# **Research Article**



# Quantification of Mineral Element, Vitamins and Identification of Betacarotenoid in HPLC of Mukia maderaspatana (Linn.) Cogn. Fruits

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#### ABSTRACT

This study aimed to determine the mineral composition, vitamins and quantification of beta carotene in HPLC *M.maderaspatana* fruits to explore its uses as a source of food industry. The concentration of various minerals (K, Ca, Mg and Na) and trace elements (Fe and Zn) were recorded by using an atomic absorption spectrophotometer. The mineral elements analysis showed a significantly higher amount of potassium (512.17mg/100g), phosphorous (192.83mg/100g), calcium (180.2 mg/100g), sodium (164.28 mg/100g) and iron (6.86 mg/100g) in *M.maderspantna* fruit. It contained provitamin A (18.85mg/g100g), vitamin B1, (0.01mg/100g), vitamin B2 (0.01mg/100g), vitamin C(0.01 mg/100g). The βeta- carotene content through spectrophotometry and high performance liquid chromatography HPLC analysis showed that 88ppm of betacarotene present in *M.maderaspatana* fruits. *Mukia maderaspatana* fruits could be beneficial to food scientists, nutritionists, cancer researchers and epidemiologists.

Keywords: M.maderaspatana, vitamins, mineral element, beta carotene.

### INTRODUCTION

utritional informational is used increasingly by public agencies and agricultural industries to promote fresh produce. Consumers are looking for variety in their diets, and are aware of the health benefits of fresh fruits and vegetables. Of special interest are food source rich in antioxidant vitamins (Vitamin A, B, C and E), Calcium (Ca), Magnesium (Mg) and potassium (K). The 2005 dietary guidelines advisory committee recommended increasing the dietary intake of provitamin A (beta carotenoids), Vitamin C, E, Ca, Mg, K and fiber. βcarotene (vitamin A precursors), is a major carotenoid important to humans<sup>12</sup>. Beta-carotene is known to have a high demand in food industry which has made global industry to face challenges especially in fulfilling customers requirement that are looking for environment friendly and natural products. Beta-carotene is one of the key products of food industry which has been widely used as nutrients and additives.

Melothria maderaspatana (Linn.) Cogn. is an annual monoecious herb, densely covered with white hairs. It is found throughout India ascending up to 1800 m in the hills. The tender shoots and bitter leaves are used as a gentle aperient and prescribed in vertigo and biliousness<sup>3</sup>. This plant has a unique place in the Siddha system of medicine. M. maderaspatana has been shown to exert hepatoprotective, antioxidant, anti-inflammatory, antiarthritic activities, immunomodulatory activity, antimicrobial activity<sup>4</sup> and hypolipidemic<sup>5</sup>. Some tribes of India (Orissa) also use this herb for the treatment of diabetes mellitus. Certain traditional medical practitioners also use the leaf-tea of this plant for alleviation of jaundice<sup>6</sup>. This plant leaf extract has also anti-arthritic properties<sup>7</sup>. Anti-oxidants agents are effective in the prevention and treatment of complex diseases, like atherosclerosis, diabetes, Alzheimer's disease and cancer. Furthermore, our earlier report showed significant antioxidant potential along with higher content of total phenols and flavonoids in *M. maderaspatana<sup>8</sup>*. The present study is to find out the mineral element, vitamins and quantification of betacarotenoid in HPLC of *Mukia maderaspatana* fruits.

# **MATERIALS AND METHODS**

#### **Collection and Preparation of Plant Material**

The *Mukia maderaspatana* fruits were collected from Coimbatore district, Tamil Nadu state, India. The fruits were cut into pieces and shade dried. The dried samples were pounded into powder using mortar and pestle. The powder obtained was kept in the laboratory and used for mineral elements analysis during the period of the research.

### **Determination of mineral elements**

Finely ground (5 g) of sample was oven dried at 60°C and was weighed into crucible. The sample was ignited into a muffle furnace for 6-8 hours at a temperature between 450°C and not exceeding 500°C, a grayish white ash was obtained. The sample was cooled on asbestos sheet and 5 cm<sup>3</sup> 1N HNO<sub>3</sub> solutions were added to it. It was evaporated to dryness on a steam bath or a hot plate at a low heat of 400°C for 15 min. until a perfectly white or grayish white ash is obtained.

