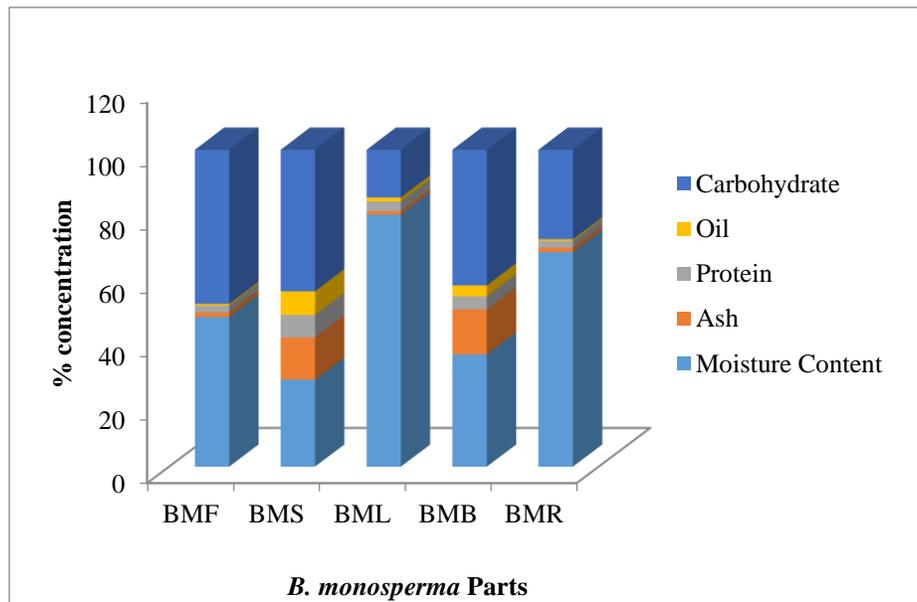


FIG. 1. Percentage nutritional composition of *B. monosperma* parts (per 100 g of dry extract).

TABLE 1. Emission lines for the selected elements.

Elements	Symbols	Emission line (nm)	DL*/ppb
Silver	Ag	328.068	0.20
Arsenic	As	193.696	0.80
Boron	B	249.677	0.20
Barium	Ba	233.527	0.03
Beryllium	Be	313.107	0.02
Bismuth	Bi	223.061	0.03
Calcium	Ca	317.933	0.04
Cadmium	Cd	228.802	0.07
Cobalt	Co	228.616	0.07
Chromium	Cr	267.716	0.25
Copper	Cu	327.393	0.25
Iron	Fe	238.204	0.20
Magnesium	Mg	285.213	0.04
Manganese	Mn	257.610	0.03
Molybdenum	Mo	202.031	0.20
Nickel	Ni	231.604	0.37
Lead	Pb	220.353	1.40
Selenium	Se	196.026	0.20
Strontium	Sr	407.771	0.07
Vanadium	V	292.464	0.07
Zinc	Zn	206.200	0.20

\*DL: Instrument Detection Limit

## Results

The result of the study showed that the leave has more moisture content followed by the root while the % ash was more in the bark than other parts of BM (FIG. 1). TABLE 2 is the result of the proximate analysis of the BM and the result shows that the seed has 100% loss on drying 1ml of the sample. Concentration of minerals was found to differ in different parts of BM viz BMR has high concentration of Ba ( $222.42 \pm 0.42$ ), Ca ( $1404.12 \pm 22.20$ ), Mg ( $4286.88 \pm 113.40$ ), and Sr ( $222.12 \pm 99.48$ ) compared to other parts of BM while BMS has higher concentration of Cu ( $20.16 \pm 0.01$ ), Fe ( $557.74 \pm 4.30$ ). BML was found to contain least concentration of Zn ( $8.72 \pm 3.12$ ) and Ca ( $297.60 \pm 18.48$ ) but high in B ( $26.30 \pm 0.10$ ). Mo ( $20.23 \pm 0.05$ ) was found to be high in BMB. In all the BM parts analyzed, Cd, Co and Se were below detectable limit (TABLES 3 and 4). FIG. 2 is the figure showing the distribution of the five major minerals in BM. TABLES 5-11 is the table of the estimated % contribution to recommended dietary allowance (RDA) for some of the minerals.

