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Nutritional, Phytochemical, and Antioxidant Evaluation of White Mulberry (*Morus alba*) Leaf Powder-infused Green Tea

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Abstract

Background: Mulberry (*Morus alba* L.) leaves have long been used in traditional medicine for their diuretic, hypoglycaemic, and hypotensive properties. The leaves are rich in bioactive compounds, making them valuable for therapeutic and nutritional applications. **Aim:** To prepare and standardise white Mulberry Leaf Powder (MLP)–infused plain green tea using different drying techniques and to evaluate its sensory, nutritional, phytochemical, and antioxidant properties. **Methods:** White mulberry leaves were dehydrated using three methods: hot air oven drying, shadow drying, and roasting. The dried leaves were powdered and prepared in three variations (1 g, 2 g, and 3 g). Each variation was infused in water at 100°C for 1 min (water-to-material ratio of 100:1, 2, and 3 ml/g), cooled to 35°C, drained, and consumed. Sensory evaluation was conducted based on colour, flavour, taste, and overall acceptability to identify the best drying treatment. **Results:** Proximate analysis was performed using standard procedures, and antioxidant activity was assessed using the 1,1-diphenyl-2-picryl hydrazyl **(**DPPH) free radical scavenging method. Sensory evaluation revealed that shadow-dried samples received lower scores and were rejected. A statistically significant difference ($p<0.05$) was observed between hot air oven–dried and roasted samples for nutritional parameters. The roasted MLP-infused plain green tea (T2MLPPGT) showed superior levels of ash, carbohydrates, protein, fibre, iron, phosphorus, calcium, vitamin C, and tannins. It also exhibited higher antioxidant activity. **Conclusion:** Roasted white MLP–infused plain green tea demonstrated better sensory acceptability, improved nutritional composition, and enhanced antioxidant activity, indicating its potential as a refreshing and health-promoting herbal beverage for Indian consumers.

Major Findings: Roasted MLP-infused green tea had significantly higher mineral, fibre, protein, tannin, and antioxidant (DPPH) content than the hot-air-oven-dried variant, with better overall sensory acceptability, supporting its potential as a functional beverage.

Keywords: Drying, Green Tea, Mulberry, Nutrients

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1. Introduction

Tea, being the most consumed beverage by two-thirds of the world's population, is derived from the processed leaf of *Camellia sinensis*. Tea types, depending on the processing of the leaf or the development of the leaf, are black, green, or oolong types. These are the main types of tea, which differ in the process of production of tea according to the different processes of drying and fermentation. Green tea is prepared using young tea leaves, and the leaves are then sold for consumption without undergoing the fermentation process after withering, steaming, or pan-firing, drying, and grading. During the production of the tea, the tea leaves need

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to be subjected to the process of pan firing to ensure that the tea leaves do not undergo the fermentation process due to the enzymes naturally present in the tea leaves. The tea leaves need to be subjected to the fermentation process for a few hours before they are then subjected to the process of smoke firing, flame firing, or steaming to prepare black tea. Oolong tea is prepared through the partial oxidation of the tea leaves, similar to the oxidation of the tea leaves during the preparation of green or black tea. Green tea contains polyphenolic compounds such as epigallocatechin-3-gallate, epigallocatechin, epicatechin-3-gallate, and epicatechin. Flavonols, including quercetin, kaempferol, myricetin, and their glycosides/flavonoid glycosides, are also found in green tea. A cup of green tea contains 250 to 350 mg of solids, of which 30 to 42 per cent is catechins, while 3 to 6 per cent is caffeine[1,2].

Mulberry (*Morus alba* L.) is a perennial, woody plant that belongs to the family *Moraceae*. It is a flowering and deciduous plant that grows in Asia, Europe, and America[3-5]. Mulberry has been cultivated in China since ancient times[6]. During that period, apart from using the leaves of the mulberry plant to rear silkworms, the leaves were used as a traditional Chinese medicine because of their efficiency in treating liver, lung, dryness syndrome, wind heat syndrome, and vision impairment symptoms[7]. The amount of nutritional and functional components in mulberry leaves was significantly impacted by the cultivar, time of harvest, and degree of maturity[8-11]. Compared to mature leaves, the upper leaves had more active compounds[12]. Tender shoots from eight mulberry cultivars were examined for ascorbic acid, carotenoid, total soluble protein, total soluble sugar, fructose, and sucrose contents. The research results demonstrated that several mulberry cultivars clearly differ in a few nutrients and taste components. According to Hu *et al.*[12], mature leaves from mulberry cultivars had DNJ (1-deoxynojirimycin) contents ranging from 0.1341 to 1.472 mg/g DW. Depending on the cultivars, mulberry leaves had different phenolic concentrations[13].

In recent years, the development of biotechnology has greatly increased the level of public knowledge about mulberry leaves. From a chemical composition point of view, mulberry leaves contain nutrients such as crude protein content (27.6-37.4%), soluble carbohydrates (5.87-15.0%), and crude fibre content (9.90-13.9%). Mulberry leaves are also rich in other nutrients such as 1-deoxynojirimycin, phenolic acids, flavonoids, alkaloids, and GABA[14-16]. Mulberry leaves are therefore considered a food resource that has a lot of development potential and are currently being utilised in the production of different kinds of food products[17,18]. The nutritional composition of MLs (Mulberry Leaves) varies in different studies. This could be attributed to different factors such as variety, genetics, environment, ecology, and plant harvest. In the early studies done on MLs, it was established that fresh MLs contain moisture content (71.13-76.68%), crude protein content (4.72-9.96%), total ash content (4.26-5.32%), NDF (Neutral Detergent Fiber) content (8.15-11.32%), crude fat content (0.64-1.51%), carbohydrates (8.01-13.42%), and energy content (69-86 kcal/100g)[19].

