UNDER PEER REVIEW

Evaluation of physcio-chemical parameters of flavour blended mulberry leaf powder

**Abstract** 

Herbal tea is often combined to create delicious taste with unique flavours and mainly enjoyed as a natural caffeine-free alternative to standard teas. In the present study, mulberry leaves based herbal tea were developed with the combination of cumin, mint and ginger. The selected spices and herbs were subjected to drying conditions to obtain maximum retention of bioactive compounds. The herbal combination developed was packed as dip tea bags. Moisture, Protein, pH, Titratable acidity, Antioxidants, Total soluble solids and 1-DNJ. Antinutrient content like Tannins and Flavonoids were investigated by standard methods. The nutrient analysis revealed that flavour blended mulberry tea powder had moisture content of (7.8 %), with higher protein content of (23g), a pH value of (5.8), titratable acidity of (1.66 %), total soluble solids of (8.10 °Bx), 1-DNJ (0.132 mg/g) (1-Deoxynojirimycin) whereas, tannins with 2.34 (mg TAE/g) and flavonoids with 32.56 (mg RE/g).

Keywords: Mulberry herbal tea, 1-Deoxynojirimycin (1-DNJ), Bioactive compounds Flavonoids, Antioxidants

### 1. Introduction

Tea is an aromatic non-alcoholic beverage with a functional food effect and prepared by pouring boiled water into the fresh or cured leaves of *Camellia sinensis*. It is reported that tea consumption started about five thousand years ago in China and India. Traditionally, tea was consumed to improve blood flow, eliminate toxins and improve resistance to diseases while several epidemiological studies have linked tea consumption to the reduction of

cardiovascular disease risk, diabetes, cholesterol level, arthritis, osteoporosis and dental carriers. Tea is categorized into several types such as white, green, oolong, black, puerrh, scented and herbal teas (Odoh *et al.*, 2022)

Spices and herbs had been used as food and medication for centuries. In the renovation of the high quality of human existence, it plays a widespread role by providing an abundant source of antioxidants and medicinal constituents (Craig, 1999). Nutraceutical has been defined as "food or part of food" which offers scientific or health benefits such as disease prevention and treatment (Dureja, *et al.*, 2003).

Herbal teas are actually mixtures of many ingredients and are extra-accurately referred to as 'Tisanes.' Tisanes consist of mixtures of dried leaves, seeds, grasses, nuts, barks, fruits, flowers or other botanical elements that give them their taste and provide the benefits of herbal teas (Killedar *et al.*, 2017). Green teas are usually produced from younger leaves, leaf buds and internodes of the tea plant Camellia sinensis or Camellia assamica. It contains caffeine and it has been used to increase alertness.

Therefore, herbal teas were developed using mulberry leaves to improve the flavour, aroma and health promoting properties that could substitute commercial green tea. The herbs specifically *Murraya koenigii* leaves, *Moringa oleifera* leaves, *Phyllanthus emblicaberries*, *Zingiber officinale* rhizome, *Apium graveolens* leaves, *Ocimum basilicum* leaves had been used to develop a healthy ideal and safe tea appropriate for all age groups.

#### 2. Material and methods

Fresh leaves of the mulberry variety V1 were harvested from the Department of Sericulture, GKVK, Bengaluru, in the early morning. Only the top, tender, fresh leaves were selected for the study. The leaves were shade-dried to retain their nutrients and bioactive compounds and subsequently stored in polythene bags for further use.

To maximize the retention of bioactive compounds, specific processing techniques were standardized for the selected spices and herbs. Cleaned and sliced ginger was spread thinly on butter paper and dried using a tray drier. Fresh mint (*pudina*) leaves were cleaned, spread thinly on butter paper, and dried in a similar manner. The cumin (*jeera*) seeds were lightly roasted to enhance their flavor and aroma and then ground into a fine powder.

