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NUTRITIONAL PROFILING OF *PERILLA FRUTESCENS* (L.) BRITTON FOUND INDIGENOUS IN MANIPUR

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Abstract

***Background**: Perilla frutescens (L.) Britton, a traditional herb belonging to the Lamiaceae family, is extensively cultivated in Manipur, a Northeastern State of India. **Objective:** This study aimed to investigate the proximate composition, essential oil content, and fatty acid composition of two cultivar variety of Perilla frutescens (L.) Britton. **Methods**: The proximate composition, including protein, total carbohydrates, and total fats, essential oil content, and fatty acid composition of Brown and white Perilla frutescens leaves and seeds were analyzed. **Results**: The results revealed that the leaves of White and Brown Perilla are rich in protein, total carbohydrates, and total fat content. The seeds contained significant amounts of essential oil, healthy fats, carbohydrates, and protein. Fatty acid composition analysis revealed high levels of alpha-linolenic acid (omega-6) and linolenic acid (omega-3) in the seed. **Conclusion**: These findings suggest that Perilla frutescens (L.) Britton is an excellent source of essential nutrients, supporting its potential health benefits.*

1. INTRODUCTION

Perilla frutescens (L.) Britton commonly known as *Perilla* or wild sesame. Locally known as "Thoiding" in Manipur is an essential aromatic herbaceous plant belonging to the family Lamiaceae. It is widely cultivated and utilized in various parts of Asia for its culinary, medicinal and therapeutic properties. Among its diverse varieties *Perilla frutescens* is indigenous to Manipur, a region in Northeast India known for its rich biodiversity and unique ethnobotanical practices. The plant holds significant cultural role, nutritional and medicinal importance in the local traditions of Manipur [1].

Figure 1: A-Brown *Perilla frutescens* Leaves. B- White *Perilla frutescens* leaves. C- Brown *Perilla frutescens* seeds. D- White *Perilla frutescens* seeds

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Two prominent cultivar varieties of Perilla, Brown *Perilla frutescens* (L.) Britton and White *Perilla frutescens* (L.) Britton, are predominantly grown in Manipur. In Manipur *Perilla frustescens* is widely distributed and has been an integral part of the local flora, used by various ethnic communities for its medicinal, culinary, and cultural significance [1].

In Manipur, the seeds of *Perilla frutescens* are a valued ingredient in various local dishes. The grounded, roasted seeds are used in a traditional salad called "Singju." Mature seeds are also consumed in different forms, such as:

1. "Thoiding Kangshu": a delicious item made by grinding the seeds and mixing them with fresh ginger (Fig:2 A)
2. "Kabok": a local aliment composed of roasted mature Perilla seeds, concentrated sugar cane juice, and popped rice (Fig:2 B)
3. "Kangsubi": another popular and delicious aliment made from Perilla seeds (Fig: 2 C).

Figure 2: A- Thoiding kangshu, B -Kabok, C- Kangsubi

Interestingly, despite the seeds being a common ingredient in Manipuri cuisine, the edibility and nutritional value of the leaves remain largely unknown to the local population.

Recently *Perilla* has garnered attention for its exceptional nutritional and phytochemical profile. Nutritional profiling of indigenous plants like *Perilla* is essential to understand their role in traditional diets and their potential as functional foods. The seeds of *Perilla* are particularly valued for their high oil content which is rich in omega 3 fatty acids antioxidants and essential micronutrients. Furthermore, the leaves and stems are widely used as condiments, herbal remedies, in folk medicine [2].

Despite its versatile applications and nutritional benefits, limited scientific research has been conducted on the biochemical and nutritional composition of *Perilla frutescens* found in Manipur.

The Chinese Pharmacopeia 2010 recognizes the dried parts of *Perilla frutescens*, including the stems, leaves, and ripe fruits, as therapeutic agents for various health conditions. Traditionally, *Perilla frutescens* has been used as a natural herbal remedy to alleviate symptoms associated with: Mental health: depression, anxiety, Respiratory issues: asthma, coughs, allergies, Infectious diseases: cold, fever, chills, Digestive problems: intestinal disorders, General well-being: headaches, stuffy nose, intoxication. This versatile herb has been valued for its medicinal properties, addressing a range of health concerns. Its traditional uses highlight its potential as a natural remedy for various ailments [3].