One of the vital protein sources of the plant world, mulberry leaf protein, has tremendous potential for development. The three major benefits of MLP indicate a promising market opportunity. The benefits are as follows: The high yield of mulberry leaf protein indicates a cost-effective opportunity to produce various plant-based beverages and proteins. The technology for the extraction of protein is already developed and in use for emulsification. The unique bioactive properties of mulberry leaf protein indicate a promising opportunity for nutraceutical and functional food products for improving metabolism. The substantial yield and low cost of mulberry leaves in China provide a good opportunity to produce mulberry leaf protein[20,21]. What is more, mulberry leaves possess high antioxidant capacity. Statistical data reveal that in India, the consumption of mulberry leaf tea is at a minimum. It is rich in various phytochemicals and antioxidant properties with respect to numerous disease conditions. The study aims to incorporate MLP in tea (plain) to prepare and standardise white mulberry leaf (*Morus alba*) powder–infused plain green tea and to evaluate its sensory characteristics, nutritional composition, phytochemical profile, and antioxidant activity.

2. Materials and Methods

The methodology is discussed under the following headings:

2.1 Selection and Procurement of White Mulberry Leaves

White mulberry leaves are rich in nutrients with high antioxidant properties. The mulberry plant ensures the

presence of valuable phyto-nutrients in all its parts. The leaves procured for the study were very fresh and bright green and were collected from the sericulture field situated in the Palani region. The mulberry leaves used for the study have obtained authentic certification from the Botanical Survey of India, located in Coimbatore District.

2.2 Steps Involved in the Processing of White Mulberry Leaves into Powder

The collected mulberry leaves were washed thoroughly with water. Then the leaves were dehydrated using three drying techniques, such as a hot air oven, shadow drying, and roasting methods. The steps involved in processing mulberry leaves into powder are represented in Figure 1.

2.3 Preparation of White MLP Infused Plain Green Tea

The processed white MLP, which was dehydrated under three different drying techniques such as Hot air oven (T_1H), Roasting (T_2R), and shadow drying (T_3S), was categorised into three varying proportions, such as 1g (V_1), 2g (V_2), and 3g (V_3). Each of the three varying proportions V_1, V_2, V_3 of white MLPs, dehydrated under the three different drying techniques, was then immersed in water at a temperature of 100°C for 1 min (water and material ratio of 100: 1, 2, 3 ml/g), then immediately cooled at room temperature 35°C for 1 min, drained, and consumed. One of the studies showed that mulberry leaf extract was prepared according to Katsube *et al.* with minor modifications[22].

2.4 Standardisation of white MLP Infused Plain Green Tea

The white MLPs dehydrated under 3 drying techniques were standardised with 3 three varying proportions of mulberry powder: 1 g, 2 g, 3 g (V_1, V_2, V_3) by infusing into 100 ml of boiling water for a minute, and after complete infusion, it was then drained and consumed. The prepared mulberry plain tea was further standardised by sensory evaluation using sensory attributes like colour, flavour, taste, and overall acceptability to determine the best variation out of the three drying treatments adopted in the study. Based on panellists' evaluation of sensory traits, the MLP dehydrated under hot air oven drying and roasting methods have been selected for experimental analysis, whilst the shadow-dried samples were rejected by the panellists due to lower sensory scores (Table 1).

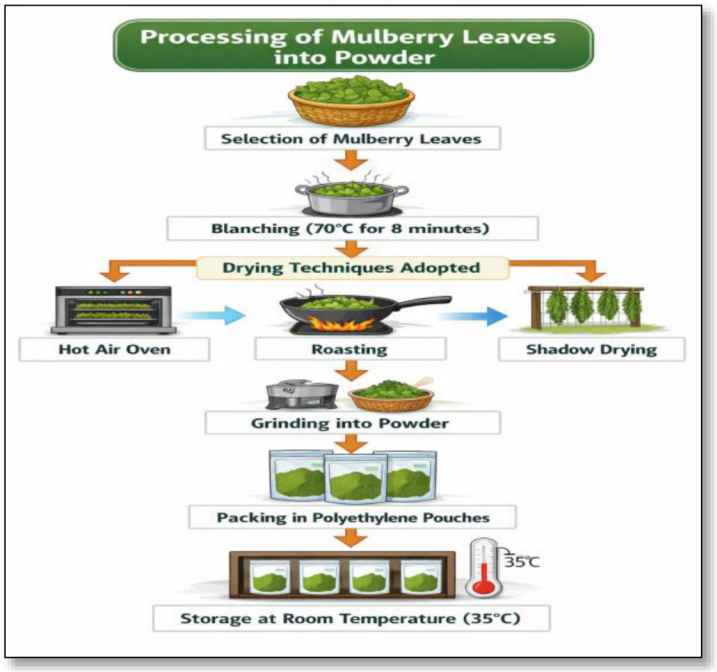


Figure 1. Steps involved in the processing of mulberry leaves into powder.

