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Nutritional Value and Elemental Analysis of Katphala (*Myrica esculenta* Buch-Ham)

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ABSTRACT: *Myrica esculenta* (Family: Myricaceae) commonly known as Kaiphala or Katphala is a widely used medicinal plant in Ayurveda. Traditionally its leaves are used in the treatment of fever, catarrh of mucous membrane, asthma, diarrhoea and bronchitis, its paste is applied to cuts and wounds. In spite of its numerous medicinal attributes, no published work is available till date on nutritional values and elemental analysis of leaves. The nutrient and antinutrient compositions of the leaves were investigated and compare the evaluated data with reported nutritional parameters of fruit. The result of proximate analysis shows that Myrica fruit had higher moisture content, crude lipids and carbohydrate contents values of 72.33±0.23, 4.93±0.06 %and 78.03±0.14 % while those of the leaves had 3.54±0.11 %, 1.38±0.54 % and 46.19±0.21 %. The total ash, crude fibre and crude protein contents in the leaves were higher values of 8.3±0.28 %, 22.45±0.17 % and 10.55±0.22 % while the fruit had 2.18±0.02 %, 5.22±0.08 % and 9.62±0.03 %. The antinutrient parameters evaluated in the leaves were phytic acid (2.46±0.16) %, saponin content (1.76±0.25 %), alkaloidal contents (0.28±0.06) and oxalate content (0.27 ± 0.10) %. The predominant mineral elements in the leaf powder according to ICP-MS were Ca (42.59 mg/100g), Mg (14.82 mg/100g), and Na (12.41 mg/100g). Lead and arsenic contents were not detected in the leaves and fruits of Myrica. Hence the outcome of this study revealed that *M. esculenta* leaves could be a valuable nutraceutical supplement and a cheapest source of essential nutrients to the human diet.

Keywords: Macronutrients; proximate analysis; elemental analysis; oxalate content and ICP-MS.

INTRODUCTION: Plants are used as medicine to maintain human health from ages[1] and are also major natural sources of medicinal compounds in current pharmacopoeias.[2] The World Health Organization reports that up to 80% of people still rely primarily on traditional medicines.[3] The medicinal value of these plants due to the presence of nutrients, minerals and a variety of phytochemicals. These nutrients and minerals are essential for physiological functions of human body. Nutrients and biochemical compounds such as carbohydrate, protein and lipid play an important role in satisfying human needs for energy and life process[4].

Myrica esculenta Buch.-Ham. *syn. Myrica nagi F.* (Myricaceae), commonly known as 'Box Berry', 'Kaiphal', 'Katphala' is an important medicinal tree distributed all along outer Himalaya from Ravi (Punjab) eastwards to Assam, Khasia, Jaintia, Shimla, Bengal, Naga and Lushai hills at altitudes of 900-2100m. *M. esculenta* is a small to moderate sized, evergreen, dioecious tree indigenous to subtropical temperate region.[5-9] Trees of Myrica grow well in nitrogen depleted soils and are common associates of Pine (*Pinus sp.*) and Oak (*Quercus leucotrihophora*). They are also found in mixed forests and in agricultural and marginal lands.[10] *M. esculenta* known for edible fruit and other by-products is a potential income-generating species in the sub-Himalayan region.[11] As per the Ayurvedic literature, various parts of this plant is used in gulma (abdominal tumors), jvara (fever), arsa (piles), grahani (irregular bowel function), pandu roga (anemia), hrillasa (nausea), mukha roga (oral disorders), kasa (cough), svasa (dyspnea), agnimandhya (indigestion), aruchi (anorexia) and kantharoga (ears, nose, and throat disorders).[12]