Perilla seed oil is a rich source of essential fatty acids, including α-linolenic acid (54-64%) and linoleic acid (14%). Perilla seeds and their oils have been widely used in traditional nutritional and medicinal formulations. Biological analysis of *Perilla* seeds has revealed a range of beneficial effects, including: - Anti-cancer, Anti-diabetic, Anti-asthma, Antimicrobial, Anti-inflammatory Antioxidant, Cardio protective [4].

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This study aims to comprehensively analyze the nutritional profile of *Perilla frutescens* (L.) Britton, an indigenous plant species in Manipur. By examining its nutrient composition, essential oil content, and essential fatty acid content, this study seeks to highlight the potential of *Perilla* as a nutrient-dense food source.

The findings of this study will contribute to a broader understanding of underutilized plants in sustainable agriculture, food security, and neutraceutical development, while preserving the ethnobotanical heritage of Manipur. This research will also provide valuable insights into the nutritional and phytochemical properties of *Perilla,* which can have significant implications for its potential uses in food, pharmaceutical, and cosmetic industries.

2. MATERIALS AND METHODS

2.1 Sample Collection

The Plant sample of the *Perilla frutescens* (L.) Britton were collected during the harvesting season from Khongman, Imphal East district of Manipur. It was identified and authenticated at Institute of Bio resources and Sustainable Development (IBSD) Takyel, Manipur.

The fresh leaves were washed under running tap water, shade dried at room temperature, and powdered for further quantitative analysis. Some of the fresh samples were stored in 4 ^{o}C. Similarly, the seeds were shade dried at room temperature and powdered for further quantitative analysis.

2.2 Total Ash content

Total ash content was estimated by using the ignition method described by AOAC [6].

Ignition Method-

Sample Preparation: About 2 g of the sample were weighed accurately and transferred it to a porcelain crucible. Ignition: The crucible were heated over a low flame, by gradually increasing the temperature, until the substance is thoroughly charred. Ashing**:** The crucible were then transferred to a muffle furnace at 600 ± 25°C. The substance were ashed for 2 hours or until the ash is white or nearly white. Cooling and Weighing: The crucible were then removed from the furnace and were allowed to cool in a desiccator. Then the crucible and ash were weighed and were calculated.

Gravimetric Calculation. Calculate the percentage of total ash as follows:

Total Ash (%) = [(Weight of ash / Weight of sample) x 100]

2.3 Total Protein content by Kjedahl method

The Total protein were estimated using Kjedahl method described in The Indian Standard (IS) 7219 [6].

Reagents and Apparatus- Copper sulfate solution- Potassium sulfate- Sodium hydroxide (NaOH)- Sulfuric acid (H2SO4)- Ammonia (NH3)- Distilled water- Kjeldahl flask- Digestion apparatus- Distillation apparatus- Burette

Sample Preparation: About 1 g of the sample were weighed and were grinded into a fine powder. The power sample were mixed with 10 g of potassium sulfate. Digestion**:** Then the sample mixture were transferred to a Kjeldahl flask. 25 mL of sulfuric acid were added to it. Then 1-2 mL of copper sulfate solution were added. Then the mixture were digested at 420°C for 2 hours. Distillation and Titration: the digested mixture were distilled using a distillation apparatus. And the distillate were collected in a receiver containing 50 mL of boric acid solution. The distillate were then titrated with 0.1 N sulfuric acid using a burette.

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The percentage of nitrogen were calculated using the following formula:

Nitrogen (%) = [(Volume of sulfuric acid x Normality of sulfuric acid x 14) / Weight of sample] x 100

Percentage of protein were calculated using the following formula:

Protein (%) = Nitrogen (%) x 6.25

2.4 Total Carbohydrates content by Phenol Sulphuric Acid method.

The Total Carbohydrates content were estimated using the phenol sulphuric method described by The Association of Official Analytical Chemists (AOAC) 986.25 [5].

