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## Transformation of pomegranate (*Punica granatum* (L.)) leaf waste into a functional beverage and its phytochemical and antioxidant properties

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Pomegranate leaves, once valued in traditional medicine for their health benefits, are now often seen as waste in pomegranate cultivation. Renewed interest in their medicinal properties could support more sustainable agriculture. This study aims to explore the bioactive properties of leaves from four pomegranate cultivars and formulate a herbal tea blend. Samples were collected from authenticated plants cultivated under comparable soil and climatic conditions to ensure consistency and reliability in the study. Leaf samples were screened for phytochemicals and antioxidants to select a superior variety for herbal tea formulation. The total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity, mineral composition, and caffeine content determination and sensory evaluation were conducted using established methodologies. The data were analyzed utilizing ANOVA and the Friedman test using R and SPSS software. The results indicated that pomegranate leaves from all four varieties contained significant levels of TPC, TFC, and antioxidants (DPPH and ORAC) and favourable low caffeine content, demonstrating their suitability as raw materials for herbal tea production. Among the tested blends, a 60 : 40 ratio of pomegranate leaf to black tea received the highest scores across all sensory attributes. Additionally, this blend exhibited a lower caffeine content (0.82% w/w) than commercial black tea (2.23% w/w), establishing it as a healthier, low-caffeine option rich in antioxidants. The findings prove that pomegranate leaf waste can be effectively utilized in the innovative herbal tea industry.

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### Sustainability spotlight

While pomegranate leaves have long been valued for their intrinsic properties, they are often overlooked and categorized as waste. Our research aims to investigate the physical, chemical, and biological properties of various pomegranate varieties grown in Sri Lanka. By transforming these underutilized leaves into a wellness tea, we are pleased to support the United Nations Sustainable Development Goal 3, which promotes health and well-being. This initiative not only seeks to reduce waste but also to enhance the use of local resources for the benefit of our communities.

## 1 Introduction

The growing global interest in functional beverages has sparked a significant focus on the development of sustainable and health-enhancing herbal alternatives.<sup>1</sup> Among these alternatives, pomegranate (*Punica granatum* L.), commonly referred to as the “fruit of paradise”, is indigenous to Central Asia and exhibits adaptability to a wide range of climatic conditions.<sup>2</sup> Furthermore, pomegranate fruit, peel, leaves and membranes exhibit a rich phytochemical profile, containing significant bioactive compounds such as anthocyanins,

ellagic acid, penicillin, punicalagin, pedunculagin, and various flavanols.<sup>3–5</sup> Meanwhile, the application of salicylic acid as a foliar spray has been found to enhance the phytochemical contents of pomegranate leaves.<sup>6</sup> Owing to its abundant bioactive compound content, pomegranate has played a crucial role in traditional medicinal practices, including Ayurveda, for centuries.<sup>7</sup> Currently leaves of pomegranate are mainly considered waste despite their rich phytochemical contents and bioactive compounds. Previous studies have shown that pomegranate leaves possess a range of beneficial properties,<sup>8</sup> including antioxidant, anti-inflammatory, and antimicrobial effects, and potential neurological benefits. Additionally, they may help in the prevention of various cancers, such as breast, lung, bladder, and skin cancers.<sup>9–11</sup> Additionally, pomegranate leaf tea enhances cerebral microcirculation, promotes bile excretion,

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increases intestinal motility, and reduces gastric acid secretion, thereby lowering the risk of gastric ulcers. It also effectively lowers blood lipid levels and regulates lipid metabolism.<sup>12</sup> Sri Lanka cultivates four principal commercial pomegranate cultivars: Kalpitiya hybrid, Nimali, Daya, and Nayana.<sup>3,7</sup> Despite the considerable leaf biomass produced by pomegranate plants, known for their richness in bioactive compounds, this valuable resource remains largely untapped and underutilized.<sup>13</sup> Herbal tea is regarded as a functional beverage, widely popular due to its therapeutic and healing properties.<sup>14</sup> This study explores the bioactive properties of the four pomegranate cultivars to formulate a potent herbal tea blend that promotes wellness.

## 2 Materials and methods

### 2.1 Chemicals and reagents

Folin–Ciocalteu's reagent, gallic acid,  $C_{15}H_{10}O_7 \cdot 2H_2O$  (quercetin), 6-hydroxy-2-5-7-8-tetramethylchroman-2-carboxylic acid (Trolox), 1-1-diphenyl-2-picryl-hydrazyl (DPPH), potassium persulphate, 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH), monosodium dihydrogen orthophosphate, disodium monohydrogen orthophosphate, aluminium chloride, and methanol were purchased from Sigma-Aldrich, USA. All the other chemicals and reagents were of analytical grade and all the analyses were carried out using high-throughput 96-well microplate readers (SpectraMax Plus384, Molecular Devices, USA, and SPECTRA max-Gemini EM, Molecular Devices Inc, USA).