Table 1. Standardisation of white MLP infused plain green tea

Drying methods	Ingredients	Variation V_1 (infusion in 100 ml water)	Variation V_2 (infusion in 100 ml water)	Variation V_3 (infusion in 100 ml water)
Hot air oven	Mulberry Leaf Powder (MLP)	1 g	2 g	3 g
Roasting		1 g	2 g	3 g
Shadow		1 g	2 g	3 g

2.5 Sensory Evaluation of White MLP Infused Plain Green Tea

Sensory evaluation of plain green tea samples was conducted to determine the preference rating of tea for the flavour, taste, colour, and general acceptability. Tea samples of 1 g, 2 g, and 3 g tea were infused with 100ml of fresh boiling water for 5 min, after which the powder was transferred to a 100 mL tea tasting porcelain bowl for quality assessment. However, the threshold sensitivity test was conducted for the panellists before the commencement of the evaluation. Subsequently, the tea samples were provided one after another to the panellists. Sensory evaluation was conducted in the panel room at controlled temperatures and relative humidity. The panel room was completely free from food/chemical odours, unnecessary sounds, and mixing of daylight. The judges were provided with the prescribed questionnaire for the sensory evaluation.

2.6 Physico-chemical Analysis of White MLP

Physicochemical composition of the white MLP comprising moisture and ash was quantified following official procedures. Moisture was estimated by heating the sample to a constant weight at 105 °C using an oven. Total ash was estimated by burning the samples in a muffle furnace at 550 °C for 4 hours to remove all the organic materials[24].

2.7 Chemical Analysis of White MLP Infused Plain Green Tea

The chemical composition of fresh white mulberry (*Morus alba* L.) leaves and dehydrated MLP tea was evaluated using official standardised analytical methods[23,24]. Crude fibre content was determined gravimetrically by subjecting sample residue to sequential acid and alkali boiling followed by ignition in a furnace[23]. Crude protein content was measured using the Kjeldahl digestion method, where digested nitrogen was distilled, titrated, and multiplied by a conversion factor of 6.25[24,29]. Total carbohydrate content was calculated by subtracting the combined sum percentages of moisture, protein, fat, ash, and fibre from 100%[25]. For mineral analysis, samples were first wet-digested using a concentrated nitric acid and perchloric acid mixture in a 4:1 ratio. Calcium and iron levels were measured directly from the digested solution using Atomic Absorption Spectrophotometry (AAS), whereas phosphorus content was measured spectrophotometrically at 470 nm using the ammonium molybdate-vanadate colorimetric method[8,24]. Finally, ascorbic acid content in the aqueous extracts was estimated by titrating sample extracts against a standard 2,6-dichlorophenolindophenol (DCPIP) dye solution until a rose-pink endpoint persisted[23,30].

2.8 Phytochemical Analysis

2.8.1 Qualitative Phytochemical Analysis of White MLP Infused Plain Green Tea

The Phytochemical screening of crude ethanol and aqueous extracts of Mulberry plant samples was carried out. The screening of methanolic extracts of Mulberry plant samples using the Soxhlet extraction method revealed the presence of alkaloids, carbohydrates, reducing sugars, cardiac glycosides, phenolic compounds, flavonoids, tannins, and resins in fruit and stem extracts of the Mulberry plant. The absence of carbohydrates was noted in stem extracts. The leaves of the Mulberry plant contained flavonoids, quinones, and tannins[25].

2.8.1.1 Tannins

Plant material (200 mg) was extracted with 10 mL of distilled water and filtered. A 2 mL aliquot of the filtrate

was treated with 2 mL of 5% ferric chloride solution, which gave a blue-black precipitate, indicating the presence of Tannins[31,32].

2.8.1.2 Alkaloids

Plant material (200 mg) was extracted with 10 mL of methanol and filtered. The filtrate was acidified with 1% hydrochloric acid, heated, and treated with 6 drops of Wagner's reagent. Gave a brownish-red precipitate, indicating the presence of alkaloids[31,32].

2.8.1.3 Saponins

The plant filtrate was diluted with 5 mL of distilled water and shaken vigorously for 2 min to assess foam persistence, and frothing persisted, indicating the presence of saponins[31,32].

Cardiac Glycosides: A 2 mL aliquot of the filtrate was combined with 1 mL of glacial acetic acid, 1 mL of ferric chloride solution, and 1 mL of concentrated sulfuric acid. Green-blue colour indicated the presence of cardiac glycosides[31,32].

2.8.1.4 Steroids and Terpenoids

Plant material (200 mg) was extracted with 10 mL of chloroform and filtered. A 2 mL aliquot of the filtrate was treated with 2 mL of acetic anhydride and 2 mL of concentrated sulphuric acid. A blue-green ring indicated the presence of terpenoids[31,32].

2.8.1.5 Flavonoids

Plant material (200 mg) was extracted with 10 mL of ethanol and filtered. A 2 mL aliquot of the filtrate was treated with concentrated hydrochloric acid and magnesium ribbon fragments. A pink-to-tomato red colour indicated the presence of flavonoids[31,32].