A standardized recipe for flavor-blended mulberry leaf herbal tea was developed. The optimized formulation included 4 g of mulberry leaf powder and 0.6 g each of ginger, cumin, and mint powders. This blend was developed after testing various combinations to ensure an optimal balance of taste and health benefits.

All analyses were performed in triplicate to ensure data reliability. Data were analyzed using Excel, OP Stat (14.139.252) and SPSS software. Analysis of variance (ANOVA) was conducted using one-way ANOVA under a Completely Randomized Design (CRD). Critical difference and F-tests were used to determine levels of significance (LOS). The methodology aligns with the approach described by Baljeet *et al.* (2010).

#### 3. Results and discussion

After the development of products, the highly acceptable flavour blended mulberry leaf powder along with its corresponding control were weighed, homogenized and dried samples were stored in air tight bags for further chemical analysis. Physical, proximate and antinutritional parameters of control and best accepted variation of flavoured mulberry herbal tea was computed. The results are presented in (Table 1, 2, 3 and 4).

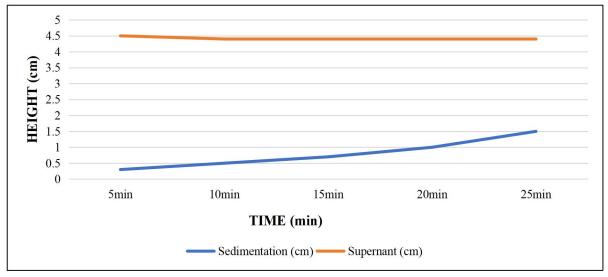
Table 1: Sedimentation rate of flavour blended mulberry herbal leaf powder

Time	Sedimentation (cm)	Supernant (cm)
5min	0.3	4.5
10min	0.5	4.4
15min	0.7	4.4
20min	1.0	4.4
25min	1.5	4.4

Fig 1: Sedimentation and supernant test curve Vs time

The Fig.1 presents a comparison between sedimentation and supernatant levels over a period of 25 minutes. The X-axis denotes time in minutes, ranging from 5 to 25 minutes, while the Y-axis indicates the height of sedimentation and supernatant in centimeter

The blue line represents the sedimentation curve, which showed a steady increase from nearly 0 cm at 5 minutes to about 1.5 cm by 25 minutes. This trend indicates that particles are progressively settling out of the solution, forming a sediment layer at the bottom.



In contrast, the orange line represents the supernatant curve, which starts high at approximately 4.5 cm and remains relatively stable, with a slight decrease to about 4.4 cm over the same period. This stability suggests that while sedimentation occurs, the volume of the clear liquid above the sediment does not significantly change (Table 1 and Fig. 2).

As sedimentation height increases lesser the homogenization, hence less appealing to the consumers. So, within 10 min consumption of flavour blended mulberry tea is best to avoid the wastage.

Table 2: Colour properties of fresh leaves and flavour blended mulberry leaf tea powder

Ingredients	Colours
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	L*	a*	<b>b</b> *
Fresh leaves	48.23±2.42	-13.41±0.63	24.85±1.24
Flavour blended mulberry leaf powder	31.26±1.56	14.13±0.70	17.03±0.85

Brightness ( $L^*$ ), red-green ( $a^*$ ) and blue-yellow ( $b^*$ ), (+L) vs. darker (-L), red (+a) vs. green (-a), and yellow (+b) vs. blue (-b) hues, respectively.

Colour is an important parameter for consumer acceptance of any food/ beverage product. If more the colour retention more the minerals present in it.

The Table.2 compares the colour properties of fresh mulberry leaves and shade-dried flavoured mulberry leaf powder using the Minolta chroma meter colour space. For fresh leaves, the lightness value ( $L^*$ ) was 48.23, indicating moderate brightness, with a negative  $a^*$  value of -13.41, showing a green hue, and  $b^*$  value of 24.85, indicated a strong yellow hue. In contrast, the shade-dried flavoured blended mulberry leaf tea powder had a lower  $L^*$  value of 31.26, indicating it is darker. It's  $a^*$  value shifts to 14.13, suggesting a change from a green to a red hue, while the  $b^*$  value decreases to 17.03, indicating a less intense yellow colour compared to fresh leaves.