### 2.2 Collection of leaf samples of pomegranate leaves and black tea

Different varieties of pomegranate leaves, including Nimali, Daya, Kalpitiya hybrid, and Nayana, were collected from the Kalpitiya Agriculture Research Station located in Kandakuliya, Sri Lanka. Additionally, commercial tea was purchased from the local market. Fresh pomegranate leaves were carefully separated from the pruned twigs and washed thoroughly with clean water to eliminate any dirt or debris. Following this, the leaves underwent a withering process. Each variety of pomegranate leaves was ground separately using a small-scale leaf grinder. The resulting ground leaves were then fermented for a duration of 24 hours at a controlled temperature of 25 °C, achieving a moisture content of approximately 60–70%, which is optimal for enzymatic reactions. After fermentation, the samples were dried 80 °C for 4 hours. Once dried, the ground samples were graded and stored at room temperature in airtight polythene bags until they were required for extraction.

### 2.3 Sample preparation

Two grams of each sample were extracted using 70% methanol (MeOH) and allowed to soak overnight. The extract was then filtered through Whatman No. 1 filter paper and subsequently centrifuged at 3000 rpm for 15 minutes.

### 2.4 Quantification of antioxidants and antioxidant activity

#### 2.4.1. Determination of total polyphenolic content (TPC).

The total polyphenol content (TPC) of pomegranate leaves was assessed using the Folin–Ciocalteu method as described by Abeysekera *et al.*<sup>15</sup> In this procedure, 110  $\mu$ L of a freshly prepared Folin–Ciocalteu reagent, diluted 10 times, was added to the samples. Following this, 20  $\mu$ L of the sample was incorporated, and the absorbance was measured at 765 nm using a 96-well microplate reader. Subsequently, 70  $\mu$ L of a 10% sodium carbonate solution was added, and the mixture was incubated at room temperature ( $25 \pm 2$  °C) for 30 minutes before taking the absorbance reading again at 765 nm. To establish a standard curve, seven different concentrations of gallic acid (0.015, 0.031, 0.062, 0.125, 0.25, 0.5, and 1 mg mL<sup>-1</sup>) were used. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight (DW).

#### 2.4.2. Determination of total flavonoid content (TFC).

The TFC of pomegranate leaves was determined by the aluminum chloride colorimetric method as described by Wang *et al.*<sup>16</sup> with some slight modifications. Briefly, a 100  $\mu$ L sample was read at 415 nm. Then, 100  $\mu$ L of a 2% aluminum chloride solution was added and incubated at room temperature ( $25 \pm 2$  °C) for 10 minutes, after which the absorbance was read at 415 nm. Three different concentrations of 100  $\mu$ L of quercetin were used as standards, and the results were expressed as milligrams of quercetin equivalents (QE) per gram of dry weight (DW).

**2.4.3. DPPH radical scavenging activity.** The free radical scavenging assay was performed according to the standard method<sup>15</sup> with slight modifications, using Trolox as the standard. Reaction volumes of 200  $\mu$ L were prepared, which included 80  $\mu$ L of DPPH radical, 50  $\mu$ L of the sample, and 60  $\mu$ L of distilled water. The mixture was incubated at room temperature ( $25 \pm 2$  °C) for 15 minutes, and the absorbance was measured at 517 nm. The results were expressed as milligrams of Trolox equivalents (TE) per gram of dry weight (DW).

**2.4.4. Oxygen radical absorbance capacity (ORAC).** The ORAC radical scavenging assay was performed as described by Abeysekera *et al.*<sup>15</sup> with slight modifications. The assay was conducted at 37 °C (pH 7.4), and a blank sample was run in parallel. Trolox standard (0.75  $\mu$ g mL<sup>-1</sup> in phosphate buffer), fluorescein (4.8  $\mu$ M), and APPH (40 mg mL<sup>-1</sup> in phosphate buffer) solutions were prepared prior to use in a phosphate buffer (75  $\mu$ M, pH 7.4). A reaction volume of 200  $\mu$ L was used, containing 100  $\mu$ L of 4.8  $\mu$ M fluorescein and 50  $\mu$ L of the sample, which were pre-incubated at 37 °C for 5 minutes. After this, 50  $\mu$ L of APPH (40 mg mL<sup>-1</sup>) was added, and the mixture was placed on a fluorescence microplate reader (Ex. 494 nm and Em. 535 nm). The decay of fluorescein was recorded at 1-minute intervals over 35 minutes. The results were expressed as milligrams of Trolox equivalent (TE) per gram of dry weight (DW).

### 2.5 Sensory evaluation

Sensory evaluation of three different herbal teas was conducted with the participation of 30 untrained panelists. The



tea samples were assessed based on several attributes, including color, aroma, taste, appearance, mouthfeel, after-taste, and overall acceptability. A 5-point hedonic scale was employed, where 1 indicated “dislike extremely” and 5 denoted “like extremely”.<sup>17</sup> Each sample was coded randomly to eliminate bias and served in 30 mL portions at approximately 65 °C during the tasting session. To minimize the impact of astringent taste, panelists were instructed to rinse their mouths with warm water before evaluating each new sample.<sup>18</sup>

## 2.6 Determination of caffeine content

The caffeine content of the final products was determined using the ISO 14502-2: 2005 (E) method. This internationally recognized protocol involves extracting caffeine from the sample using a specific solvent and quantifying it through high-performance liquid chromatography (HPLC). The caffeine concentration is quantified by comparing the sample's chromatographic peak with that of a known caffeine standard, ensuring accurate determination of the caffeine content in the final product.