2.8.2 Quantitative Phytochemical Analysis of white MLP Infused Plain Green Tea

Quantitative estimation of major phytochemical constituents in aqueous Quantitative estimation of major phytochemical constituents in aqueous extracts of white MLP-infused plain green tea formulations was carried out using spectrophotometric techniques. Total tannin content was determined using Folin-Denis colorimetric method where 1.0 mL of sample extract was mixed with 75 mL distilled water, 5.0 mL of Folin-Denis reagent, and 10.0 mL of saturated sodium carbonate (Na_2CO_3) solution to make up to 100 mL; where tannin-like compounds reduce phosphotungstomolybdic acid in alkaline medium to form molybdenum blue complex and absorbance was read at 700 nm after 30 min of incubation in dark and values were calculated as mg Tannic Acid Equivalents per gram (mg TAE/g) using standard graph of tannic acid (0–100 µg/mL). Total phenolic content was determined using Folin-Ciocalteu reagent at 765 nm and was expressed as mg Gallic Acid Equivalents per gram (mg GAE/g), whereas total flavonoid content was estimated using $AlCl_3$ colorimetric technique at 415 nm and was expressed as mg Quercetin Equivalents per gram (mg QE/g)[33].

2.9 Antioxidant (DPPH Free Radical Scavenging Activity) of Plain Green Tea

The ability of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals to be scavenged by the mulberry leaf tea extract was determined using the spectrophotometric method described by Brand-Williams *et al*[37]. An aliquot (100 µL) of the mulberry leaf tea extract was added to 3.9 mL of the 0.1 mM methanolic DPPH solution. This mixture was then vortexed vigorously and allowed to stand for 30 minutes in the absence of light. The absorbance was read at 517 nm using a UV-Visible spectrophotometer against a methanol blank. Ascorbic acid/BHT was used as the positive control standard[34].

3. Results

3.1 Comparative Sensory Evaluation of White MLP Infused Plain Green Tea Across Different Processing Treatments

In the present study, there were three dehydration treatments- hot air oven drying, roasting, and shadow drying methods- were carried out to make the mulberry leaves moisture-free and fine enough to grind into powder form to prepare plain tea. Based on this treatment process, there were three plain tea samples with respect to each dehydration treatment adopted for the mulberry leaves as follows: Concerning the sensory attributes of the plain tea samples, the test sample scored the highest for all attributes like taste (3.12), colour (3.12), flavour (3.64), and overall acceptability (3.28). Regarding the T_1H sample, all the variations of the samples V_1, V_2, and

V_3 have scored almost the highest values. However, V_2 has the highest score based on consumer acceptability with respect to the sensory attributes. Regardless of the varying proportions (V_1, V2, V_3) of infusion of T_2R MLPs used for the tea preparations. V_2 has been selected as the best variation in terms of sensory properties according to consumer preference. Irrespective of the varying proportion of infusion of MLP, which was dehydrated under the shadow drying method, variation 2 has scored the highest in terms of sensory properties such as taste (3.24), colour (3.28), flavour (3.36) and overall acceptability (3.76), as given in Table 2 and Figure 2.

Table 2. Sensory evaluation of samples processed by different drying methods at various concentrations

Criteria	T1H (Hot air oven)			T2R (roasted)			T3S (shadow dried)		
	V1 (1g)	V2 (2g)	V3 (3g)	V1 (1g)	V2 (2g)	V3 (3g)	V1 (1g)	V2 (2g)	V3 (3g)
Taste	3.00±1.09	3.12±1.04	2.68±1.28	2.68±1.02	3.40±1.08	2.72±1.20	3.08±1.20	3.24±0.95	3.08±1.38
Colour	3.12±1.09	3.36±1.35	3.08±1.28	3.08±1.41	2.90±1.42	2.84±1.40	3.24±1.20	3.28±1.48	2.92±1.57
Flavour	3.48±0.82	3.64±0.56	3.08±1.07	3.48±0.82	3.56±0.82	3.28±1.02	3.32±0.85	3.36±0.75	3.00±1.00
Overall Acceptability	3.12±0.72	3.28±0.67	3.08±1.03	3.04±0.97	3.40±0.88	2.68±0.60	2.88±0.60	3.76±0.77	3.04±1.05

Values are Mean±stdv, V_1 - Variation1 (1g), V_2 - Variation 2(2g), V_3 - Variation 3(3g), (T_1HMLPPGT) - Hot air oven-dried MLP incorporated into plain green tea, (T_2RMLPPGT) - Roasted MLP incorporated into plain green tea, (T_3SMLPPGT) - Shadow-dried MLP incorporated into plain green tea.