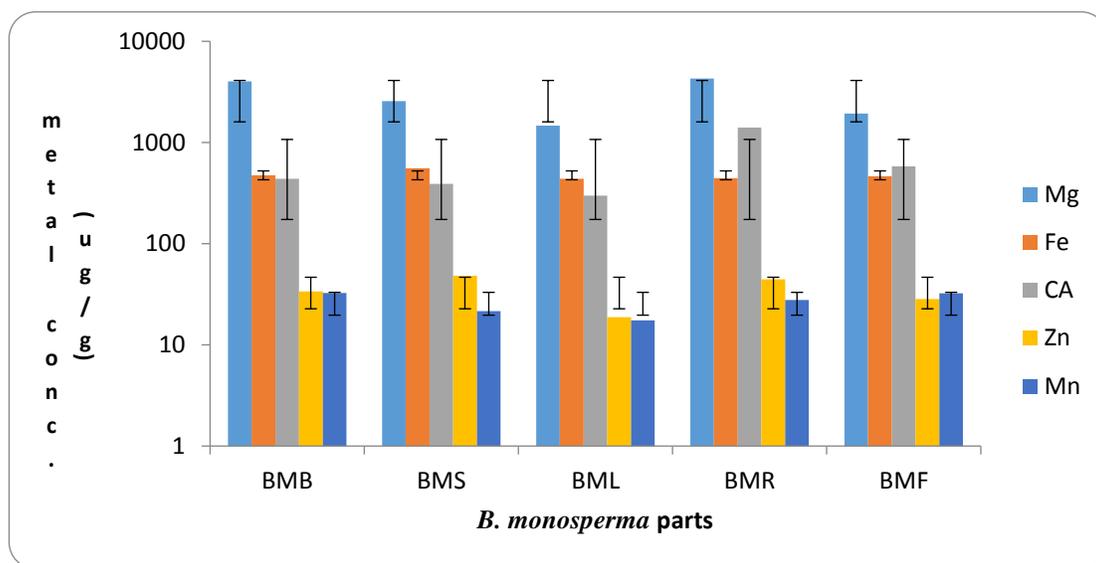


FIG. 2. Concentration of five major elements in different parts of *B. monosperma*.

TABLE 2. Proximate analysis of different parts of BM.

Physicochemical properties	Plant parts				
	BMF	BMS	BML	BMB	BMR
Physical state	Fine powder				
Colour	Yellowish	Whitish	Green	Ash	Brown
Taste	Pungent	No taste	pungent	No taste	Pungent
% loss on drying (1ml)	97.6	100	97.4	97.2	97.3
% Ash content	11.2	9.95	13.6	10.3	7.70
% Ash value (water)					
Sol.	41	40	56	33	44
Insol.	59	60	44	57	56
pH					
Sol.	71	79	63	56	58
Insol.	29	21	37	44	42

TABLE 3. Concentration (mg/100 g) of different minerals in BM parts.

Elements	BM PARTS				
	BMB	BMS	BML	BMR	BMF
Ag	ND	0.12 ± 0.0	0.18 ± 0.006	0.12 ± 0.0	0.12 ± 0.0
As	16.71 ± 0.51	13.90 ± 0.44	11.25 ± 0.15	17.34 ± 0.78	13.62 ± 0.06
B	16.55 ± 0.25	26.30 ± 0.10	20.14 ± 1.06	19.62 ± 1.86	19.62 ± 1.50
Ba	55.57 ± 1.63	54.60 ± 1.44	54.24 ± 1.44	222.42 ± 0.42	84.84 ± 0.60
Be	ND	ND	ND	ND	ND
Bi	2.73 ± 0.03	2.40 ± 0.36	2.52 ± 0.24	2.16 ± 0.12	2.34 ± 0.06
Ca	437.88 ± 1.34	391.32 ± 2.16	297.60 ± 18.48	1404.12 ± 22.20	581.46 ± 25.98
Cd	ND	ND	ND	ND	ND
Co	ND	ND	ND	ND	ND
Cr	8.42 ± 0.22	7.08 ± 0.36	9.60 ± 0.12	9.60 ± 0.48	10.38 ± 0.66
Cu	6.71 ± 0.01	20.16 ± 0.01	9.36 ± 0.24	7.32 ± 0.36	10.02 ± 1.26
Fe	474.19 ± 9.93	557.74 ± 4.30	438.89 ± 2.17	444.10 ± 2.06	465.64 ± 11.02
Mg	4019.20 ± 15.09	2555.88 ± 42.00	1463.28 ± 52.20	4286.88 ± 113.40	1938.48 ± 165.00
Mn	32.64 ± 0.24	21.54 ± 0.06	17.46 ± 0.18	27.84 ± 1.08	32.28 ± 0.96
Mo	20.23 ± 0.05	2.76 ± 0.12	1.26 ± 0.30	4.92 ± 0.12	1.50 ± 0.18
Ni	6.24 ± 0.00	12.18 ± 0.66	7.14 ± 0.18	7.98 ± 0.30	7.92 ± 0.72
Pb	3.15 ± 0.15	3.60 ± 0.48	4.32 ± 0.12	5.04 ± 0.48	6.48 ± 0.01
Se	ND	ND	ND	ND	ND
Sr	7.34 ± 0.26	10.02 ± 0.18	16.32 ± 1.08	222.12 ± 99.48	23.52 ± 1.08
V	0.87 ± 0.05	0.71 ± 0.03	0.45 ± 0.03	1.02 ± 0.03	0.90 ± 0.60
Zn	33.83 ± 0.01	48.04 ± 0.80	8.72 ± 3.12	44.40 ± 0.84	28.38 ± 1.02