Reagents and Apparatus- Phenol solution (80%) - Sulfuric acid (H2SO4, 98%) - Glucose standard solution- Distilled water- Spectrophotometer (520 nm) - Volumetric flasks- Pipettes

Sample Preparation: 1 g of the sample were weighed accurately and the sample were grinded into a fine powder. Then the powder were mixed with 10 mL of distilled water. The mixture were heated at 100°C for 10 minutes. Phenol-Sulfuric Acid Reaction: 1 mL of the sample mixture were pipetted into a test tube and 1 mL of phenol solution were added. Then 5ml of sulphuric acid were added carefully to it. The solution were then mixed well and the reaction were allowed to proceed for 10 minutes. Spectrophotometric Measurement: The absorbance of the sample were measured at 520 nm using a spectrophotometer. A blank solution using distilled water was prepared and were its absorbance were measured. The net absorbance was calculated by subtracting the blank absorbance from the sample absorbance.

Standard Curve: a series of glucose standard solutions (0-1 mg/mL) were prepared and the phenol-sulfuric acid reaction were performed and spectrophotometric measurement for each standard solution were done. Standard graph has been plot against the glucose concentration to obtain a standard curve.

Calculation. Determine the glucose equivalent of the sample using the standard curve $y=0.6465x +0.0124$, $r^2=0.997$

Total Carbohydrates (%) = (Glucose equivalent x 100) / Sample weight

2.5 Total Fat content by Soxhlet method

The total fat content were estimated using the Soxhlet extraction method described in The Association of Official Analytical Chemists (AOAC) 920.39 [7].

Reagents and Apparatus- Ether (diethyl ether or petroleum ether) - Soxhlet extraction apparatus- Extraction thimble- Fat extraction flask- Desiccator- Balance

Sample Preparation: About 2-5 g of the sample were weighed accurately. Then the sample were grinded into a fine powder. The powder were then mixed with a small amount of sand or silica gel to facilitate extraction. Soxhlet Extraction: the sample mixture were placed in the extraction thimble. Then the Soxhlet extraction apparatus were assembled. Then ether was added to the fat extraction flask. Then the ether were heated gently to reflux. The fat sample were extracted from the sample for 4-6 hours or until the ether is colorless. Fat Determination: The extraction thimble were then removed from the Soxhlet apparatus. The ether were then allowed to evaporate from the thimble. Then the thimble and extracted fat were weighed.

The total fat content were calculated as a percentage of the sample weight using the following formula:

Total Fat (%) = [(Weight of extracted fat / Weight of sample) x 100]

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2.6 Extraction of oil

Hydroditillation was carried out in the Clevenger apparatus for the extraction of essential oil.200g of Brown and the white *Perilla frutescens* Fresh leaves and seeds were directly immersed in 800 ml of water and were subjected to hydrodistillation for 3 h in a clevenger-type apparatus under optimal operational conditions with temperature 80˚C reduce overtime to 40˚C. The obtained essential oil was collected and dehydrated using anhydrous sodium sulfate and were stored at low temperature. [8].

The yield of essential oils in each sample was calculated according to the following formula: Yield of EO (%) = (WE0/WL) × 100%

Where, WEO is the weight of extracted Perilla leaf / seed EO (g), and WL is the weight of the leaf/seed powder (g).

2.7 Fatty acid composition Analysis

The total fatty acid composition of the Perilla seed were analyzed by using EASI-CHE-SOP-166 which is a scientific protocol for fatty acid profiling using gas chromatography (GC) [8].

Sample derivatization: The extracted fatty acids were derivatize to form fatty acid methyl esters (FAMEs) using a reagent such as boron trifluoride (BF3).

2.7.1 Preparation of Fatty acid methyl esters

In order to make fatty acids present in the seed volatile, derivatizaion was performed prior to GC-MS analysis. Methylation is the most general method of converting non-volatile fatty acids into volatile fatty acids methyl esters. Methylation of fatty acids was performed with BF3 – methanol as derivatizing reagent, which is the most accepted procedure for converting fatty acids into FAMEs. The Fatty acids were derivatized by using the boron trifluoride method as described by Hisil, 1988 [9].