Traditionally, leaves of this plant are useful remedy in fever, catarrh of mucous membrane, asthma, diarrhoea and bronchitis.[13] Leaf paste is applied to cuts and wounds.[14] Bark is widely used in the Indian systems of medicine as astringent carminative, antiseptic and in the disease supposed to be caused by deranged

phlegm, such as catarrhal, fever, cough and affection of throat.[15] Paste of the bark is applied on the chest to get relief from cough & bronchitis.[16] Fruits are utilized in food industries in Himalayas in different forms like syrups, jam, and squash.[17] Due to the high medicinal values of the leaves, bark and fruits of this medicinally important tree are imported and exported.[18]

Several valuable reviews of the ethnobotanical uses and phytochemistry of *M. esculenta* are available. Myrica is found to be a rich source of phenolic compounds, triterpenoids, saponins, glycosides. flavonoids and flavonols.[19] The leaves, in particular, have been found to contain flavonoids, tannins, diarylheptanoids, phenolic acids and flavonoids; these compounds have various biological activities, including antihypertensive, anti-inflammatory, antidiabetic, antihelmintic by using a number of in vitro and in vivo animal models, which prove the traditional utilization of leaves scientifically.[20]

The plant has tremendous therapeutic potential with every part of the plant being used medicinally. Despite vast medicinal uses of leaves, no report is known about its nutritional analysis hence the aim of this study were to determine the proximate nutritional value and elemental compositions of *M. esculenta* leaves and also to providing scientific data based on our findings in relation to its dietary and medicinal applications.

MATERIAL AND METHOD:

Collection and authentication of plant materials: Leaves of *Myrica esculenta* used for this study were collected from out skirt of Chail Chowk, Mandi, Himachal Pradesh (H.P.) in November 2016. The plant was identified, authenticated and certified (AGI/16/1245) by Prof. Suresh Kumar, Head Department of Botany, Abhilashi Group of Institutions, Mandi, H.P. Freshly collected plant material was cleaned to removed adhering dust and then dried under shade. The dried sample groundedinto powder and kept in air tight container for further studies.

Reagents: All chemicals used for the experiment were of analytical grade (Merck, Germany).

Proximate analysis: Powdered leaf sample were dried in an oven at 110^{o}C for 1hr to a constant weight for moisture determination. Ash content, crude fibre, crude protein and crude lipid were analyzed by triplicate according to AOAC method.[21] The total carbohydrates were determined by the subtraction method [100 − (% crude proteins + % crude lipids + % moisture content + % ash)].[22] The energy values (kcal/100g) were determined by multiplying the values of proteins, lipids, and carbohydrates by factors of 4, 9, and 4, respectively, and taking the sum expressed in kilocalorie .[23]

Anti-nutrient analysis:

Determination of oxalate content: For the determination of oxlate content modified titration method was used.[24, 25] One gm of pulverized sample was placed in conical flask. 75 mL of 3M H_2SO_4 was added. The solution was carefully stirred with a magnetic stirrer for 1 hour and then filtered using Whattman filter paper No.1. 25 mL of the filtrate was collected titrated against 0.1M $KMnO_4$ solution till a light pink colour that persisted for 15 seconds appeared. The oxalate content was calculated by taking 1 mL of 0.05 mol/L of $KMnO_4$ as equivalent to 2.2 mg oxalate.

Determination of phytic acid: The phytic acid content was determined as described method.[26] 2.0 g of sample was weighed into a 250 mL flask, 100 mL of 2% concentrated HCl was added and allowed to stand for 3 hrs and then filtered with Whattman filter paper No.1. 25 mL of the filtrate was placed in 250 mL of conical flask with 10 ml of 0.3% ammonium thiocyanate solution as indicator and titrated with standard Iron III Chloride solution containing 0.00195 g Iron/mL, end point observed to be brownish yellow colour persisted for 5 min. The percentage phytic acid was calculated as:

Phytic acid (%) = titre value × 0.001 95 × 1.19 × 100

Determination of alkaloidal content: Alkaloid content was determined as described method.[27] 5 g of plant extract was added with 200 mL of 10% acetic acid in ethyl alcohol and mixed. The mixture was covered and allowed to stand for 4 hrs. and then mixture was filtered with Whattman filter paper No.1. Filtrate was concentrated to a 1/4th of its original volume on a water bath. Concentrated ammonium hydroxide was added in drops to the extract until precipitation was completed. The solution was allowed to settle, washed with dilute NaOH solution and then filtered. The residue collected was dried and weighed. The alkaloid content was calculated using the equation:

$$\% \text{ Alkaloid content} = \frac{Weight\ of\ precipitate}{Weight\ of\ origional\ sample}\ X\ 100$$

Determination of saponin content: Saponin content was estimated as described by Obadoni and Ochuko.[28] 5 g of the plant sample was added to 50 mL of 20% ethyl alcohol, kept on a shaker for 30 min after that heated in a water bath for 4 h at 55ºC. The resulting

mixture was filtered and the residue re-extracted with another 200 mL of 20% aqueous ethanol. The filtrates were combined and reduced to 40 mL in a water bath at 90°C. The concentrate was transferred into a separating funnel, 20 mL of diethyl ether was added, and shaken vigorously. The upper ether layer was discarded and the aqueous (bottom) layer retained in a beaker. The retained layer was re-introduced into a separating funnel and 60 mL of n-butanol was added and shaken vigorously. Butanol extract present in the upper layer was retained while the bottom layer was discarded. The butanol layer was washed twice with 10 mL of 5% aqueous NaCl. The remaining solution was collected and heated to evaporation in a water bath, then dried to constant weight at 40°C in an oven. The saponin content was calculated using the equation:

$$\% \text{ Saponin content} = \frac{Weight\ of\ residue}{Weight\ of\ origional\ sample}\ X\ 100$$

Elemental analysis: Leaf samples were digested in concentrated HNO_3. The digest was transferred to a 25-ml volumetric flask, and the volume was adjusted to 25 ml with deionized water. A blank digest was prepared in a similar manner. The multi elemental analysis was performed by inductively coupled plasma mass spectrometry (ICP-MS; XSeries 2, Thermo Scientific).

Statistical analysis of data: The results were statistically analyzed and expressed as mean (n = 3) ± standard deviation. All experiments were performed in triplicates and the data expressed as mean ± SD using the Microsoft Excel 2010 spreadsheet.

RESULTS AND DISCUSSION:

Proximate composition: The results of proximate analysis of leaves and fruits of *M. esculenta* were shown in Table 1. There were a significant difference in the total ash and crude fiber contents in the leaves ad fruits of Myrica with the leaves having the higher values of 8.3±0.28 and 22.45±0.17 %. Sood et al. reported that moisture content and carbohydrate contents having significantly higher value than leaves were 72.33±0.23 % and 78.03±0.14 %.[20] The lipid content was also significantly high in the fruits than leaves was 4.93±0.06 %. The total energy derived from this plant leaves and fruits calculated were 239.49±0.080 and 394.98±0.012 %.

Anti-nutrient composition: The summary of anti-nutrient composition of the sample is presented in Table 2. Four anti-nutrient were studied, phytic acid content was most abundant with a percentage value of *2.46±0.16* %, which was closely followed by saponin 1.76±0.25 %. The alkaloid and oxalate content were least values 0.28± .06 % and 0.27±0.10%. There is no data reported on the antinutrient analysis of fruits.

Table 1: Proximate nutritional composition of dried *M. esculenta* leaf and fruit.

Parameter	Composition %	
	Leaf	Fruit
Moister content	3.54±0.11	72.33±0.23**
Total ash	8.3±0.28**	2.18±0.02
Crude lipid	1.38±0.54	4.93±0.06**
Crude fibre	22.45±0.17**	5.22±0.08
Crude protein	10.55±0.22	9.62±0.03
Carbohydrate	46.19±0.21	78.03±0.14**
Energy value (Kcal/100 gm)	239.49±0.080	394.98±0.012**

Values expressed as Mean ± SD, n=3
Statistically significant differences between the means of both are denoted as
***p < 0.001; *p < 0.05.*

Table 2: Anti-nutrient Composition of *M. esculenta* leaf.