TABLE 4. Comparison of measured and certified values (mean ± SD, n=3) in the CRM (white clover powder) (ERA A Waters Company).

Elements	Certified mg kg <sup>-1</sup>	Measured µg g <sup>-1</sup> in BM parts				
		BMB	BMS	BML	BMR	BMF
As	0.093 ± 0.010	16.71 ± 0.51	13.90 ± 0.44	11.25 ± 0.15	17.34 ± 0.78	13.62 ± 0.06
Co	0.178 ± 0.08	ND	ND	ND	ND	ND
Fe	244	474.195 ± 9.925	557.741 ± 4.295	438.89 ± 2.17	444.1 ± 2.06	465.64 ± 11.02
Ni	8.25	6.24 ± 0.00	12.18 ± 0.66	7.14 ± 0.18	7.98 ± 0.30	7.92 ± 0.72
Zn	25.2	33.83 ± 0.01	48.04 ± 0.80	8.72 ± 3.12	44.40 ± 0.84	28.38 ± 1.02
Se	6.70 ± 0.25	ND	ND	ND	ND	ND

Based on dry mass, \*\*Mean ± S.D, at 95% confidence interval, n=3  
 BMF: *B. monosperma* Flower; BMS: *B. monosperma* Seed; BML: *B. monosperma* Leaf; BMB: *B. monosperma* Bark BMR: *B. monosperma* Root

**TABLE 5. Dietary Reference Intake (DRI): Recommended Dietary allowance (RDA) and Tolerable Upper Intake Level (UL) of elements, compared to the average concentration of elements obtained from BMF.**

Elements	Average concentration (µg/g)	Average concentration (mg/10 g)	DRI (mg/day)		Estimated contribution to RDA (%)
			RDA	UL	
Arsenic	0.12 ± 0.0	0.0012	ND	ND	ND
Silver	13.62 ± 0.06	0.1362	ND	ND	ND
Barium	19.62 ± 1.50	0.1962	ND	ND	ND
Boron	84.84 ± 0.60	0.8484	ND	20	ND
Beryllium	ND	ND	ND	ND	ND
Bismuth	2.34 ± 0.06	0.0234	ND	ND	ND
Calcium	581.46 ± 25.98	5.8146	1000-1300	2500	0.45-0.58
Cadmium	ND	ND	ND	ND	ND
Cobalt	ND	ND	ND	ND	ND
Chromium	10.38 ± 0.66	0.1038	0.024-0.035	ND	296.57-432.50
Copper	10.02 ± 1.26	0.1002	0.9	8.0	11.13
Iron	465.64 ± 11.02	4.6564	8-15	45.0	31.04-58.21
Magnesium	1938.48 ± 165.00	19.3848	310-320	350	6.06-6.25
Manganese	32.28 ± 0.96	0.3228	1.6-3.0	9.0	10.76-20.18
Molybdenum	1.50 ± 0.18	0.0150	0.045	2.00	33.33
Nickel	7.92 ± 0.72	0.0792	ND	1.0	ND
Lead	6.48 ± 0.01	0.6480	ND	ND	ND
Selenium	ND	ND	0.055	0.40	ND
Strontium	23.52 ± 1.08	0.2352	ND	ND	ND
Vanadium	0.90 ± 0.60	0.0090	ND	ND	ND
Zn	28.38 ± 1.02	0.2838	8-11	34.0	2.58-3.55