The sample was later cooled on asbestos sheet and 10  $\text{cm}^3$  1N HCl was added and the solution filtered into 50 cm3 volumetric flask. The crucible and filter paper were washed with additional 10cm<sup>3</sup> portion of 0.1N HCl three times to make up to the volume with 0.1N HCl solution. The filtrate was stored for Na, P, K, Ca, Mg, Fe and Zn



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determination using Atomic Absorption Spectrophotometer<sup>9</sup> (AOAC 1990).

# **Determination of vitamins**

Vitamins analyses were carried out using the methods <sup>9</sup>(AOAC 1990).

# **Determination of Beta carotenoids in HPLC**

The chromatographic measurements were performed using Gemini – C18-Reverse phase column - 15cm. The mobile phase consist of Pump A – Acetonitrile; Pump B – Methanol at 50:50 ratios. The separation was performed by gradient elution at a total flow rate of 1 mL/min. The injection volume was  $20\mu$ l and elutes were detected at 454nm. Betacarotene were as standard for identification and quantification in the *M.maderaspatana*. All the standard and sample solutions were filtered through 0.45 membrane filter and injected by auto sample.



**Figure 1:** Spectrum of Standard Beta carotene at 454nm *vs* Spectrum of Beta carotene of *Mukia maderaspatana* at 454nm



**Figure 2:** Chromatogram of Standard Beta carotene at 454nm *vs* Chloroform extract of *Mukia maderaspatana* at 454nm

# Statistical analysis

Descriptive statistics were performed by using Microsoft Excel 2007 to calculate mean and standard errors for mineral contents of fruit sample.

# **RESULTS AND DISCUSSION**

Minerals play several important roles in human physiology and biochemistry as co-factors for enzymes and are related to energetic efficiency, fertility, mental stability and immunity<sup>10</sup>. The results regarding the mineral and trace elements level Mukia maderaspatana fruit studied show that the M.maderaspatana fruit contain the magnesium (38.7 ± 0.52mg/100g). Sponias mombin fruit contains the higher value of magnesium (465.0±21.21mg/100g) than the Mukia maderaspatana<sup>11</sup>. Magnesium plays a major role in relaxing muscles along the airway to the lung thus allowing asthma patients to breathe easier. The daily value for Mg is 400mg. It plays fundamental roles in most reactions involving phosphate transfer, believed to be essential in the structural stability of nucleic acid and intestinal absorption while deficiency of magnesium in man is responsible for severe diarrhoea, hyper-tension, cardiomyopathy, migraines, arterio sclerosis and stroke<sup>11</sup>.

# Table 1: Mineral Element of M.maderaspatana fruits

Mineral Element	M.maderaspatana fruits
Sodium	164.28 ± 0. 03
Phosphorus	192.83 ± 0.41
Potassium	512.17 ± 0.05
Zinc	0.05 ± 0.10
Calcium	180.2± 0.33
Magnesium	38.7 ± 0.52
Iron	$6.86 \pm 0.08$

Sodium and potassium perform important biochemical functions as in acid-base balance, nerve impulse mediation and cell membrane Na/K channels and pumps. However, compelling evidence have implicated high dietary sodium intake in the development of cardiovascular disease, importantly hypertension and that increased intake of dietary potassium is beneficial in control<sup>12</sup>. pressure relation to blood In M.maderaspatana fruits contain sodium 164.28 ± 0.02 mg/100g and potassium (512.17 ± 0.05mg/100g). The sodium level of some commercial fruits vary between 6 -28 mg/100g<sup>12</sup>. Potassium is essential for the ability of skeletal and smooth muscles to contract.

High concentrations of sodium (Na) were present in *Mukia maderaspatana* ripe fruits (164.28  $\pm$  0.02 mg/100g). The sodium level of some commercial fruits varies between 6 – 28 mg/100g (Table 2)<sup>13</sup>.

Potassium has the highest concentration (512.17  $\pm$  0.05mg/100g). These results were higher than the fig and mulberries fruits<sup>14</sup>. Potassium is essential for the ability of skeletal and smooth muscles to contract. Because of this, an adequate intake of potassium is important for regular digestive and muscular functioning.