## 2.7 Determination of mineral composition

The mineral content was analyzed utilizing inductively coupled plasma atomic emission spectrometry (ICP-AES), in accordance with the methodology outlined by Musa Özcan *et al.*<sup>19</sup> with modifications implemented as per internal procedures. The concentration levels were quantified using an ICP-AES instrument operating under defined conditions.

## 2.8 Determination of ash content

Ash content was determined by standard procedures (AOAC, 925.19-1925).

## 2.9 Determination of moisture content

Moisture content was determined by standard procedures (AOAC, 2007).

## 2.10 Determination of pH content

A pH meter (A215, Thermo Scientific, Korea) was used to assess the pH of each sample. Fresh standardization solutions of pH 4.01 and 6.86 were used to calibrate before using and the readings were obtained in quadruplicate for each sample.<sup>20</sup>

## 2.11 Data analysis

All experimental measurements were performed in quintuplicate and are reported as the average  $\pm$  standard error. For the non-parametric sensory data, the Friedman test was employed with a 95% confidence interval utilizing SPSS software (version 22) to statistically compare the treatments and evaluate significant differences in sensory perception. Conversely, parametric data were analyzed at a 95% confidence interval using R software (version 4.3.3).

# 3 Results and discussion

## 3.1 Phytochemical contents of leaves from different pomegranate varieties

As demonstrated in Table 1, leaves of all pomegranate varieties exhibited a marked content of polyphenols and flavonoids which are highly influential for the quality of herbal teas.

Among the cultivars analyzed, the cultivar Kalpitiya hybrid demonstrated significantly higher total phenolic content ( $225.89 \pm 3.94$  mg GAE per g), followed by the cultivars Nayana, Nimali, and Daya, respectively. The order of increasing total phenolic content (TPC) is as follows: Daya < Nimali < Nayana < Kalpitiya hybrid. Similarly, the flavonoid content exhibited a different sequence, with Daya < Kalpitiya hybrid < Nimali < Nayana. It is important to note that the TPC and total flavonoid content (TFC) of black tea were lower than those observed in pomegranate leaves, except for the cultivar Daya. This finding underscores the potential benefits of incorporating pomegranate leaves to enhance the value of tea products. Additionally, our results surpass the findings of Zhang *et al.*,<sup>21</sup> who reported variations in TPC in two pomegranate cultivars at various maturity stages. Furthermore, our study indicates a notably higher flavonoid content when compared to the results of Kolar *et al.*,<sup>22</sup> who documented a flavonoid content of 7.69 mg RE per g in pomegranate leaves. Such variations can be attributed to the differences in cultivar types and the specific soil and climatic conditions under which they are grown.

Table 2 demonstrates the antioxidant capacities of leaves of the four different pomegranate cultivars and black tea determined by DPPH and oxygen radical absorbance capacity.

As illustrated in Table 2, all four cultivars tested displayed a remarkable level of antioxidant activity, highlighting their potential health benefits. Among these, the leaf extracts of cultivar Daya were particularly noteworthy, exhibiting the most favorable EC<sub>50</sub> value, which indicates a lower concentration required to achieve a 50% reduction in DPPH radicals. This suggests that cultivar Daya possesses superior DPPH antioxidant activity compared to the other cultivars tested. Following closely were the Kalpitiya hybrid, Nayana, and Nimali cultivars, each showing varying levels of activity but significantly lower

Table 1 Phytochemical content of leaves from different pomegranate varieties and black tea<sup>a</sup>

Name of the cultivar	Phytochemical contents	
	TPC (mg GAE per g)	TFC (mg QE per g)
Nayana	$141.36 \pm 4.19^b$	$21.71 \pm 0.13^a$
Nimali	$113.59 \pm 0.93^c$	$20.73 \pm 0.09^a$
Daya	$99.41 \pm 2.29^e$	$9.06 \pm 0.61^d$
Kalpitiya hybrid	$225.89 \pm 3.94^a$	$17.60 \pm 0.17^b$
Black tea (D1)	$106.50 \pm 1.70^d$	$11.32 \pm 0.13^c$

<sup>a</sup> All values are means of four replicates  $\pm$  standard error (SE). Values in the same row with different letters (a, b, c, d, and e) are significantly different ( $P < 0.05$ ). TPC, total polyphenolic content; TFC, total flavonoid content; GAE, gallic acid equivalents; QE, quercetin equivalents.