ND: Not Determined; BMF: *B. monosperma* Flower. Source: FAO/WHO (Codex Alimentarius Commission).; WHO, Annette Dickinson. Council for Responsible Nutrition, 2002

**TABLE 6. Dietary Reference Intake (DRI): Recommended Dietary allowance (RDA) and Tolerable Upper Intake Level (UL) of elements, compared to the average concentration of elements obtained from BMS.**

Elements	Average concentration (µg/g)	Average concentration (mg/10 g)	DRI (mg/day)		Estimated contribution to RDA (%)
			RDA	UL	
Arsenic	0.12 ± 0.0	0.0012	ND	ND	ND
Silver	13.90 ± 0.44	0.1390	ND	ND	ND
Barium	26.30 ± 0.10	0.2630	ND	ND	ND
Boron	54.60 ± 1.44	0.5460	ND	20	ND
Beryllium	ND	ND	ND	ND	ND
Bismuth	2.40 ± 0.36	0.0240	ND	ND	ND
Calcium	391.32 ± 2.16	3.9132	1000-1300	2500	0.30-0.39
Cadmium	ND	ND	ND	ND	ND
Cobalt	ND	ND	ND	ND	ND

Chromium	7.08 ± 0.36	0.0708	0.024-0.035	ND	202.29-295.00
Copper	20.16 ± 0.01	0.2016	0.9	8.0	22.4
Iron	557.74 ± 4.30	5.5774	8-15	45.0	37.18-69.72
Magnesium	2555.88 ± 42.00	25.5588	310-320	350	7.99-8.24
Manganese	21.54 ± 0.06	0.2154	1.6-3.0	9.0	7.18-13.46
Molybdenum	2.76 ± 0.12	0.0276	0.045	2.00	61.33
Nickel	12.18 ± 0.66	0.1218	ND	1.0	ND
Lead	3.60 ± 0.48	0.0360	ND	ND	ND
Selenium	ND	ND	0.055	0.40	ND
Strontium	10.02 ± 0.18	0.1002	ND	ND	ND
Vanadium	0.71 ± 0.03	0.0071	ND	ND	ND
Zn	48.04 ± 0.80	0.4804	8-11	34.0	4.37-6.01

ND: Not Determined; BMS: *B. monosperma* Seed. Source: FAO/WHO (Codex Alimentarius Commission). (2001) WHO (1998), Annette Dickinson. Council for Responsible Nutrition, 2002

**TABLE 7. Dietary Reference Intake (DRI): Recommended Dietary allowance (RDA) and Tolerable Upper Intake Level (UL) of elements, compared to the average concentration of elements obtained from BML.**

Elements	Average concentration (µg/g)	Average concentration (mg/10 g)	DRI (mg/day)		Estimated contribution to RDA (%)
			RDA	UL	
Arsenic	0.18 ± 0.006	0.0018	ND	ND	ND
Silver	11.25 ± 0.15	0.1125	ND	ND	ND
Barium	20.14 ± 1.06	0.2014	ND	ND	ND
Boron	54.24 ± 1.44	0.5424	ND	20	ND
Beryllium	ND	ND	ND	ND	ND
Bismuth	2.52 ± 0.24	0.0252	ND	ND	ND
Calcium	297.60 ± 18.48	2.9760	1000-1300	2500	0.23-0.30
Cadmium	ND	ND	ND	ND	ND
Cobalt	ND	ND	ND	ND	ND
Chromium	9.60 ± 0.12	0.0960	0.024-0.035	ND	274.29-400.00
Copper	9.36 ± 0.24	0.0936	0.9	8.0	10.4
Iron	438.89 ± 2.17	4.3889	8-15	45.0	29.26-54.86
Magnesium	1463.28 ± 52.20	14.6328	310-320	350	4.57-4.72
Manganese	17.46 ± 0.18	0.1746	1.6-3.0	9.0	5.82-10.91
Molybdenum	1.26 ± 0.30	0.0126	0.045	2.00	28.00
Nickel	7.14 ± 0.18	0.0714	ND	1.0	ND
Lead	4.32 ± 0.12	0.0432	ND	ND	ND
Selenium	ND	ND	0.055	0.40	ND
Strontium	16.32 ± 1.08	0.1632	ND	ND	ND
Vanadium	0.45 ± 0.03	0.0045	ND	ND	ND
Zinc	8.72 ± 3.12	0.0872	8-11	34.0	0.79-1.09