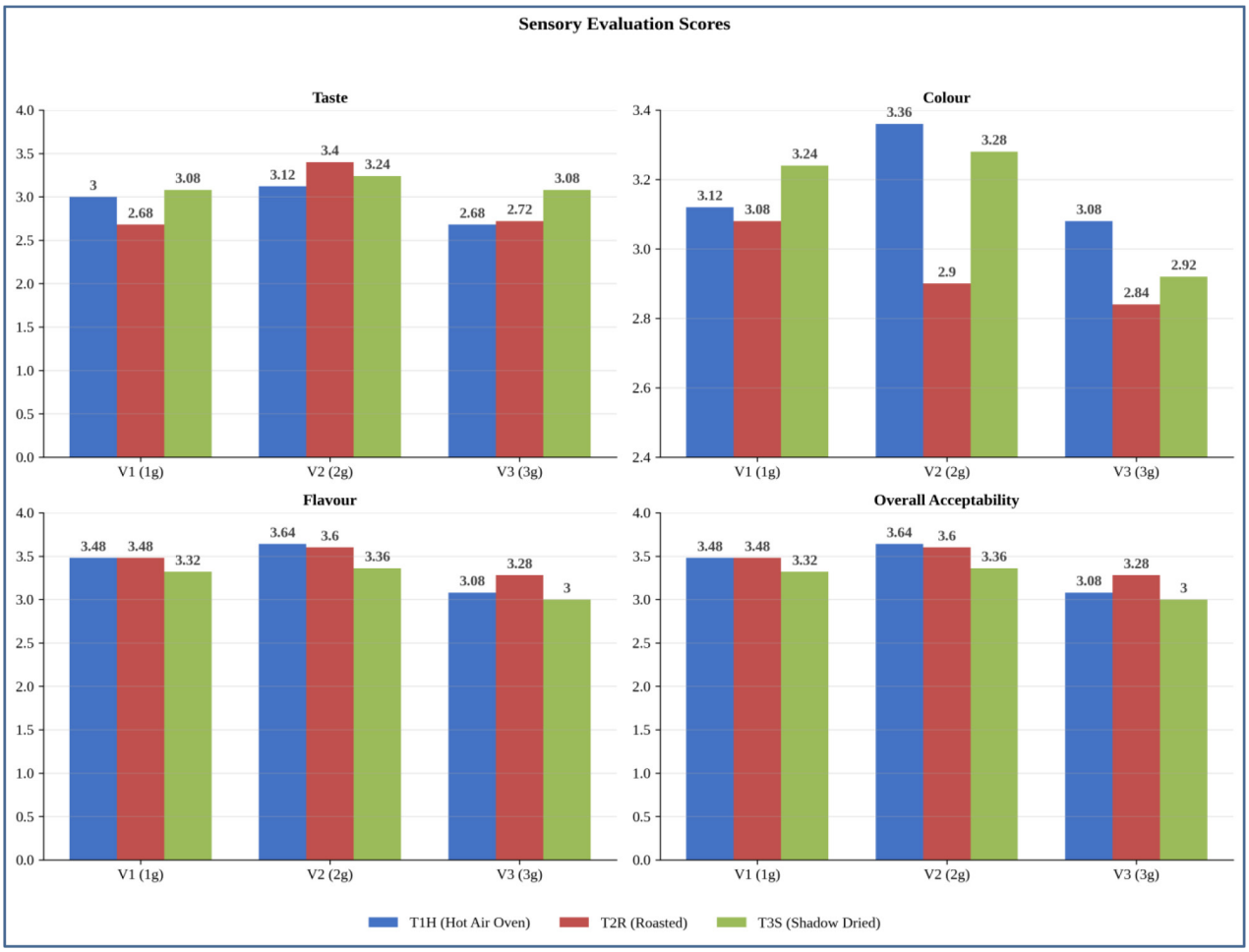


Figure 2. Sensory evaluation of samples processed by different drying methods.

3.2 Evaluation of Physicochemical Parameters of White MLP and Infused Plain Green Tea (MLPPGT)

The physicochemical constituents, like moisture and ash, were analysed for the MLP using standard procedures[23]. pH level was assessed for plain tea samples to determine the acidic or alkaline nature of the samples.

3.2.1 The Moisture and Ash Level of the White MLP

Irrespective of the drying techniques adopted, there was no significant difference was observedbetweenT_1HMLP (4.5) and T_2RMLP (4.6) at $p<0.005$. Also, the moisture content was lower in the dehydrated powders, which could be stored for a longer period and used instantly to prepare green tea, even during non-seasonal times. Generally, ash content reflects the total mineral profile of any food. The ash content of both T1 and T_2R samples consists of higher values, which contribute nutritional and therapeutic significance with respect to ailments. Regarding the ash content of the MLP sample, a significant (5% level) difference was noted between T_1H and T_2R samples, with higher values possessed by the T_2R sample, as presented in Table 3.

3.3 Physicochemical Properties of MLPPGT

3.3.1 pH value of White MLP-infused Plain Green Tea

The p^H value represents the acidic or alkaline nature of the food samples, which generally depends on the concentration of hydrogen ions. The p^H value of T_1HMLPPGT and T_2RMLPPGT remained almost the same, with no significant difference. This was observed statistically at $p<0.05$; MLP plain tea was obtained from the two treatments (T_1HMLPPGT and T_2RMLPPGT), 7.8 and 7.9, respectively. The values of the T_1HMLPPGT and T_2RMLPPGT ranged between 7.8 and 7.9, respectively. These represent the alkaline nature of the plain tea, which was preferred by consumers with greater acceptance in terms of sensory traits. The pH value of the white MLP is presented in Table 4.

3.3.2 Chemical Properties of MLP Infused Plain Green Tea (MLPPGT)

The chemical analysis of the mulberry leaf plain tea reveals nutrients such as carbohydrate, protein, calcium, and crude fibre, along with minerals like iron, phosphorus, and ascorbic acid. The carbohydrate content of the T_1HMLPPGT and T_2RMLPPGT samples was 42 g and 30 g, respectively. There was a significant difference was observed between the two samples ($p<0.05$). Whereas the greatest carbohydrate values were possessed by T_2RMLPPGT due to the application of drying. Crude protein content was calculated as N × 6.25 by the Kjeldahl Method.