Table 2 Antioxidant content of leaves from four different pomegranate varieties<sup>a</sup>

Name of the cultivar	Antioxidant contents	
	DPPH EC <sub>50</sub> (mg TE per g DW)	Oxygen radical absorbance capacity (ORAC) (mg TE per g DW)
Nayana	51.06 ± 0.13 <sup>b</sup>	292.79 ± 3.54 <sup>a</sup>
Nimali	52.34 ± 0.32 <sup>a</sup>	267.71 ± 6.78 <sup>b</sup>
Daya	31.35 ± 0.35 <sup>d</sup>	216.16 ± 2.73 <sup>c</sup>
Kalpitiya hybrid	46.15 ± 0.39 <sup>c</sup>	262.71 ± 2.31 <sup>b</sup>
Black tea (D1)	23.13 ± 0.10 <sup>e</sup>	182.28 ± 2.62 <sup>c</sup>

<sup>a</sup> All values are means of four replicates ± standard error (SE). Values in the same row with different letters (a, b, c, d and e) are significantly different ( $P < 0.05$ ). DPPH, 1,1-diphenyl-2-picryl-hydrazyl; TE, trolox equivalents.

than cultivar Daya. The findings of our study align with those of Balamurugan *et al.*<sup>23</sup> who reported that the DPPH value of pomegranate leaves varied between 16.90 and 79.13  $\mu\text{g mL}^{-1}$ . Aldhanhani *et al.*<sup>24</sup> discovered that there are varying levels of phytochemicals and antioxidant capacities present in the leaves of *Ziziphus mauritiana* and *Ziziphus spina-christi*. In contrast, when evaluating the ORAC values, cultivar Nayana emerged as the standout cultivar, achieving the highest significant ORAC value. This indicates its exceptional capacity to scavenge free radicals over a specific time frame. The ORAC values for the other cultivars followed in descending order with Nayana, Nimali, Kalpitiya hybrid, and Daya (Table 2), respectively. The findings highlight the differences in antioxidant capacities across the various cultivars, indicating their potential benefits for health and nutrition. As presented in Table 2, the black tea sample showed a significantly lower DPPH value ( $23.13 \pm 0.10$ ), although its ORAC value was notably lower than that of pomegranate leaves. This suggests that combining pomegranate leaves with black tea could be a promising candidate for producing value-added herbal teas; the presence of high antioxidant capacity and polyphenol content may enhance the astringency and bitterness of tea and plant flavonoids are an important part of the diet because of their effect on human nutrition.<sup>25</sup>

An analysis of both micro- and macro-mineral components, alongside other phytochemicals, is essential for the development of functional herbal beverages.<sup>26</sup> In this study, samples from four recommended pomegranate cultivars were subjected to a thorough compositional analysis of 24 key macro- and micro-minerals. The results of this analysis are presented in Table 3 and variations of the four major minerals are presented in Fig. 1. As indicated in Table 3, the pomegranate leaves from all four cultivars are significantly enriched with essential minerals, including potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), zinc (Zn), zinc (Zn) and iron (Fe), all of which are vital for physiological functions. Furthermore, it is noteworthy that principal toxic elements such as mercury (Hg), cadmium (Cd), silver (Ag), titanium (Ti), and lead (Pb) are either absent or present in negligible amounts, thereby reflecting safer levels for consumption. The results of the present study indicate the comparatively marked levels of favourable minerals compared to previous studies.<sup>27</sup>

Table 3 Mineral content of leaves from different pomegranate cultivars<sup>a</sup>

Minerals	Mineral contents (mg kg <sup>-1</sup> )			
	Kalpitiya hybrid	Nimali	Nayana	Daya
Li	0.18	0.27	0.18	0.12
Be	ND	ND	ND	ND
B	18.90	16.40	24.5	16.2
Na	227	230	366	332
Mg	4410	3912	3376	3596
Al	31.90	30.91	33.2	30.5
Si	39.21	37.0	40	39.5
K	9636	12 569	12 221	8674
Ca	18 694	16 653	12 155	13 181
Ti	ND	ND	ND	ND
V	0.11	0.08	0.09	0.08
Cr	3.21	3.10	2.7	3.1
Mn	38.0	50.91	26.1	19.8
Fe	32.36	35.26	30.8	44.8
Co	ND	ND	ND	ND
Ni	0.85	1.24	ND	ND
Cu	7.0	5.90	6.0	5.6
Zn	8.71	10.37	10.6	10.5
As	0.11	0.11	ND	0.13
Se	ND	ND	ND	ND
Mo	1.0	0.67	0.63	0.97
Ag	ND	ND	ND	ND
Cd	ND	ND	ND	ND
Sn	0.26	0.24	0.21	0.21
Sb	ND	ND	ND	ND
Ba	3.926	3.235	2.233	3.445
Hg	ND	ND	ND	0.056
Ti	ND	ND	ND	ND
Pb	0.16	0.31	0.16	0.21

<sup>a</sup> ND-not detected.

### 3.2 Sensory evaluation of herbal tea formulations

Upon the formulation of functional beverages that integrate essential nutrients and bioactive compounds, the subsequent step involves the assessment of their organoleptic properties. This evaluation encompasses key attributes such as color, taste, aroma, mouthfeel, and overall acceptability.<sup>26,27</sup> In this investigation, formula 235, comprising 40% black tea and 60% pomegranate leaf, exhibited a significantly superior color attribute compared to formula 180 (50% black tea and 50%