ND: Not Determined; BML: *B. monosperma* Leaf. Source: FAO/WHO (Codex Alimentarius Commission). (2001); WHO (1998), Annette Dickinson. Council for Responsible Nutrition, 2002

**TABLE 8. Dietary Reference Intake (DRI): Recommended Dietary allowance (RDA) and Tolerable Upper Intake Level (UL) of elements, compared to the average concentration of elements obtained from BMB.**

Elements	Average concentration (µg/g)	Average concentration (mg/10 g)	DRI (mg/day)		Estimated contribution to RDA (%)
			RDA	UL	
Arsenic	ND	ND	ND	ND	ND
Silver	16.71 ± 0.51	0.1671	ND	ND	ND
Barium	16.55 ± 0.25	0.1655	ND	ND	ND
Boron	55.57 ± 1.63	0.5557	ND	20	ND
Beryllium	ND	ND	ND	ND	ND
Bismuth	2.73 ± 0.03	0.0273	ND	ND	ND
Calcium	437.88 ± 1.34	4.3788	1000-1300	2500	0.34-0.44
Cadmium	ND	ND	ND	ND	ND
Cobalt	ND	ND	ND	ND	ND
Chromium	8.42 ± 0.22	0.0842	0.024-0.035	ND	240.57-350.83
Copper	6.71 ± 0.01	0.0671	0.9	8.0	7.46
Iron	474.19 ± 9.93	4.7419	8-15	45.0	31.61-59.27
Magnesium	4019.20 ± 15.09	40.1920	310-320	350	12.56-12.97
Manganese	32.64 ± 0.24	0.3264	1.6-3.0	9.0	10.88-20.40
Molybdenum	20.23 ± 0.05	0.2023	0.045	2.00	449.56
Nickel	6.24 ± 0.00	0.0024	ND	1.0	ND
Lead	3.15 ± 0.15	0.0315	ND	ND	ND
Selenium	ND	ND	0.055	0.40	ND
Strontium	7.34 ± 0.26	0.0734	ND	ND	ND
Vanadium	0.87 ± 0.05	0.0087	ND	ND	ND
Zn	33.83 ± 0.01	0.3383	8-11	34.0	3.08-4.23

ND: Not Determined; BMB: B.monosperma Bark. Source: FAO/WHO (Codex Alimentarius Commission); WHO, Annette Dickinson. Council for Responsible Nutrition, 2002

**TABLE 9. Dietary Reference Intake (DRI): Recommended Dietary allowance (RDA) and Tolerable Upper Intake Level (UL) of elements, compared to the average concentration of elements obtained from BMR.**

Elements	Average concentration (µg/g)	Average concentration (mg/10 g)	DRI (mg/day)		Estimated contribution to RDA (%)
			RDA	UL	
Arsenic	0.12 ± 0.0	0.0012	ND	ND	ND
Silver	17.34 ± 0.78	0.1734	ND	ND	ND
Barium	19.62 ± 1.86	0.1962	ND	ND	ND
Boron	222.42 ± 0.42	2.2242	ND	20	ND
Beryllium	ND	ND	ND	ND	ND
Bismuth	2.16 ± 0.12	0.0216	ND	ND	ND
Calcium	1404.12 ± 22.20	14.0412	1000-1300	2500	1.08-1.40
Cadmium	ND	ND	ND	ND	ND
Cobalt	ND	ND	ND	ND	ND
Chromium	9.60 ± 0.48	0.0960	0.024-0.035	ND	274.29-400
Copper	7.32 ± 0.36	0.732	0.9	8.0	8.133