Parameters	Values %
Phytic acid	2.46±0.16
Saponin	1.76±0.25
Alkaloids	0.28±0.06
Oxalate	0.27±0.10

Values expressed as Mean ± SD, n=3 on dry weight basis.

Mineral Composition: Table 3 represents the mineral composition of both the leaves and fruits of Myrica. Calcium, magnesium, sodium, manganese, zinc, and iron contents were significantly higher in the leaves than fruits. Their values were 42.59 mg/100g, 14.82 mg/100g, 12.41 mg/100g, 3.45 mg/100g, 2.32 mg/100g, and 1.71 mg/100g. Potassium content was reported slightly higher value in the fruits than leaves 6.86 mg/100g. Arsenic and lead contents were not detected in the leaves and fruits both.

The shelf-life and stability of any food component is determined by its moisture content. The low moisture content indicates that food products have a long shelf life and reduced microbial contamination.[29] The ash content evaluated from this leaves was relatively indicating that its richness in mineral nutrients. These minerals act as inorganic co-factors in metabolic processes.[30] The percentage of dietary fibre content was relatively high of the sample. The relatively high fibre content prevents constipation by facilitates peristaltic movement and aids the absorption of certain minerals in the gut and reduces cholesterol absorption.[31, 32] High fibre intake could lead to a decline the incidence of

diseases associated with metabolic disorders.[33] The lipid content in the sample was very less and it was lowest nutritional composition evaluated in this study. Dietary lipids enhance the taste of food by preserving its flavours.[34] Excess intake of dietary lipids increases the risk of cardiovascular diseases, aging, and cancer. In this concern, the low crude lipids content of *M. esculenta* leaves indicates that it could prevent certain chronic diseases associated with lipids in human beings. The crude protein content in the sample was third highest proximate composition of the sample

Table 3: Measured mineral composition of *M. esculenta* leaf and fruit (mg/100 g).

Minerals	mg/100 g DW	
	Leaf	Fruit
Calcium	42.59±0.020**	4.63±0.06
Magnesium	14.82±0.025*	8.4±0.20
Potassium	6.86±0.011	7.75±0.11*
Phosphorous	0.91±0.01	0.24±0.25
Sodium	12.41±0.015**	0.81±0.013
Zinc	2.32±0.005**	0.216±0.0016
Copper	0.17±0.005*	0.004±0.0002
Manganese	3.45±0.011**	0.032±0.0001
Iron	1.71±0.005**	0.404±0.0021
Lead	ND	ND
Arsenic	ND	ND

Values expressed as Mean ± SD, n=3 on dry weight basis.
ND: non determined
Statistically significant differences between the means of both are denoted as
***p < 0.001; *p < 0.05.*

The relatively high protein level of *M. esculenta* leaves could make it a useful supplement to diets. Dietary proteins play an important role in the manufacturing and safe guarding of certain organic materials necessary for the smooth functioning of the human body.[35] Proteins also serves the purpose of enzymatic catalyst and mediates metabolic and energy regulation.[36] Carbohydrate content was the highest nutritional composition. The high carbohydrate content makes it rich source of energy and this could be used to enhance the energy content of diets.[34] The overall energy derived from *M. esculenta* leaves sample calculated was 239.49±0.080 kcal/100g which is below the recommended daily energy value. Therefore *M. esculenta* as a low energy food source maybe very helpful in weight management programmes as used by traditional practioners.