2.7.2 Gas Chromatography/mass spectrometry Analysis (GC/MS)

Derivatized fatty acids methyl esters were analysed by using a Shimadzu GC-2010 equipped with a Shimadzu GCMS-QP2010 Plus mass selective detector having HP- MS capillary column (30m x 0.25mm, film thickness 0.25 µm). The column oven initial temperature was 140°C, programmed at 4°C/min to final oven temperature 240°C and held for 10 min at this temperature, injector temperature was 270°C. Helium was used as carrier gas with column flow rate 1.21 ml/min and the split ratio 1:20. For GC/MS detection, an electron ionization system with ionization energy of 70eV was used, Ion source temperature was 230°C and Interface temperature was 280°C.The components were identified by comparing their relative retention times and mass spectra with those of standards [9].

3. RESULT AND DISCCUSION

3.1 Proximate nutritional composition

The proximate nutritional composition has been shown in Table 1, Leaf sample has the higher ash content compared to the seeds. White *Perilla* leaf has the highest ash content of 13.72 ±0.1 and the Brown *Perilla* seed has the lowest of 2.80±0.1. Ash content provides vital information towards the nutritional value of food. Ash content is the inorganic noncombustible mineral content in a sample. Previous study revealed that the main mineral present in the *Perilla* are calcium, iron, phosphorous, magnesium [4].

White *Perilla* has more protein content than the Brown *Perilla*. White *Perilla frutescens* seed has the highest protein content of 20.60 ±0.1 and the Brown *Perilla* leaf has the lowest of 18.09 ±0.1. Previous research showed that the main amino acids found in Perilla plant are (Tyrosine, Phenylalanine, Methionine, Histidine, glutamate, leucine, aspartate, arginine) [5].

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The plant *Perilla* showed very high content of carbohydrates. Carbohydrates content were estimated by calculating the glucose equivalent from the Standard curve using glucose as standard Figure 3. Brown *Perilla* has more carbohydrates content as compared to the white *Perilla*. Brown *Perilla* seed has the highest carbohydrates content of 74.09±0.5 and the least was white *Perilla* seed of 30.45 ±0.1. This is due to the high amount of dietary fiber present in the *Perilla* seeds. Fiber is an essential part of a healthy diet. High fiber diets have been linked to health benefits such as improved metabolism and heart health. Overall dietary fibre helps in gut health.

The seeds of the *Perilla* plant also showed high fat content. Brown *Perilla* seed showed high fat content of 45.91±0.5 which is followed by the white *Perilla* seed of 42.17±0.1 and the least was observed in white *Perilla* leaf of 3.36±0.1. *Perilla* seed content many healthy fat and essential fatty acids.

Table 1: Nutritional Value (Proximate Composition) Of Leaf and seed sample of Brown and White *Perilla frutescens* (L.) Britton

SL.NO.	Sample	Total Ash content %	Total Protein content %	Total Carbohydrate %	Total Fat content %
1.	Brown *Perilla frutescens* Leaf	13.29±0.1	18.09±0.1	50.12±0.5	5.23±0.1
2.	Brown *Perilla frutesecens* Seed	2.80±0.1	18.53±0.1	74.09±0.5	45.91±0.1
3.	White *Perilla frutescens* leaf	13.72±0.1	20.10±0.1	48.75±0.5	3.36±0.1
4.	White *Perilla frutescens* Seed	3.22±0.1	20.60±0.1	30.45±0.1	42.17±0.1

Experiments were performed in triplicates and the data are represented as mean ± SD