Iron	444.10 ± 2.06	4.4410	8-15	45.0	29.61-55.51
Magnesium	4286.88 ± 113.40	42.8688	310-320	350	13.40-13.83
Manganese	27.84 ± 1.08	0.2784	1.6-3.0	9.0	9.28-17.40
Molybdenum	4.92 ± 0.12	0.0492	0.045	2.00	109.33
Nickel	7.98 ± 0.30	0.0098	ND	1.0	ND
Lead	5.04 ± 0.48	0.0504	ND	ND	ND
Selenium	ND	ND	0.055	0.40	ND
Strontium	222.12 ± 99.48	2.2212	ND	ND	ND
Vanadium	1.02 ± 0.03	0.0102	ND	ND	ND
Zn	44.40 ± 0.84	0.4440	8-11	34.0	4.04-5.55
ND: Not Determined; BMR: <i>B. monosperma</i> Root. Source: FAO/WHO (Codex Alimentarius Commission). [27]; WHO, Annette Dickinson. Council for Responsible Nutrition, 2002					

## Discussion

These minerals are essential in the body system for disease prevention, control and may account for the ethnomedicinal effectiveness of most medicinal plants in the treatment and management of several disease conditions. Calcium (Ca) is an important element that plays a major role in bones, teeth, muscular system and heart functions. Calcium is necessary for the coagulation of blood, the proper functioning of the heart and nervous system and the normal contraction of muscles [27-29]. Daily Ca intake lower than the recommended could have serious negative effects for human health such as onset of osteoporosis, very common in menopausal women, hypertension, colon and breast cancer [30,31]. Calcium (Ca) is important for growth and maintenance of strong bones, muscular function, synthesis of enzymes, and normal physiological function of the body etc., [32]. Calcium, which contributes about 2% towards body weight, was found to be high in both soil and fruits. Calcium in the fruits is believed to delay ripening and it helps to reduce biological aging or degradation of the fruit.

Magnesium (Mg) serves as a cofactor for enzymes activation and biological structure promoter [33]. Magnesium deficiency is correlated with the impaired function of many enzymes utilizing high energy phosphate bonds, as in the case of glucose metabolism [21]. The recommended dietary intake of Mg is about 310-320 mg per day for females and 400-420 mg per day for males. Magnesium is important for human health because it regulates many physiological processes, such as bone growth, blood pressure, protein and nucleic acids metabolism, neuromuscular transmission and muscle contraction. Besides, Mg acts as a cofactor of many enzymes and has an important role in reducing asthma. Magnesium deficiency can increase the risk of osteoporosis, mainly in menopausal women, the risk of atherosclerosis and lead to oxidative stress [34].

Manganese (Mn) is an important modulator of cells functions and play vital role in the control of diabetes mellitus [35]. Chromium (Cr) has been shown to participate in sugar metabolism and possible in the prevention of diabetes [36]. Nickel (Ni) serves as a cofactor of important antioxidant enzymes such as superoxide dismutase [37]. Nickel is not in detectable limits which may be accounted for the less toxicity of these medicinal plants in traditional medicine. The results are in line with Taylor et al. [38] who reported the elemental composition of various medicinal plants of Nepal. Copper (Cu) is a very powerful pro-oxidant and catalyzes the oxidation of unsaturated fats and oils as well as ascorbic acid [39].

Zinc is essential to all organisms and has an important role in metabolism, growth, development and general well-being. It is an essential co-factor for a large number of enzymes in the body [21]. Selenium has an important role in thyroid hormone metabolism, as an essential component of the three deiodinase. Also the selenium deficiency has been reported to be correlated with thyroid dysfunctions [40]. It is obvious from above discussion that minerals both micro and macro play a vital

role in variety of functions in human body and a proper balance has to be maintained for the well-being. Lack, insufficient or excess intake of any of them can result to deleterious health effect. Other elements such as As, Cd, and Pb, which are known as non-essential/toxic elements also accumulate in these plants [41,42] through environmental contamination. These non-essential/toxic metals are said to be non-biodegradable and bioaccumulates in the body to cause toxic effect with time. More importantly they exert diverse toxicological effects in animal and human systems. Pb is a multi-organ toxicant affecting virtually all the organs of the body. Screening of different parts *B. monosperma* in this study indicates the presence As and Pb but within the permissible limit, however, with increased use of *B. monosperma* in everyday activities both industrial, agricultural and domestics, chances are abound of the contamination of these toxic metals in the earth crust through bioaccumulation and non-biodegradable system and therefore continuous screening of the plants is required.

