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## Quantification of nutrient and phytoconstituent in fresh and dehydrated *Piper betel* Leaf

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### Abstract

*Piper betel* leaf of its green heart shaped leaves belongs to the family Piperaceae rich in macro and micro nutrients, vitamins, phytochemicals, antioxidant and anticancer activity. In this study macro and micronutrients of fresh and dehydrated betel leaves were analyzed. Carbohydrate content of fresh and dehydrated *Piper betel* leaf ranged from 6g to 12g, protein had 2.63g to 3.21g and fat ranged from 1g to 1.8g of fresh and dehydrated *Piper betel* leaf. Fiber composition in dehydrated and fresh *Piper betel* leaf ranged from 7.64g to 9.7g. Micronutrients include calcium, phosphorus,  $\beta$  carotene possess higher activity in dehydrated than fresh *Piper betel* leaf and iron showed identical results that is 3mg. Quantification of phytochemical analysis are done using aqueous and ethanolic extract of fresh and dehydrated *Piper betel* leaf. Total antioxidant activity of aqueous and ethanolic extract of fresh and dehydrated *Piper betel* leaf were analyzed.

**Keywords:** Fresh *Piper betel* leaf, dehydrated *Piper betel* leaf, antioxidant activity, phytochemicals, ethanolic dehydrated *Piper betel* leaf, total phenols

### Introduction

Betelvine is the most important useful asexually propagated cash crop having various cultivars and it belongs to Piperaceae family. It is originated from Malaysia but is distributed extensively in South and Southwest China. This crop is usually cultivated in India, Sri Lanka, Malaysia, Thailand, Taiwan and other Southeast Asian countries [1]. *Piper betel* or Betel vine deep green heart shaped leaves is cultivated in hotter and damper part of country following the traditional methods about 55,000 hectares with an annual production worth about Rs 9000 million [2]. Stems are supposed to be useful in treating indigestion, bronchitis, constipation, coughs and asthma and the whole plant also used for many other purposes such as foods and spices, fish bait, fish poison, hallucinogens, insecticides, oils, ornaments, perfumes, antiwormal and anti-infectious agent because of its pungent taste. Leaves are rich in many nutrients like water, energy, protein, fats, fiber, calcium and iron etc. and the antioxidants present are flavonoids, tannins, saponins alkaloids, terpenoids [2]. The plant extract also showed highest inhibitory effect against proliferation of MCF – 7 cells, with increased activities of catalase and superoxide dismutase [3]. The leaves were chewed by singers to improve their voice. The fruit of *Piper betel* employed with honey as a remedy for cough. Leaf extract is reported to inhibit male reproductive competence. The leaves possess antifertility on male rats and antimotility effects on washed human spermatozoa. *Piper betel* showed hypotensive, cardio tonic, smooth and skeletal muscles relaxant actions [4].

Antioxidants are compounds capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. It is involved in the defense mechanism of the organism against the pathologies associated to the attack of free radicals [6].

Animal studies have shown that the topical application of betel leaf extract,  $\beta$ -carotene and  $\alpha$ -tocopherol was effective reducing the tumor formation significantly in both Swiss mice, Swiss bare mice, while hydroxychavicol was highly effective only in Swiss bare mice [6]. Antioxidants are compounds capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. It is involved in the defense mechanism of the organism against the pathologies associated to the attack of free radicals [7].

## Materials and Methods

### Collection of samples

The betel leaf was collected from the local markets of Coimbatore, Tamil Nadu during the month of December 2019, January 2020, February 2020 and March 2020. The *Piper betel* leaf was authenticated by Dr. C. Murugan, Scientist 'E' – in – charge, Botanical Survey of India, Southern Regional Centre, Government of India, Coimbatore. The betel leaf was authenticated as *Piper betel* L. belongs to the family PIPERACEAE.

### Preparation of Sample

Fresh, green, undamaged leaves are taken, washed in running water, chopped into small pieces, weighed and then they were dried. The temperature was maintained at 120 °C and the leaves were sufficiently dried till they became crisp and brittle to touch. The leaves took 1.5 hours for complete drying. The dehydrated sample kept in a beaker and stored in ambient temperature for further use.