The saponin content in *M. esculenta* was low and within the safe limit, since an amount below 10% is not hazardous to the body.[37] High saponin levels in human and animal diets have been implicated in growth impairment; decrease the bioavailability of nutrients and inhibition of biochemical reactions that promote breakdown of ingested proteins.[38] Alkaloids are one of the most efficient therapeutic bioactive components in plants. For instance, consumption of high tropane alkaloids will cause rapid heartbeat, paralysis and in fatal case, lead to death. Uptake of a high dose of tryptamine alkaloids will lead to the staggering gait and death.[39] Other toxic effect of alkaloids includes disruption of the mucus membrane in the gastrointestinal tract.[40] The alkaloid content recorded in this study was quite low, attenuating the fear of anti-nutrient activity. The phytate content of *M. esculenta* leaf sample was low. Phytate chelates with metal ion such as calcium, copper, zinc, magnesium and iron to form an insoluble complex that are not readily absorbed from gastro intestinal tract (GIT).[41,42]A dietary phytate content of 1% - 6% over a long period decrease zinc bioavailability.[43]

Oxalate form chelate with toxic metals such as lead and mercury and show antioxidant effect. The presence of oxalate in foods causes irritation in the mouth and could diminishes the absorption of calcium and increase the risk of kidney stone.[44, 45] The concentrations of anti-nutrients (saponin, oxalate, phytate and alkaloids) recorded in this study were however within the safe limit and may not elicit toxic effect when consumed.

Minerals are considered to be essential in human nutrition for the overall physical and mental health, as well as important constituents of nerve cells, bones, teeth, tissues, muscles, and blood. Table 3 represents the result of mineral composition available in *M. esculenta*. The results revealed that leaves of this are a good source of macro and micro both minerals. The minerals evaluated are in the order Ca>Mg>Na> K> Mn>Zn> Fe>P >Cu.

Calcium play a vital role in muscle contraction, neurological function, blood clotting, neurological examination, regulation of cell permeability and osteoporosis. It is also needed for building and strong bones and teeth.[46, 47] Thus, *M. esculenta* is considered to be a natural cure for osteoporosis. Furthermore Magnesium plays an important role in enzyme activity and prevents from heart disease. It also plays an important role in formation and function of bones, muscles, nerve transmission and immune system. Magnesium plays a vital importance in strengthening of β-cells functions thus preventing onset of diabetes.[48] Sodium is very important mineral element involved in the transmission of nerve impulse as well as maintenance

of osmotic pressure of body fluids.[49] Deficiency of sodium cause dehydration and muscle cramps.[50] Potassium plays an important role in water as well as acid-base balance in the body. It is also responsible for maintaining cardiac rhythm, nerve action and functioning of muscles. Deficiency of potassium cause muscle paralysis.[45]

Four micro elements (Fe, Cu, Zn, Mn) were present in the sample. Manganese serves as a cofactor of various enzymes involved in metabolism essential for reproduction function, growth, and skeletal development. It also plays an important role in the metabolism of carbohydrates, proteins, cholesterol and also involved in formation of urea.[51, 52]

Iron is an important constituent of Hemoglobin (Hb) and carries oxygen in the blood. It plays an important role in formation of tendons and ligaments. Deficiency of iron cause anaemia, weakness, poor resistance o infection and in female may cause infertility.[53] Therefore, *M. esculenta* could be used as a potent source to improve the anemic condition of a patient. Zinc a vital traces element found in many enzymes, needed for brain development, normal growth, bone formation, wound healing, sperm production and sexual maturation.[54] Therefore this sample can be a good zinc supplement for people with infertility challenges. Copper is a part of various enzymes such as cytochrome oxidase, lysyl oxidase, ceruloplasm required for metabolism of iron in blood, it also involved in erythropoiesis, erythrocyte function and survival. Copper deficiency can cause cardiac disorders, anemia and neutropenia.[50]

CONCLUSION: The study revealed that *M. esculenta* had high crude fibre, ash content, calcium, magnesium, manganese and iron contents. The antinutrient contents were within acceptable limit and may not affect the absorption of other nutrients. *M. esculenta* is rich in many macro and micro nutrients and can therefore serve as a supplement to prevent many mineral deficiencies. *M. esculenta* should therefore be considered a plant with great potential in the food, nutritional and pharmaceutical industries. Further studies on toxicity of *M. esculenta* are on-going to ascertain its possible adverse effects and to confirm some of the ethnopharmacological claims.

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