TABLE 10. The upper intake levels (ULs) of elements for most individuals [42].

Age	As ( $\mu\text{g d}^{-1}$ )	Ca ( $\text{mg d}^{-1}$ )	Cu ( $\mu\text{g d}^{-1}$ )	Fe ( $\text{mg d}^{-1}$ )	Mg ( $\text{mg d}^{-1}$ )	Mn ( $\text{mg d}^{-1}$ )	Ni ( $\text{mg d}^{-1}$ )	Se ( $\mu\text{g d}^{-1}$ )	Zn ( $\text{mg d}^{-1}$ )	Cr ( $\mu\text{g d}^{-1}$ )
15-50	ND	3000	8000	45	350	10	1	400	35	ND
50	ND	2500	10000	45	350	11	1	400	40	ND
ND: Not Determinable										

TABLE 11. Dietary Reference Intake (DRIs): Recommended intakes for individuals [42].

Life stage group	Ca ( $\text{mg d}^{-1}$ )	Cr ( $\mu\text{g d}^{-1}$ )	Cu ( $\mu\text{g d}^{-1}$ )	Fe ( $\text{mg d}^{-1}$ )	Mg ( $\text{mg d}^{-1}$ )	Mn ( $\text{mg d}^{-1}$ )	Se ( $\mu\text{g d}^{-1}$ )	Zn ( $\text{mg d}^{-1}$ )
Children	800	15	440	10	130	1.5	ND	5.0
Adults	1000	35	900	8	420	1.9-2.2	55	11
ND: Not Determinable								

Potassium is important as diuretic and it takes part in ionic balance of the human body and maintains tissue excitability. Potassium is the principal intercellular cation and also considered as a very important constituent with the extracellular fluids. Potassium ions are concerned with the transmission of electrical impulse in the nerve and in maintaining the fluid balance of the body. Venketaraman and Gopal Krishanan [43] reported maximum concentration of Ca, Fe and K in nine plants traditionally used for jaundice and concluded that high concentration of K in the medicinal plants could be related to the diuretic action of drugs prepared from plants. The main function of iron is in the transport of oxygen to the tissues (haemoglobin) and is also involved in the processes of cellular respiration.

Magnesium is present in bones in association with calcium and phosphate and the rest in soft tissues and body fluids. In muscles and other tissue intracellular magnesium ions function as activator for many of the enzymes involved in carbohydrate metabolism and synthesis of nucleic acids (DNA and RNA). Magnesium also acts as an important binding agent of ribosomal particles where protein synthesis takes place. Increase in extracellular concentration of magnesium depresses skeletal muscle contraction [44,45]. On the other hand, low magnesium concentration causes increased irritability of the nervous system, peripheral vasodilatation and cardiac arrhythmias as earlier reported by Indrayan et al. [22].

Zinc is an essential component of a number of enzymes present in animal tissue including alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase and procarboxypeptidase. It is also essential for the normal growth and reproduction and helps in the process of tissue repair and wound healing. Zinc deficiency causes growth retardation and skin lesions reported

by Srinivasan et al. [46]. The lower amount of zinc accumulation in the plants is due to its less absorption from the soil. Phosphate ions are the major anions of intracellular fluids, phospholipids and the coenzyme NAD and NADP and especially of ATP and other high energy compounds. It helps in the process of ossification of bones by getting deposited in form of calcium phosphate.