### Extraction of Sample

The fresh and dehydrated samples were extracted with aqueous and ethanol solutions using mortar and pestle and then filtered. The sample is kept in an electric shaker for 24 hrs and stored in a dark place to get the crude extract and it is stored in refrigerator for further use.

### Nutrient Analysis of fresh and dehydrated *Piper betel* leaf

Proximate are used in the analysis of biological materials as a decomposition of a human consumable good into its major

constituents. The proximate composition includes moisture, ash, carbohydrate, protein, fat and micronutrients include calcium, iron, phosphorus,  $\beta$  carotene were analyzed using standard procedure.

### Quantification of phytochemicals

Phenolic compounds that contained in the plants have redox properties and the properties allow them acting as antioxidants. The phytochemical analysis of total phenols and vitamin c were analyzed using aqueous and ethanolic extract of fresh and dehydrated *Piper betel* leaf using general laboratory procedure.

### Total antioxidant activity of *Piper betel* leaf

The aqueous and ethanolic extract of *Piper betel* leaf were analyzed using ascorbic acid as a standard. They reported that the total anti-oxidant capacity of the *Piper betel* leaf extract showed the reducing power of the compound is associated with electron donating capacity and serves as an indicator of anti-oxidant activity<sup>[8]</sup>.

## Results and Discussion

### Macronutrient Content of Fresh and Dehydrated Betel leaf

The proximate composition of Moisture, Ash, Carbohydrate, Protein, Fat and Crude Fiber of fresh and dehydrated samples were analyzed. Table 1 showed the macronutrient composition of fresh and dehydrated *Piper betel* leaf.

**Table 1:** Macronutrient Content of Fresh and Dehydrated Betel leaf (g/100g)

Nutrients	Fresh	Dehydrated	Icmr Value (100 G)
Moisture (%)	86.27±0.1	9.55±0.1	85.4
Carbohydrate(g)	6±0.2517	12±0.2517	6.1
Protein (g)	2.63±0.1	3.21± 0.02	3.1
Fat (g)	1.0 ± 0.1528	1.8± 0.0764	0.8
Crude Fiber (%)	9.7± 0.0361	7.64± 0.0076	2.3
Total Ash (g)	0.8± 0.1	11.6± 0.1	----

From this table 1 it was observed that the moisture content of fresh betel leaves was higher with the value of 86.27% than dehydrated leaves (9.55%) because those leaves are dried at 110 °C for 1.5 hrs are done using moisture analyser. Moisture can be lost due to drying but it helps to increase the shelf life. ICMR recommendation for moisture of fresh betel leaf is 85.4%. They reported that fresh leaves of Kariyele variety had 83.86% moisture and Ambadiyele had 85.13% moisture. Then, dehydrated sample had 13.53% of moisture for Kariyele variety and 12.66% for Ambadiyele variety<sup>[9]</sup>. They reported that fresh leaves had higher moisture content than the dehydrated leaf.

Carbohydrate content of dehydrated leaves had 20g was higher than the fresh leaves i.e.6g. ICMR value of Betel leaves for carbohydrate is 6.1<sup>[12]</sup>. According to Mazumder *et al.*, (2016) who reported that fresh *Piper betel* leaves contains carbohydrate 0.5-6.10% per 100g<sup>[10]</sup>. The nutrient composition in the dried betel leaves powder contain 63.92% of carbohydrate. The protein content can be determined by Lowry's method, dehydrated sample had 3.21g was higher than fresh betel leaves i.e. 2.63g, because drying process applies heat on the product, this might lead to the degradation and decomposition of valuable nutrients