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**Table 2:** Determination of vitamins of *M.maderaspatana* 

 fruits

Vitamins	<i>Mukia maderaspatana</i> (mg/100g)
Provitamin A (Beta carotene)	18.85
Vitamin B1 (Thiamine)	0.01
Vitamin B2 (Riboflavin)	0.01
Vitamin C (Ascorbic acid)	0.01

In *M.maderaspatana* fruit contain phosphorus  $192.83 \pm 0.04 \text{ mg/g}$ . Phosphorus is needed for bone growth, kidney function and cell growth. It also plays a role in maintaining the body acid alkaline balance.

Similarly calcium is a major component of bone and assists in tooth development (Brody 1994). In the *M. maderaspatana* calcium contain (180.2  $\pm$  0.33mg/g). *Cordia dichotoma* fruit contains the higher value of calcium 615.4  $\pm$  0.30 mg/100g than the *Mukia maderaspatana*<sup>15</sup>. The recommended daily calcium intake for adult range from 1000 mg to 1500 mg. It is also recommended to take supplements with food to aid in absorption. Compared with other metals the calcium ion and most of its compound have low toxicity.

The quantity of iron in *Mukia maderaspatana* fruits contain (6.86  $\pm$  0.08 mg/100g). Iron content lower than the *Solanum torvum* fruits 5.22 mg/100 gm<sup>16</sup>. An adequate level of Fe is required for haemoglobin formation in blood, while excessive intake can result in hemochromatosis. Iron containing enzymes and proteins participate in many oxidations and in transport.

### Vitamins

Table 2 showed the determination of vitamins of *Mukia maderaspatana* fruits. Among the vitamins, Provitamin A found to be higher amount (18.85 mg/100g) than the vitamin B composition. Provitamin A (Beta carotenoid) have been credited with other beneficial effects to human health enhancement of the immune response and reduction of the risk of degenerative diseases such as cancer, cardiovascular diseases, cataract and muscular degeneration.

# **Quantification of Beta Carotenoid in HPLC**

The proposed method is a simple, precise and sensitive method for estimation of beta carotenoids from the M.maderaspatana fruits. The retention time of betacarotene was about 35.65 min. The chromatograms of standard betacarotene and test sample are shown in fig. 1 and 2 respectively. The average betacarotene content in test sample was 88ppm. A sufficient intake of beta-carotene is important as it functions as a safe source of vitamin A, helping the body to reach the vitamin A levels that are essential for normal growth and development, good vision and eye health, a strong immune system and healthy skin. Beta carotene is an antioxidant, contributing to protecting the body against the damaging effects of free radicals, which can potentially increase the risk of developing certain diseases, including cardiovascular diseases and cancer. The method provides for good resolution and separation of betacarotene. Table 1 and 2 indicates betacarotene peak area and peak height which shows the reliability and suitability of the method. Hence this method can be easily used for the standardization of the active constituent betacarotene in the M.maderaspatana fruits and its marketed antioxidant forms.

Peak number	Retention time (min)	Peak area	Peak height	Name of the compound
1	35.65	2792658.4	47195.6	Beta carotene

Table 3: Chromatogram of Standard Beta carotene at 454nm

Table 4: Chromatogram of Chloroform extract of Mukia maderaspatana at 454nm

Peak number	Retention time (min)	Peak area	Peak height	Name of the compound
1	35.36	607933.8	6964.5	Beta carotene

### **Calculation**

20µL of sample contains 3425 µg of raw material concentration.

$\beta$ carotene percentage	Area of  Area of s	sample X standard	Concentration of standard  Concentration of sample	X Purity of standard
607933.8 = X 2792658.4	1.43  3425	X 97% = 0	).0088% = 88 ppm	



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# CONCLUSION

Our study revealed that essential mineral elements and vitamins are needed for growth, production of bones, teeth, hair, blood, nerves, skin, enzymes and hormones. The healthy function of nervous transmission, blood circulation, fluid regulation, cellular integrity, energy production and muscle contraction are influenced by essential elements and too little of any essential element can lead to deficiency disease and too much of it any can be toxic. The scheme of obtaining  $\beta$ -carotene standard from *M.maderaspatana* fruits demonstrated that the purity of  $\beta$ -carotene standard obtained and quantified by HPLC was 88 ppm. This method can be used to evaluate fruit quality in the functional food industry. These fruits can be explored as an alternative food for malnutrition population in developing countries.

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