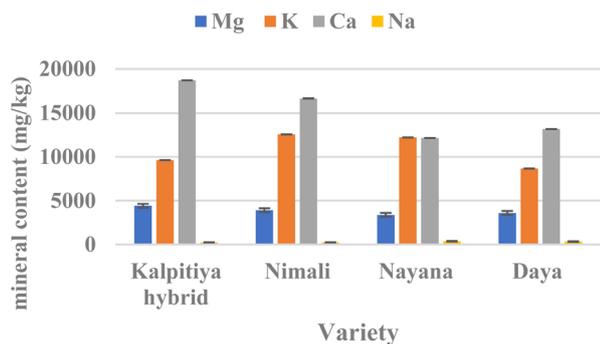


Fig. 1 Variation of four major minerals in four recommended pomegranate cultivars grown in Sri Lanka.

pomegranate leaf) and formula 330 (20% black tea and 80% pomegranate leaf). Furthermore, all other evaluated parameters were markedly higher in formula 235, suggesting that the combination of 60% pomegranate leaf with 40% black tea yielded greater consumer preference relative to the other two formulations (Table 4 & Fig. 2).

**3.2.1. Comparison of the herbal tea blend vs. commercial tea.** The results of the sensory evaluation comparing the new herbal tea blend and commercial tea are given in Table 5 and illustrated in Fig. 3.

Table 4 Sensory attributes of herbal tea formulations<sup>a</sup>

Sensory attribute	180	235	330
Colour	3.33 <sup>b</sup>	4.36 <sup>a</sup>	2.23 <sup>c</sup>
Aroma	2.83 <sup>b</sup>	3.93 <sup>a</sup>	4.43 <sup>a</sup>
Taste	3.30 <sup>b</sup>	4.53 <sup>a</sup>	2.10 <sup>c</sup>
Mouth feel	3.70 <sup>a</sup>	4.33 <sup>a</sup>	1.93 <sup>b</sup>
After taste	3.36 <sup>a</sup>	4.16 <sup>a</sup>	2.23 <sup>b</sup>
Overall acceptability	3.50 <sup>b</sup>	4.73 <sup>a</sup>	2.13 <sup>c</sup>

<sup>a</sup> 180 (50% black tea: 50% pomegranate leaf); 235 (40% black tea: 60% pomegranate leaf); 330 (20% black tea: 80% pomegranate leaf). The mean values denoted with different letters along the rows are significantly different ( $p < 0.05$ ).

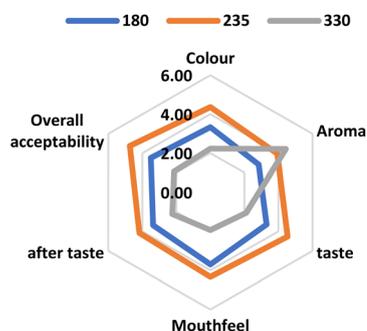


Fig. 2 The sum of the ranks performed by the Friedman test for formula 180 (50% black tea: 50% pomegranate leaf); 235 (40% black tea: 60% pomegranate leaf); 330 (20% black tea: 80% pomegranate leaf).

After selecting the optimal tea blend, it was compared to 100% black tea (sample no. 135) and 100% pomegranate leaf tea (sample no. 287). The results clearly demonstrated significant differences ( $p < 0.05$ ) across all sensory attributes evaluated. Among the samples, the one with the highest mean scores for these attributes was found to be the most preferred. Notably, sample no. 362, which combined 40% black tea and 60% pomegranate leaf, and sample no. 135 (100% black tea) did not show a statistically significant difference in terms of color, mouthfeel, and overall acceptability (Fig. 4).

However, both of these samples received more favorable evaluations compared to the 100% pomegranate leaf tea (sample no. 287). In the direct comparison between the black tea and the herbal tea blend, the herbal blend outperformed in aroma, taste, and aftertaste. Furthermore, while the overall acceptability of the herbal tea blend was on par with that of the black tea, it showed enhanced characteristics in taste and aroma. Despite the herbal tea blend being rated within an acceptable range, the 100% pomegranate leaf tea (sample no. 287) did not meet consumer preferences and was deemed unacceptable.

Following a comprehensive selection process, the most nutritious and widely accepted tea blend was identified. This blend underwent rigorous analysis to assess its phytochemical and antioxidant properties, specifically focusing on total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, and ORAC (Oxygen Radical Absorbance Capacity) values. A comparative evaluation was conducted against both commercial black tea, which enjoys widespread consumption, and the pomegranate leaf herbal tea blend, recognized for its health-promoting attributes. The results of this evaluation are presented in Fig. 5, which visually summarizes the data. The findings unequivocally indicate that all tested parameters for the selected herbal tea blend exceed those of both the commercial black tea and the 100% pomegranate leaf tea. This underscores the potential of the selected blend as a more beneficial choice for consumers seeking beverages rich in antioxidants. Additionally the previous studies conducted by Manyou Yu *et al.*<sup>28</sup> reported that pomegranate leaves consist of more than 111 chemical compounds, while Kolar *et al.*<sup>22</sup> found that the antioxidant activity of different parts of pomegranate ranged from 49.3 to 602 mg TAE per g.