It has been noted that the protein value of plain MLP tea is 9.5 g in a hot air oven-dried sample and 11.5 g in roasted samples. Also, a significant difference was noted at $p<0.05$. Statistically, higher values of protein were observed in roasted samples. Also noted that the fibre value of MLP was 29.6 g in the hot air oven-dried sample and 56.8 g in the roasted samples. Statistically, the greater values of crude fibre were observed in roasted samples. The two different samples (T_1HMLPPGT and T_2RMLPPGT) showed a statistically significant difference at ($p<0.005$).

The calcium content of hot air oven-dried MLP tea was 782 mg, and roasted mulberry leaf tea was 850 mg, respectively. A statistically significant difference was noted at $p<0.005$, with greater calcium content noted in T_2RMLPPGT; due to the heat-sensitive nature of minerals, the values might be reduced in the hot air oven-dried sample. The iron content of hot air oven-dried mulberry leaf plain green tea was 25.3 mg and roasted mulberry leaf plain green tea was 33.16 mg, respectively. A statistically significant difference was noted ($p<0.005$), with greater iron content noted in T_2RMLPPGT, due to the heat-sensitive nature of

Table 3. The moisture and ash level of the white MLP

Parameter	T1HMLP (hot air oven)	T2RMLP (roasted)	pvalue (t-test)	Significance
Moisture (%)	4.54 ± 0.50	4.60 ± 0.70	0.400	NS
Ash (%)	12.30 ± 0.23	14.60 ± 0.57	0.006	*

pvalue<0.05, Values - means±std, *- significant NS - not significant, T_1HMLP- Hot air oven MLP, T_2RMLP- Roasted MLP

Table 4. pH value of the white mulberry leaf plain green tea

Criteria	pH	pvalue (t-test)
T_1HMLPPGT	7.8±0.28	0.4^{NS}
T_2RMLPPGT	7.9±0.05	

pvalue<0.05, Values - means±std, *- significant NS- not significant.

the studies that assessed the amino acid content of MLP utilised acid hydrolysis. This process fails to detect tryptophan. Future studies could improve the amino acid composition profile of MLP using alkaline hydrolysis[26,27]. A well-balanced amino acid composition profile is a major factor in assessing the nutritional value of a protein, as shown in Table 5.

Table 5. Chemical properties of MLP-infused Plain Green Tea (MLPPGT)

Parameter	T1HMLPPGT (Hot Air Oven)	T2RMLPPGT (Roasted)	pvalue (t-test)	Significance
Carbohydrate (g)	42 ± 0.34	30 ± 0.28	0.002*	*
Protein (g)	9.5 ± 0.5	11.5 ± 0.86	0.02*	*
Crude Fibre (g)	29.6 ± 0.34	56.8 ± 0.72	0.0004*	*
Calcium (mg)	782 ± 2.51	850 ± 0.76	0.0001*	*
Iron (mg)	25.3 ± 0.57	33.16 ± 0.76	0.003*	*
Phosphorus (mg)	753 ± 2.8	795 ± 5	0.006*	*
Ascorbic Acid (mg)	137.3 ± 2.51	158 ± 2	0.0002*	*

pvalue<0.005, Values- means±std, *-significant NS- not significant, T_1HMLPPGT- Hot air oven MLP incorporated plain tea, T_2RMLPPGT- Roasted MLP incorporated into plain tea.

Table 6. Phytochemical analysis of white MLP-infused plain green tea

Photochemical	White MLP plain green tea	
	T_1HMLPPGT	T_2RMLPPGT
Tannin	+++	+++
Saponins	+	+
Alkaloids	++	++
Flavanoids	+++	+++
Terpenoids	++	++
Sugars	_	_
Phytosterols	+	+
Glycosides	_	_
Phenol	+++	+++

+ Low amount, ++ Moderate amount, +++ High amount , - Absence

minerals; the values might be reduced in the hot air oven-dried sample. The phosphorus content of hot air oven-dried mulberry leaf plain tea was 753 mg, and roasted mulberry leaf tea was 795 mg, respectively. The ascorbic acid content of hot air oven-dried mulberry plain green tea was 137.3 mg, and roasted mulberry leaf tea was 158 mg, respectively. A statistically significant difference was noted at p<0.005, with greater ascorbic acid content noted in T_2RMLPPGT. At present, all

3.4 Phytochemical analysis

The photochemical profile of the MLP-infused plain green tea was determined qualitatively and quantitatively.

3.4.1 Qualitative photochemical analysis of MLPPGT

With respect to the qualitative analysis, photochemical analysis of the plain MLP-infused plain green tea showed a greater chance of the presence of tannins, flavonoids, and phenols. The phytochemicals alkaloids, phytosterols and saponins showed less presence and terpenoids and flavonoids had only moderate presence and glycosides and sugars were absent, as shown in Table 6.

3.4.2 Quantitative Analysis of Photochemical

3.4.2.1 Tannins

Tannins are the antioxidant phytochemicals present in mulberry leaves. These antioxidants play a vital role in pharmaceutical applications. The amount of tannins present in T_2RMLPPGT was 143.00 mg, and in T_1HMLPPGT it was 134.60 mg. A statistically significant difference was observed at p<0.05, with higher tannin content in T_2RMLPPGT. The reduction could be due to the volatility and heat sensitivity of the antioxidant phytochemicals. The quantitative analysis

Table 7. Tannin content in white MLP infused in Plain Green Tea

Criteria	Tannins(mg)	pvalue (t-test)
T_1HMLPPGT	134.60±0.51	0.006
T_2RMLPPGT	143.00±1	

pvalue<0.05, Values- means±std, * - significant NS - not significant, T_1HMLPPGT- Hot air oven MLP incorporated plain tea T_2RMLPPGT- Roasted MLP incorporated into plain tea

Table 8. Free radical scavenging activity of white MLP infused in plain green tea

Criteria	DPPH (%)	pvalue (t- test)
T_1HMLPPGT	91.03±0.05	0.03*
T_2RMLPPGT	94.00±0.2	

pvalue<0.05, Values- means±std, * - significant NS - not significant, T_1HMLPPGT - Hot air oven MLP incorporated plain tea T_2RMLPPGT- Roasted MLP incorporated into plain tea.