within the leaves<sup>[11]</sup>. ICMR recommendation of protein is 3.1g. The fresh leaves had 3.49 g of protein for Kariyele variety followed by Ambadiyele had 3.06 proteins per 100g. Ambadiyele dehydrated powder was found that 12.07g of protein and Kariyele had 13.47g of protein per 100g<sup>[9]</sup>. Fat of fresh leaves had 1.0g while dehydrated leaves were higher than fresh leaves i.e.1.8. ICMR recommendation of fat is 0.8. They reported that fresh betel leaves contain fat 0.4 -1.0% per 100g<sup>[10]</sup> whereas Chauhan and Aishwarya (2016) reported that nutrient composition in the dried betel leaves powder of fat contain 1.10%<sup>[11]</sup>. Analysis of fat sources is performed as an attempt to determine fat composition, quality. Common measures of fat quality include color, fatty acid profile, free fatty acid content, degree of unsaturation, saponification value and impurities including moisture and insoluble. From this result, 9.7 g of crude fiber is present in fresh betel leaf whereas in dehydrated sample had 7.64<sup>[13]</sup>. Fresh leaves had higher content of fiber than dehydrated due to drying it may be lost. ICMR recommended 2.3g of fiber content for betel leaves. They reported that 1.96 g of crude fiber in Kariyele variety followed by 2.03 g of crude fiber in Ambadiyele variety whereas in dehydrated sample had 5.2g of crude fiber in

Kariyele variety and 6.5 g in Ambadiyele variety [9]. Crude fiber is the residue of plant materials remaining after solvent extraction followed by digestion with dilute acid and alkali. Crude fiber consists largely of cellulose (60-80%) and lignin (4-6%) plus some mineral matter.

These Fibers are beneficial in treating or preventing constipation, hemorrhoids, diverticulosis, coronary heart diseases, and some type of cancer.

Total ash content of fresh leaves had 0.8 g while dehydrated sample had higher content i.e. 11.6 g [14]. The nutrient composition in the dried betel leaves powder contain ash (6.87%). Ash helps to determine the amount and type of minerals in food because the amount of minerals can determine physiochemical properties of foods, as well as retard the growth of microorganisms [11].

### Micronutrient Composition of Fresh and Dehydrated *Piper betel* leaf

Mineral is a chemical element required as an essential nutrient by organisms to perform functions necessary for life. Calcium, Iron, Phosphorus of fresh and dehydrated samples were analyzed. It helps to build and protect your bones. Phosphorus is the second most plentiful mineral in your body. It plays an important role in how the body uses carbohydrates and fats.

**Table 2:** Micronutrient Composition of Fresh and Dehydrated *Piper betel* leaf (mg/100g)

Nutrients	Fresh	Dehydrated	Icmrvalue
Calcium (mg)	55± 0.1528	110± 0.1528	230
Iron (mg)	3±0.264	3±0.264	10.6
Phosphorus (mg)	65± 0.2082	95± 0.1528	40
β Carotene (µg)	1,375 ± 0.3606	2062 ± 0.3215	—

The results revealed that the 110 mg of calcium and 95 mg of phosphorus content of dehydrated betel leaf was higher

**Table 3:** Quantification of Fresh and Dehydrated *Piper betel* Leaf (PER100ML)

Phytochemicals	Standard (mg)	Fresh		Dehydrated	
		Aqueous	Ethanol	Aqueous	Ethanol
Total Phenols (mg)	(Pyrogallol) 0.09 ± 0.015	0.95 ± 0.02	0.81± 0.0252	0.86± 0.025	0.95± 0.076
Vitamin C (mg)	(Ascorbic Acid) 0.067 ± 0.00152	33.5 ± 0.045	67 ± 0.2082	46.9 ± 0.076	84± 0.1756

From this table 3, it showed that there are slight changes in Phenolic content of betel leaf possess higher results in both ethanolic dehydrated and aqueous fresh followed by aqueous dehydrated. Ethanolic fresh showed poor result than other extracts [19]. According to Abraham *et al.*, 2012, the ethyl acetate extract (852.3 mg GAE/g dw) of *P. Betel* had the highest phenolic content because of the presence of catechin, morin and quercetin through HPLC analyses more than 3 fold and 16 fold higher than the hexane (266.92 mg GAE/g dw) and methanol extract (52.25 mg GAE/g dw),

than fresh leaves had 55mg of calcium [15] and 65mg of phosphorus [16]. ICMR recommendation of calcium is 230 mg and phosphorus had 40mg of *Piper betel* leaf. The iron content of both fresh and dehydrated samples is same i.e. 3 mg [17] whereas recommendation of ICMR is 10.6 mg. Kariyele and Ambadiyele variety of dehydrated betel leaves of minerals include 2018.8 mg and 2894.2 mg of calcium followed by 213 and 242.3 mg of Phosphorus followed by 23.15mg and 40.98 mg of iron in Dehydrated *Piper betel* Leaf [9].