### 3.3 Comparison of caffeine content of pomegranate herbal tea with that of commercial black tea

Caffeine is widely recognized as a central nervous system (CNS) stimulant, whereas herbal teas generally contain minimal or no caffeine. In a recent study, we assessed the caffeine content of a prepared pomegranate tea blend, which registered a notably low concentration of 0.82% (w/w), in contrast to a standard tea sample that exhibited a caffeine level of 2.23% (w/w). Nehlig *et al.*<sup>29</sup> reported that the average caffeine concentrations in lemon, apple, and rosehip teas were  $28.54 \pm 0.75$ ,  $25.40 \pm 0.64$ , and  $22.87 \pm 0.54$  parts per million (ppm), respectively. However, our findings indicate that the caffeine content in the pomegranate tea



Table 5 Sensory attribute herbal tea blend, black tea and 100% pomegranate leaf

Sensory attribute	135	287	362
Colour	3.9667 <sup>a</sup>	2.5667 <sup>b</sup>	4.3000 <sup>a</sup>
Aroma	3.6000 <sup>b</sup>	3.2000 <sup>b</sup>	4.4333 <sup>a</sup>
Taste	3.3667 <sup>b</sup>	2.2333 <sup>b</sup>	4.5000 <sup>a</sup>
Mouth feel	3.6667 <sup>a</sup>	2.0667 <sup>b</sup>	4.3667 <sup>a</sup>
After taste	3.3667 <sup>b</sup>	2.2667 <sup>c</sup>	4.1333 <sup>a</sup>
Overall acceptability	3.5000 <sup>a</sup>	2.5667 <sup>c</sup>	4.7333 <sup>a</sup>

Formula 135 (100% tea); 287 (100% pomegranate); 362 (pomegranate: tea in 60 : 40 ratio); 135 (100% tea).

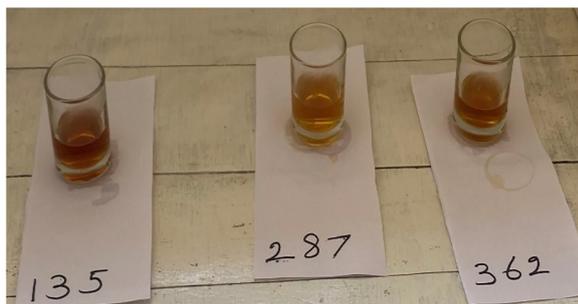


Fig. 3 Three pomegranate tea blends prepared for sensory evaluation (135 (100% tea); 287 (100% pomegranate); 362 (pomegranate: tea in a 60 : 40 ratio)).

blend is notably lower than that in other herbal teas. This is significant for individuals who prefer a low caffeine tea option.

### 3.4 Physicochemical parameters of the pomegranate tea blend

Physicochemical parameters, including pH, moisture, and ash content, are critical factors in the assessment of herbal tea, as they significantly influence its shelf life, safety, efficacy, and bioactivity (Builders *et al.*<sup>30</sup>). In our thorough investigation, we determined a moisture content of 7.25%, a pH value of 4.47, and an ash content of 5.91%. To contextualize these findings, the moisture content of herbal tea generally ranges from 3.0%

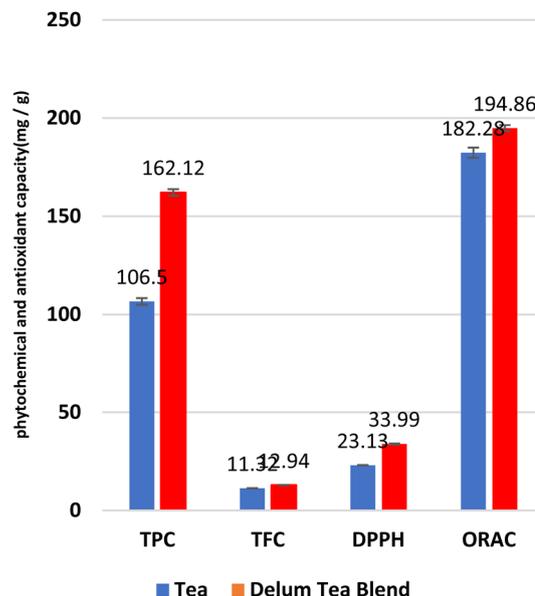


Fig. 5 Comparison of major phytochemical contents and antioxidant capacities of the pomegranate herbal tea blend and commercial tea.

to 12.5%, while pH levels are typically between 3.0 and 10.0. Additionally, ash content in herbal teas usually varies from 4.2% to 6.6%. The results of our study fall well within these established safety parameters, thereby affirming the quality and stability of the herbal tea analyzed.

## 4 Conclusion

This study focused on converting pomegranate leaf waste into a wellness beverage by evaluating four commercially cultivated pomegranate cultivars for their phytochemicals and bioactivity. We prepared different blends of black tea and active pomegranate leaves, ultimately determining that a 60 : 40 ratio of pomegranate leaf to black tea was the best. This blend was analyzed for total phenolic content, total flavonoid content, antioxidant capacity (DPPH and ORAC), caffeine content, physicochemical parameters (pH, ash content, and moisture content), and sensory attributes. The results indicated that the blend meets the standards for a herbal tea with low caffeine. Thus, pomegranate waste offers valuable, nutritious raw materials for producing herbal tea.