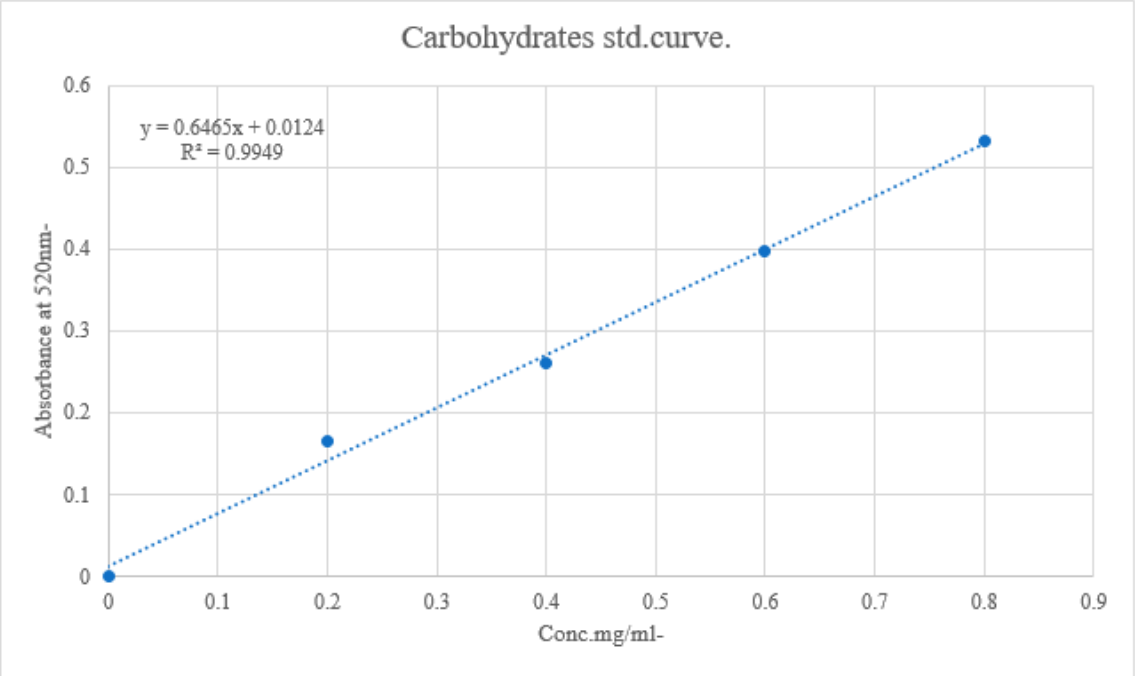

Figure 3: Standard curve for carbohydrates estimation using glucose

3.2 Essential oil content

The seed samples of *Perilla frutescens* exhibited higher oil yield percentages compared to the leaf samples. Among the samples, the Brown *Perilla* seed demonstrated the highest oil yield percentage of 47.2 ± 1.0%, followed by the White *Perilla* seed with 40.4 ± 0.8%. In contrast, the leaf samples of both the Brown and White *Perilla* plants showed relatively low oil yield percentages of 1.6 ± 1.5% and 2.0 ± 1.0%, respectively.

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The oil extracted from *Perilla frutescens* seeds is characterized by its light yellow color and distinctive aromatic smell. Rich in bioactive compounds, this oil has potential applications in various fields, including medicine, cosmetics, and the food industry [9].

Essential oils are a rich source of bioactive compounds, possessing anti-oxidative and anti-microbial properties. These oils have been utilized in traditional medicine for their therapeutic benefits. Essential oils, also known as volatile odoriferous oils, are aromatic oily liquids extracted from various plant parts, including leaves, peels, barks, flowers, buds, seeds, and more [10].

Table 2: Essential oil content (%) Of leaf and seed sample of *Perilla frutescens* (L.) Britton

Sl.No.	Sample	Essential oil yield (%V/W)
1.	Brown *Perilla frutescens* Leaf	1.6 ±1.5
2.	Brown *Perilla frustescens* seed	47.2± 1.0
3.	White *Perilla frutescens* leaf	2.0±1.0
4.	White *Perilla frutescens* seed	40.4±0.8

Experiments were performed in triplicates and the data are represented as mean ± SD.