These changes suggested that the drying and flavouring process darkens the leaves, shifts their colour balance from green to red, and reduces their yellow intensity, due to chemical changes such as chlorophyll degradation and pigment concentration.

The result aligns with studies reported by Sarkhel *et al.* (2022) found that fresh mulberry leaves had  $L^*$  (49.50),  $a^*$  (-12.76) and  $b^*$  (24.85), whereas for shade dried mulberry leaves had  $L^*$  (31.2),  $a^*$  (14.13) and  $b^*$  (17.03).

The findings correlate with studies reported by Roshanak *et al.* (2015) analysed several drying treatments such as sun, shade, oven (60 °C, 80 °C, 100 °C), microwave and freeze-drying for the drying of green tea leaves. Results showed that the colours for the shade dried leaves were  $L^*$  (21.60),  $a^*$  (-1.19) and  $b^*$  (8.50), respectively.

#### Proximate composition of flavour blended mulberry leaf powder

The proximate composition like moisture, protein of control and highly accepted flavoured mulberry herbal tea had been analysed and depicted in Table.3

It was found that Mulberry leaf tea powder with 4g and 0.6 of ginger, jeera and pudina each had moisture (7.8 %), Protein (23g), anti-oxidants 32 (RSA %), pH (5.8), Titratable acidity (1.66 %), Total soluble solids (8.20 °Bx) and 1-DNJ (0.132 mg/g) and control mulberry tea powder only had moisture (7.4%), Protein (21.3g), anti-oxidants 30.1 (RSA %), pH (5.6), Titratable acidity (2.77 %), Total soluble solids (6.80 °Bx) and 1-DNJ (0.130 mg/g).

There was non-significant difference between control and flavoured mulberry tea powder for moisture, protein, anti-oxidants, titratable acidity and 1-DNJ, expect pH and Total soluble solids. Flavoured mulberry tea powder and control had on par values for proximate composition. Probably due to incorporation at smaller per cent, there was no variation between control and flavoured mulberry leaf powder.

**Moisture content:** The total moisture content in flavour blended mulberry herbal tea (FB-MTP) powder was 7.8 per cent. The findings are similar with studies reported by Srivastava *et al.* (2009) that in dried mulberry leaf powder, moisture ranged from 5.11 to 7.24 per cent. It suggests that it has enhanced water retention capabilities, likely due to the presence of hydrophilic compounds, which could contribute to a fuller mouthfeel.

#### **Protein content:**

The total protein content in flavour blended mulberry herbal tea powder was 23g. Similar trend was reported by Vu *et al.* (2011) to determine the crude protein content of mulberry leaves. The results showed that the protein content was around 22.30 per cent in control. FB-MTP indicates a richer supply of amino acids or bioactive peptides, potentially contributing to a creamier texture and enhanced nutritional value in the tea.

#### **Anti-oxidant content:**

The total anti-oxidant content in flavour blended mulberry herbal tea powder was 32 per cent. The results are consistent with study reported by Jantwal *et al.* (2018) found that the powdered dry mulberry leaves had antioxidant activity ranging from (30.41 to 50.7 %), whereas fresh mulberry leaves had an activity range of (27.52 to 45.53 %). FB-MTP reflects

a higher concentration of bioactive compounds, which provide greater health benefits by neutralizing free radicals.

## pH and titrable acidity:

The pH of flavour blended mulberry herbal tea powder was 5.8. Gaxiola *et al.* (2017) studied drying processes at temperature of 60, 80, 100 and 120 °C of agave leaves and dried agave leaf has pH values from 4.75 to 4.98.