Beta Carotene is an antioxidant that converts to vitamin A and plays a very important role in health. It plays a crucial role in the body's fight against free radicals. It also improves cognitive function, promoting good skin health, contributing to lung health, preventing cancer and also reducing macular degeneration. β – carotene of dehydrated leaf had higher content of 2062 µg than fresh leaves had 1375 µg [18]. They reported that β – carotene of Kariyele and Ambadiyele variety of betel leaf are 3220µg and 3716µg of fresh leaves. Kariyele dehydrated powder was found that β carotene had 5440µg whereas Ambadiyele had 6693 µg [9].

### Quantification of Fresh and Dehydrated *Piper betel* Leaf

Phenolics play an important role in plant development, particularly in lignin and pigment biosynthesis. They also provide structural integrity and scaffolding support to plants. Phenols are readily absorbed following inhalation, ingestion or skin contact, and are widely distributed in the body, can cross the placenta, and have been found in human breast milk. Vitamin C is also known as ascorbic acid and it is used to prevent scurvy. It is an essential nutrient involved in the repair of tissue, formation of collagen, absorption of iron, immune system; wound healing, maintenance of cartilage, bones, teeth and the enzymatic production of certain neurotransmitters.

whereas aqueous extract (47.72 mg GAE/g dw) shows lower phenolic content [12]. For Vitamin C, Ethanolic dehydrated showed maximum result than other extract and it is followed by ethanolic fresh, aqueous dehydrated. Aqueous fresh showed lower content of Vitamin C. From this, Ethanolic Dehydrated showed better results than other extracts [20]. The abundantly available betel leaves are nutritive and dehydrated betel leaves are rich source of micronutrients. Abraham reported that the fresh betel leaves contain vitamin C 0.005 – 0.01% per 100g [12].

**Table 4:** Quantification of Total Antioxidant Content of Fresh and Dehydrated *Piper betel* Leaf (100G)

Phytochemicals	Standard Ascorbic Acid (mg)	Fresh		Dehydrated	
		Aqueous	Ethanol	Aqueous	Ethanol
Total Antioxidant (µg)	0.15 ± 0.01	2800 ± 0.23	4800± 0.152	8000± 0.15	15000± 0.2

From the above table 4 it was observed that ethanolic extract of dehydrated betel leaf possesses higher antioxidant activity followed by aqueous dehydrated, ethanolic fresh

and ascorbic acid using as a standard. Aqueous fresh showed lower result than other extracts. They reported that Antioxidant contents of Kariyele and Ambadiyele variety

had 19.72mg and 72.34mg<sup>[9]</sup>. Total antioxidant capacity is an analyte frequently used to assess the antioxidant status of biological samples and can evaluate the antioxidant response against the free radicals in a given disease. Antioxidants include Vitamin A, C, E, beta carotene, Lycopene, selenium.

### Conclusion

The key finding of this study exhibited that the macro and micronutrient showed higher results in dehydrated *Piper betel* leaf than fresh *Piper betel* leaf. Phytochemical analysis showed that total phenolic content was higher in ethanolic dehydrated *Piper betel* leaf than other extracts and Vitamin C showed higher activity in ethanolic dehydrated *Piper betel* leaf than other extracts were quantified. Total antioxidant activity was analyzed using ascorbic acid act as a standard and it was observed that the ethanolic extract of dehydrated betel leaf showed higher results than other extract. Betel leaf is economically feasible, readily available and it is taken by all people even children. Betel leaf with its enormous nutrient composition and it also possesses anticancer activity.

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