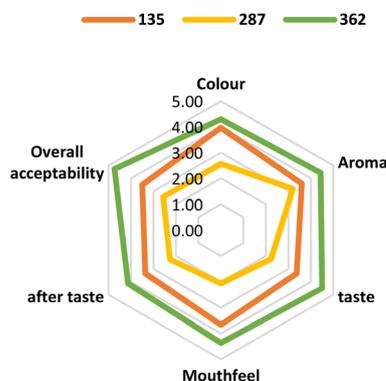


Fig. 4 The sum of the ranks performed by the Friedman test on formula 135 (100% tea); 287 (100% pomegranate); 362 (pomegranate: tea in a 60 : 40 ratio); 135 (100% tea).



## Author contributions

R. M. Dharmadasa – conceptualization, designing, supervision, writing, overall monitoring. N. G. S. Vimukthi – conducting research, data analysis, sample handling. W. M. R. S. K. Warnasooriya – supervision. H. M. U. I. Medawatta – sample analysis, supervision.

## Conflicts of interest

All authors declare that there are no conflicts of interest with other people or organizations that could inappropriately influence or bias their work.

## Data availability

All data pertaining to the current study are included in this manuscript itself. However, if further clarification is required, it can be supplied upon request.

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## References

- 1 S. H. A. Huda, N. B. Abdul Majid, Y. M. Chen, S. Adnan, M. Roszko, M. Bryła and M. Kieliszek, Exploring the ancient roots and modern global brews of tea and herbal beverages: A comprehensive review of origins, types, health benefits, market dynamics, and future trends, *Food Sci. Nutr.*, 2024, **12**, 6938–6955.
- 2 E. Stover and E. W. Mercure, The pomegranate: A new look at the fruit of paradise, *HortScience*, 2007, **42**(5), 1088–1092.
- 3 P. S. I. U Jayarathne, R. M. Dharmadasa, D. C. Abeyinghe and S. W. A. Weerawarna, Changes of physicochemical parameters, bioactive compounds, and antioxidant capacity of four different cultivars of *Punica granatum* (L.) at four distinct maturity stages, *Food Chem. Adv.*, 2024, **4**, 100640.
- 4 M. Akkawi, S. Abu-Lafi, Q. Abu-Remeleh, M. Qutob and P. Lutgen, Phytochemical screening of pomegranate juice, peels, leaves, and membranes water extracts and their effect on  $\beta$ -hematin formation: A comparative study, *Pharm. Pharmacol. Int. J.*, 2019, **7**, 193–200.
- 5 J. M. Kong, L. S. Chia, N. K. Goh, T. F. Chia and R. Brouillard, Analysis and biological activities of anthocyanins, *Phytochemistry*, 2003, **64**(5), 923–933.
- 6 H. A. Khalil, D. O. El-Ansary and Z. F. R. Ahmed, Mitigation of Salinity Stress on Pomegranate (*Punica granatum* L. cv. Wonderful) Plant Using Salicylic Acid Foliar Spray, *Horticulturae*, 2022, **8**(5), 375.
- 7 S. R. M. R. Attanayake, S. A. S. M. Kumari, W. A. P. Weerakkody, R. H. G. Ranil, A. B. Damania and P. C. G. Bandaranayake, Molecular diversity and genetic relationships among Sri Lankan pomegranate *Punica granatum* landraces assessed with inter simple sequence repeat (ISSR) regions, *Nord. J. Bot.*, 2017, **35**(4), 385–394.
- 8 Wang C., L. Shi, L. Fan, Y. Ding, S. Zhao, Y. Liu and C. Ma, Optimization of extraction and enrichment of phenolics from pomegranate (*Punica granatum* L.) leaves, *Ind. Crops Prod.*, 2013, **42**, 582–594.
- 9 J. C. B. Machado, M. R. A. Ferreira and L. A. L. Soares, *Punica granatum* leaves as a source of active compounds: A review of biological activities, bioactive compounds, food, and technological application, *Food Biosci.*, 2023, **51**, 102220.
- 10 S. Ge, L. Duo, J. Wang, G. Zhula, J. Yang, Z. Li and Y. Tu, A unique understanding of traditional medicine of pomegranate, *Punica granatum* L., and its current research status, *J. Ethnopharmacol.*, 2021, **271**, 113877, DOI: [10.1016/j.jep.2021.113877](https://doi.org/10.1016/j.jep.2021.113877).
- 11 M. Cheurfa, M. Achouche, A. Azouzi and M. A. Abdalbasit, Antioxidant and anti-diabetic activity of pomegranate (*Punica granatum* L.) leaves extracts, *Foods Raw Mater.*, 2020, **8**(2), 329–336.
- 12 L. Xiang, D. Xing, F. Lei, W. Wang, L. Xu, L. Nie and L. Du, Effects of season, variety, and processing method on ellagic acid content in pomegranate leaves, *Tsinghua Sci. Technol.*, 2008, **13**(4), 460–465.
- 13 M. Yu, I. Gouvinhas, J. Chen, Y. Zhu, J. Deng, Z. Xiang, P. O. C. Xia and A. Barros, Unlocking the therapeutic treasure of pomegranate leaf: A comprehensive review on phytochemical compounds, health benefits, and future prospects, *Food Chem.*, 2024, **22**(23), 101587, DOI: [10.1016/j.fochx.2024.101587](https://doi.org/10.1016/j.fochx.2024.101587).
- 14 C. I. Heck and E. González de Mejía, Teas and tea-based functional beverages, in *Functional and Specialty Beverage Technology*, ed. R. E. Smith and M. T. Smith, Woodhead Publishing, 2009, pp. 396–417, DOI: [10.1533/9781845695569.3.396](https://doi.org/10.1533/9781845695569.3.396).
- 15 W. P. K. M. Abeysekera, W. K. S. M. Abeysekera, W. D. Ratnasooriya and H. M. U. L. Medawatta, Antioxidant and glycemic regulatory potential of different maturity stages of leaf of Ceylon cinnamon (*Cinnamomum zeylanicum* Blume) *in vitro*, *Evidence-Based Complementary Altern. Med.*, 2019, 2693795, DOI: [10.1155/2019/2693795](https://doi.org/10.1155/2019/2693795).
- 16 L. Wang, Y. Yu, M. Fang, C. Zhan, H. Pan, Y. Wu and Z. Gong, Antioxidant and antigenotoxic activity of bioactive extracts from corn tassel, *J. Huazhong Univ. Sci. Technol.*, 2014, **34**(1), 131–136.
- 17 P. Mabai, A. Omolola and A. Jideani, Effect of drying on quality and sensory attributes of lemongrass (*Cymbopogon citratus*) tea, *J. Food Res.*, 2024, **7**, 68, DOI: [10.5539/jfr.v7n2p68](https://doi.org/10.5539/jfr.v7n2p68).
- 18 M. C. Meilgaard, B. T. Carr and B. T. Carr, *Sensory Evaluation Techniques*, CRC Press, 4th edn, 2007, DOI: [10.1201/b16452](https://doi.org/10.1201/b16452).
- 19 M. Musa Özcan, A. Ünver, T. Uçar and D. Arslan, Mineral content of some herbs and herbal teas by infusion and decoction, *Food Chem.*, 2008, **106**(3), 1120–1127, DOI: [10.1016/j.foodchem.2007.07.042](https://doi.org/10.1016/j.foodchem.2007.07.042).