FB-MTP suggests that the flavouring process does not significantly alter the tea's acidity, maintaining a mild and balanced flavour profile.

## **Total soluble solids (TSS)**

The TSS of flavour blended mulberry herbal tea powder was 8.10 per cent. Yang *et al.* 2022 found that mulberry leaf vegetable in packed in MP20 modified polyethylene, CK normal polyethylene during storage initially it had (10.5 to 11.0.3 %) and (10.55 to 11.79 %), respectively.

FB-MTP has a richer concentration of dissolved nutrients, likely enhancing the teas body and sweetness.

## 1-De-oxynojirimycin (1-DNJ)

The DNJ content in flavour blended mulberry herbal tea powder was (0.132 mg/g). Ji *et al.* (2016) found average content of DNJ was 1.53 mg/g, while the total contents of DNJ in the 29 mulberry leaf sample ranged from 0.20 to 3.88 mg/g.

Table 3: Proximate composition of flavour blended mulberry leaf powder (FB-MLP) (per 100g)

Nutrients	Control	FB-MTP	t value
Moisture (%)	7.4±0.37	7.8±0.39	1.28 <sup>NS</sup>
Protein (g/100g)	21.3±1.06	23.00±0.92	$2.09^{ m NS}$
Antioxidants (%)	30.1± 1.50	32.00±1.28	1.66 <sup>NS</sup>
pН	5.6±0.28	5.8±0.23	$0.95^{ m NS}$
Titratable acidity (%)	2.77±0.08	1.66±0.11	13.88*
Total soluble solids (°Bx)	6.80±0.34	8.10±0.41	4.51*
1-DNJ (mg/g)	0.130±0.0065	0.132±0.006	$0.18^{\rm NS}$

T test - NS-non significant, \* significant, FB-MTP- flavour blended mulberry tea powder

# Anti-nutrient content of best accepted flavour blended mulberry leaf powder

Tannins content in flavour blended mulberry herbal tea powder was 2.34 (mg TAE/g). Ramya and Chandrashekar (2020) reported comparable results. The anti-nutrient content of a chapathi mix with (5 %) mulberry leaf was phytic acid (28.2 mg), oxalates (25.67 mg) and tannin (0.606 mg).

Tannins, a type of polyphenol, bind proteins and essential minerals, reducing their digestibility and bioavailability, which can hinder nutrient absorption, especially of iron and zinc, potentially leading to deficiencies.

Flavonoids content in flavour blended mulberry herbal tea powder was 32.56 (mg RE/g). Yanfang *et al.* (2018) that examined mulberry leaves and found significant genotypic variation especially in the total flavonoid concentration, which ranged from 21.36 to 56.41 mg RE/g.

Table 4: Tannin and flavonoid content of flavour blended mulberry leaf powder

Anti-nutrients	Control	FB-MTP	t value
Tannins (mg TAE/g)	2.01	2.34	$3.40^{ m NS}$

Flavonoids(mg RE/g)	29.03	32.56	2.40 <sup>NS</sup>
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#### **Conclusion:**

On the basis of the results obtained from the present investigation showed that mulberry leaves and blending of flavours showed improved protein (23 g), antioxidant (32%), and total soluble solids (8.1 °Bx) levels, with minimal differences in moisture and 1-DNJ content. Sedimentation studies suggested consuming the tea within 10 minutes for better homogenization. Shade drying and flavour blending significantly altered the tea's colour, shifting it to a darker, redder hue due to pigment changes. Overall, the flavoured mulberry tea powder is nutritionally rich, consumer-friendly, and a promising addition to the functional beverage market that they can be proven to be an excellent source of nutraceuticals and flavouring agents. Based on these findings, it is recommended to use mulberry leaves for herbal tea preparation since it possessed high protein and anti-oxidant activity which is beneficial for human health.

## **DISCLAIMER (ARTIFICIAL INTELLIGENCE):**

Author(s) hereby declare that NO generative AI technologies such as large language models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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