3.3 Fatty acid composition of *Perilla* seed

Major fatty acids reported in Brown and the White *Perilla* seeds were alpha- linolenic acid (omega 3), linoleic acid (omega 6), palmitic acid, oleic acid and stearic acid. GC-MS examination of White and Brown *Perilla frutescens* seed shows that it contains Palmitic acid 3.40%, 3.13%, Stearic acid 1.10%, 1.32%, Oleic acid 6.15%, 4.65%, Linoleic acid 8.79 %, 6.74% and α-linolenic acid 26.20%, 26.09% respectively as major components.

Linoleic acid (omega-6) and α-linolenic acid (omega-3) are essential fatty acids crucial for human health. Omega-3 fatty acids, in particular, play a vital role in brain function, growth, and development, as well as behavioral function. Since the human body cannot produce omega-3 fatty acids, it is essential to obtain them through dietary sources.

Research has shown that omega-3 fatty acids reduce inflammation and may help lower the risk of chronic diseases, such as heart disease, cancer, and arthritis. Infants who do not receive adequate omega-3 fatty acids from their mothers during pregnancy are at risk of developing vision and nerve problems [11, 12].

Research emphasizes the importance of maintaining a balance between omega-6 and omega-3 fatty acid intake. While omega-6 fatty acids support normal immune function and clotting, excessive consumption may promote abnormal clotting and an overactive immune system [11].

Alpha-linolenic acid (omega-3) and linoleic acid (omega-6) are essential fatty acids that cannot be synthesized by the human body. As a result, they must be obtained through dietary sources. The beneficial health effects of omega-3 fatty acids have been extensively documented, with benefits related to: Cancer prevention, Inflammatory bowel disease management, Rheumatoid arthritis treatment, Psoriasis management, mental health support [13, 14].

The Institute of Medicine of the National Academies (2005) recommends a daily intake of alpha-linolenic acid (LNA) of: 1.6 g/day for men, 1.1 g/day for women. Interestingly, an individual would need to consume approximately 8 g of *Perilla* seeds (white or brown) to meet the recommended daily intake of LNA. This highlights the potential of *Perilla* seeds as a rich dietary source of essential fatty acids [15].

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Table 3: Essential Fatty acid composition of Brown and White *Perilla frutescens* seed (100g)

Essential Fatty acids	Brown *Perilla frutescens* seed Result ±LOQ	White *Perilla frutescens* seed Result ±LOQ
C 16 : 0 Palmitic acid	3.40±0.1	3.13 ±0.1
C 18 : 0 Stearic acid	1.10±0.1	1.32±0.1
C 18: 1 Oleic acid	6.15±0.1	4.65±0.1
C 18 : 2 Linoleic acid	8.79±0.1	6.74±0.1
C 18: 3 n3 Alpha –linolenic acid	26.20±0.1	26.09±0.1

Experiments were performed in triplicates and data were expressed in mean ± LOQ

4. CONCLUSION

The present study reveals that *Perilla frutescens* (L.) Britton is a rich source of protein, carbohydrates, and healthy fats, comparable in quality to sesame and chia seeds. This makes it an edible, healthy, and medicinal plant. The plant also exhibits high oil content, with its seeds containing a high amount of essential fatty acids, including alpha-linolenic acid (omega-3) and linoleic acid (omega-6), which are renowned for their numerous health benefits.

The consumption of *Perilla frutescens* as food has been associated with several health benefits, including the prevention of cardiovascular diseases, cancer, and neurodegenerative diseases. Despite its well-established ethnobotanical uses in Asian cultures, the plant's properties remain poorly documented in English literature. Its rich nutritional profile, including high carbohydrate and fatty acid content, makes it an attractive functional dietary supplement.

The findings suggest that *Perilla frutescens* is an excellent source of essential nutrients, supporting its potential health benefits. It is a nutritious and versatile food ingredient that has been used in traditional culinary practices in Northeast India. The plant's leaves, seeds, and oil provide a rich source of essential nutrients and health benefits, making it a valuable addition to a healthy diet. Further research should focus on evaluating its therapeutic applications through clinical studies and potential development as a functional food or dietary supplement.

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Ethical Approvals

This study does not involve experiments on animals or human subjects**.**

Conflict of Interest

The authors hereby declare that there are no conflict of interest.

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