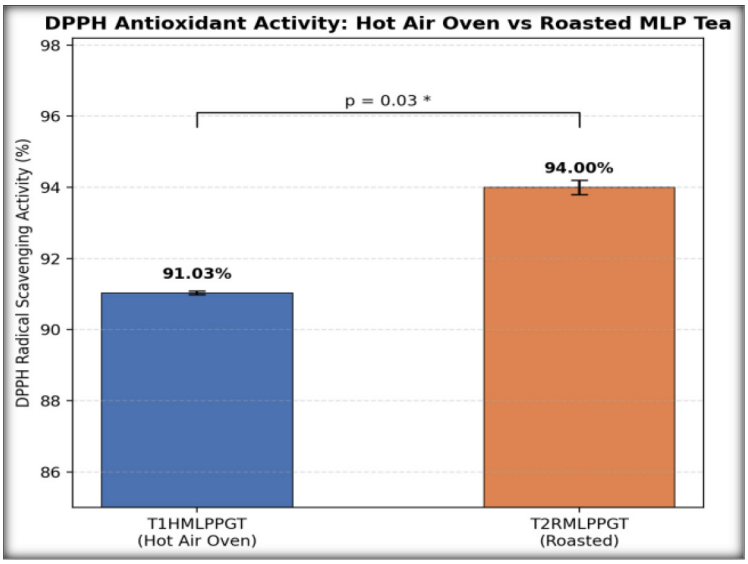


Figure 3. DPPH radical scavenging activity (%) of T1H vs T2R MLP tea.

of tannin content in MLP-infused plain green tea is presented in Table 7.

3.5 Determination of Antioxidant Activity of MLPPGT

DPPH is often employed used to assess biological sample antioxidant activity. The plant extract's propensity for providing electrons or hydrogen atoms was assessed by bleaching a purple methanol solution of DPPH, which served as a benchmark. The stable radical DPPH* is used as a reagent in this spectrophotometric test. This assay's mechanism relies on the discolouration of the DPPH free radical when it reacts with hydrogen-donating species, such as antioxidants found in plant extracts. T1HMLPPGT and T2RMLPPGT had DPPH values of 91.10 and 94.00, respectively, indicating a significant difference ($p<0.05$). According to a recent study, adding paper mulberries to dairy cows can reduce the relative abundance of harmful bacteria like *Tyzzerella*-4.28 and the *taxa Ruminococcaceae* UCG-013[28]. The free radical scavenging activity of white MLP-infused plain green tea is represented in Table 8 and Figure 3.

4. Discussion

The present study evaluated the chemical, antioxidant, and phytochemical properties of MLP infused in plain green tea and assessed its overall acceptability. Mulberry (*Morus* spp. L., *Moraceae*) leaves have long been used in traditional medicine for their diuretic, hypoglycaemic, and antihypertensive properties, largely attributed to their rich bioactive composition. The leaves are known to contain significant amounts of minerals such as calcium, iron, and phosphorus, along with vitamins like ascorbic acid, which contribute to their strong antioxidant potential. Sensory evaluation revealed that variation 2 (2 g incorporation) of both hot air oven–dried and roasted samples received higher mean scores for taste, colour, flavour, and overall acceptability compared to other variations. This

indicates that moderate incorporation levels enhanced consumer preference, making V_2 the most acceptable formulation. Physicochemical analysis showed that the moisture content of both hot air oven–dried and roasted MLPs was almost similar, indicating good shelf stability. However, ash content, which reflects total mineral composition, was higher in the roasted sample (14.60%) compared to the hot air oven- dried sample (12.30%), suggesting better mineral retention in roasted treatments. The pH values of both tea samples were nearly similar, indicating comparable acidity levels.

In terms of proximate composition, the carbohydrate content was higher in the hot air oven–dried sample (42.00 g) compared to the roasted sample (32.00 g). However, protein content was higher in the roasted sample (11.50 g) than in the hot air oven–dried sample (9.50 g). A similar trend was observed for crude fibre, with roasted samples showing significantly higher fibre content (56.80 g) compared to hot air oven–dried samples (29.60 g). This kind of difference may result from variations in the removal of moisture content, concentration of dry matter, and nutrient proportion in relation to the process undergone by the product. The differences in proximate composition in the case of mulberry leaves have been noted in many cases depending upon cultivar, maturity, and process involved. Srivastava *et al.*[29] found that dried mulberry leaves had a content of 15.31-30.91% of crude protein, 27.60-36.66% of neutral detergent fibre, and 9.70-29.64% of carbohydrate. The mineral analysis demonstrated superior values in the roasted samples. Calcium (850 mg), iron (33.1 mg), and phosphorus (795.00 mg) were higher in roasted mulberry leaf tea compared to hot air oven–dried tea.