Lead contamination occurs due to anthropogenic sources such as combustion and smelting of leaded gasoline. Lead is also toxic in plants as it impairs plant growth by damaging seed germination and root elongation. Lead concentrations in the present study ranges from 3.15 to 6.48  $\mu\text{g/g}$  with the flower having the highest concentration and the bark having the lowest. According to the Department of health, the maximum level of Pb in fruits and berries in South Africa is set at 0.20  $\text{mg kg}^{-1}$ . All the parts of BM had concentrations of Pb above this limit but were found to be below the FAO/WHO maximum permissible limit; hence they are safe for human consumption. The study showed that BML has the highest moisture content of 79.6% while the seed has the lowest (27.6%). The percentage ash, protein and oil was found more in the seed compared to the other parts of BMS (23.4, 7.04 and 7.44% respectively), the carbohydrate content was found to be highest in seed (49.34%). Most natural foods contain about 0-5% of protein while processed foods contains more than 10%. On analysis of the data, in particular the concentration of the elements in different parts of BM, it was observed that concentrations of Ca, Fe, Mg, Mn, and Zn were higher than other metals with Mg having the highest concentration followed by Fe and the lowest was Mn. This distribution of the major elements in different parts of BM is illustrated in FIG. 2. In general, the concentrations of the elements in *B. monosperma* were found to be in decreasing order of  $\text{Mg} > \text{Fe} > \text{Ca} > \text{Zn} > \text{Mn} > \text{Ba} > \text{B} > \text{As} > \text{Sr} > \text{Cr} > \text{Cu} > \text{Ni} > \text{Pb} > \text{Bi} \approx \text{Mo} > \text{V}$  while Cd, Be, Co and Se were not detected (ND) in all parts of *B. monosperma*.

Plants are not specific when taking heavy metals from the soil, either essential such as Cr, Mn, Fe, Co, Cu, Zn and Se or non-essential elements such as As, Cd and Pb. These metals have a tendency of accumulating in plants and vegetables. Some plants are able to eliminate or accumulate heavy metals to meet their metabolic demands. When the concentration (total and exchangeable) of the plant essential element in the soil is below the concentration required by the plant, the plant tends to accumulate that particular element to meet its physiological demand and when the soil concentrations (total and exchangeable) exceed its physiological demand the plant tends to exclude the excess amount. The study showed that *B. monosperma* accumulated Mg, Fe, Ca, Mn and Zn and tends to exclude the other elements to meet its physiological requirement levels. A balanced diet is one of the important parameters in support of normal growth and development of the human body. A balanced diet means eating the right types and amounts of food to supply nutrition and energy for maintenance of body cells, tissues and organs. An imbalance in the diet can lead to malnutrition; eating low amounts of certain nutrients can lead to deficiencies while eating too much can lead to toxicities. The elemental distribution in BM was compared to Dietary Reference Intakes (DRIs) (TABLE 5-11). If an average serving size of 10 g (dry mass) of BM is consumed it would contribute significantly towards the daily intake of important nutrients. Most of the nutrients did not exceed their Tolerable Upper Intake Levels (ULs). Manganese is one of the essential nutrients for nerve impulses and muscle contraction. About 10 g of BM bark, leave, root, seed and flower contributes about 240-350%, 274-400%, 272-400%, 202-295 and 296-432% respectively towards the RDA for Cr. Although the percentage contribution towards the RDA for Cr was high, the DRI for Cr (15-35 mg per day) was not exceeded. If approximately 10 g of BM seed is consumed per day the contribution to the diet for the elements Mn and Fe would be <14% and <70% for these elements, respectively. For the toxic elements, As and Pb concentrations in the plant were at safe levels for human consumption. In India, especially in rural communities and also in Ayurvedic medicine, BM has been employed extensively in health conditions without knowing the

elemental composition. This study provides information on the nutritive value of BM which indicates that it is good for health and does not have a tendency to accumulate toxic elements.

## Conclusion

The elemental concentrations as well as the nutritional value of five major parts of BM were assessed. The concentration of the elements were found to be in decreasing order of Mg>Fe>Ca>Zn>Mn>Ba>B>As>Sr>Cr>Cu>Ni>Pb>Bi≈Mo>V in all the parts of BM with the highest concentration of the As, Ba, Ca and Mg observed in BMR (17.34, 222.42, 1404.12, 4286.88 respectively) proving root to be the point of absorption of this minerals to other parts of the plants. The elemental analysis in BM showed that the plant controls the uptake of the elements to meet its physiological demands. The plant tended to accumulate Mg, Ca, Fe and Zn and tended to exclude the other elements studied. BM was found to be rich in essential nutrients and contained low concentrations of the toxic elements studied. The high concentration of most essential elements such as Mg, Fe and Ca supports the medicinal use of BM in several disease conditions and also as a tonic. In conclusion, from the present study, it was discovered that all parts of BM, exhibits same characteristic and same medicinal properties as all parts of BM contain same number of essential elements with no significant difference in the concentration of each elements.

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