This result is in accordance with the mineral richness of the mulberry leaves. The amount of calcium was found to be 786.66-2226.66 mg/100 g, while iron content in dried MLP was recorded as 19.00-35.72 mg/100 g in six Morus alba genotypes, thus suggesting that the mulberry leaves could serve as a rich source of calcium and iron from plant sources. As minerals are relatively stable at high temperatures, increased mineral contents in the roasted sample could be accounted for mainly based on differences in moisture content and concentration of total solids, rather than the generation of new minerals due to the process[29].

Vitamin C content was also higher in roasted samples (158.00 mg) than in hot air oven–dried samples (137.30 mg), indicating better retention of ascorbic acid in the roasted treatment. This result lies within the range reported earlier for dried MLP, where values for ascorbic acid between 100–200 mg/100 g were found. However, since ascorbic acid is vulnerable to heat damage, the higher concentration in the roasted sample needs to be explained in terms of a possible greater apparent retention due to the particular roasting conditions rather than an indication of the positive effect of roasting on vitamin C stability in general. Roasting of shorter duration could cause less thermal exposure in comparison to hot air oven drying, whereas the faster rate of moisture loss could also result in higher vitamin C concentrations on an analytical basis. More investigations on the standardised basis of moisture content using various time-temperature combinations are required to confirm the above mechanism. Extraction, rather than formation of new minerals, seems to be the reason for higher mineral content in the roasted samples[29].

Qualitative phytochemical screening revealed the strong presence of tannins, flavonoids, and phenolic compounds, moderate presence of alkaloids, and absence of sugars, saponins, and glycosides. This is in line with the existing phytochemistry of Morus alba leaf extract, which comprises phenolic acids and flavonoid analogs such as chlorogenic acid, rutin, isoquercitrin, astragalin, and quercetin glycosides. They have been identified as being highly important components responsible for the antioxidant properties of the mulberry leaves. The non-existence of sugar, saponin, and glycoside in the qualitative test could be a result of the extraction method used and not necessarily that they are not present in the original plant material[9]. Quantitative tannin analysis showed higher values in roasted samples. Heat processing of the tested samples was seen by Lee and Choi to result in an overall increase in the polyphenol concentration, which was measured. In their roasting test procedure, the total concentration of quantifiable polyphenols rose from 1,005.02 mg/100 g in unprocessed leaves to 1,305.72-1,433.03 mg/100 g after 1-5 min of the roasting process. It is possible that the rise in the polyphenol concentration could be attributed to disruption of cell-wall architecture in the

heated samples, which leads to easier extractability of phenolic compounds in the analysis process.

Furthermore, antioxidant activity was markedly higher in roasted MLP–infused tea (94%) compared to the hot air oven–dried sample, confirming its enhanced free radical scavenging potential. Such an outcome is physiologically reasonable since flavonoids, tannins, and phenolic acids have the potential to donate electrons or hydrogen atoms to stabilise free radicals. Nonetheless, the effect of the heating process on the antioxidants is inconsistent. According to Hu *et al.*[35], the way of drying affected the composition of flavonoids and antioxidants in mulberry leaves, with air drying and freeze drying giving better results in the conservation of flavonoid antioxidants than oven drying for 48 hours at 65°C. In this regard, the higher antioxidant activity of roasting in the current study can be attributed to the roasting temperature and time, increased release of antioxidants, and reduced total exposure to heat when compared with oven drying for an extended period.

Clinical evidence is emerging but remains comparatively limited. In a 12-week placebo-controlled study among individuals with type 2 diabetes mellitus, *Morus alba* extract supplementation significantly reduced serum insulin and malondialdehyde concentrations and increased HDL-cholesterol; however, it did not significantly improve several other measured metabolic outcomes. A more recent meta-analysis reported that mulberry interventions reduced postprandial glucose at 30 and 60 min and produced a modest reduction in HbA1c after 4–16 weeks, while changes in fasting blood glucose, fasting insulin, and lipid profiles were not consistently significant. Thus, white mulberry may have potential as a complementary food-based or nutraceutical approach, particularly for improving postprandial glycaemic control, but it should not be considered a replacement for established dietary management or pharmacotherapy for type 2 diabetes[36].

5. Conclusion

Overall, the present study demonstrates that white mulberry (*Morus alba)* leaf powder can be effectively incorporated into plain green tea to develop a nutritionally enriched, sensory-acceptable herbal beverage. Among the three drying methods trialled, roasting produced tea with the highest sensory acceptability along with superior ash, carbohydrate, protein, fibre, calcium, iron, phosphorus, vitamin C, tannin, and antioxidant (DPPH) values compared with hot-air-oven drying, while shadow-dried samples were rejected on sensory grounds. These findings indicate that roasting is the most suitable processing technique for retaining the nutritional and phytochemical quality of MLP-infused tea. Given its favourable nutrient density and antioxidant potential, roasted white MLP-infused green tea holds promise as a functional, health-promoting beverage for Indian consumers, warranting further clinical and shelf-life studies to validate these benefits.

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Author Contribution

Syed Rubina Fatima Abdul Kani: Presentation of research idea and study design, Data collection, Analysis and interpretation of the data, writing the draft of the article, data analysis, revising and editing the article. Dr M.Sivasakthi: Presentation of research idea and study design, supervising the study and approval of the final version. Radhika Kadumeni: Presentation of research idea and study design, Data collection, Analysis and interpretation of the data. All authors have read and agreed to the published version of the manuscript.