- 20 L. Lunkes and L. Hashizume, Evaluation of the pH and titratable acidity of teas commercially available in Brazilian market, *Rev. Gaucha Odontol.*, 2014, **62**, 59–64, DOI: [10.1590/1981-8637201400010000092623](https://doi.org/10.1590/1981-8637201400010000092623).
- 21 H. Zhang, M. Wang, G. Yu, J. Pu, K. Tian, X. Tang, Y. Du, H. Wu, J. Hu, X. Luo, L. Lin and Q. Deng, Comparative analysis of the phenolic contents and antioxidant activities of different parts of two pomegranate (*Punica granatum* L.) Cultivars: 'Tunisia' and 'Qingpi', *Front. Plant Sci.*, 2023, **14**, 1265018.
- 22 F. R. Kolar, S. M. Lingasur, T. M. Kumathalli and S. A. Gurikar, Comparative analysis of phytochemical constituents and antioxidant activity of two pomegranate (*Punica granatum* L.) cultivars, *Isr. J. Plant Sci.*, 2021, **69**(1–2), 100–109, DOI: [10.1163/22238980-bja10042](https://doi.org/10.1163/22238980-bja10042).
- 23 C. Balamurugan, R. Karuppasamy, C. Sivaraj, K. Saraswathi and P. Arumugam, Cytotoxic activity of leaves extract of pomegranate (*Punica granatum* L.), *J. Pharmacogn. Phytochem.*, 2020, **10**(1), 1982–1985.
- 24 A. R. H. Aldhanhani, N. Kaur and Z. F. R. Ahmed, Antioxidant phytochemicals and antibacterial activities of sidr (*Ziziphus* spp.) leaf extracts, *Acta Hort.*, 2022, **1353**, 323–332.
- 25 J. Zhishen, T. Mengcheng and W. Jianming, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, *Food Chem.*, 1999, **64**(4), 555–559.
- 26 Y. Yilmaz, I. Çelik and F. Isik, Mineral composition and total phenolic content of pomegranate molasses, *J. Food, Agric. Environ.*, 2007, **5**, 102–104.
- 27 D. Wu and D.-W. Sun, Colour measurements by computer vision for food quality control – A review, *Trends Food Sci. Technol.*, 2013, **29**(1), 5–20, DOI: [10.1016/j.tifs.2012.08.004](https://doi.org/10.1016/j.tifs.2012.08.004).
- 28 Y. Manyou, I. Gouvinhas, J. Chen, Y. Zhu, J. Deng, Z. Xiang, P. Oliveira, C. Xia and A. Barros, Unlocking the therapeutic treasure of pomegranate leaf: A comprehensive review on phytochemical compounds, health benefits, and future prospects, *Food Chem.*, 2024, **23**, 101587, DOI: [10.1016/j.fochx.2024.101587](https://doi.org/10.1016/j.fochx.2024.101587).
- 29 A. Nehlig, J. L. Daval and G. Debry, Caffeine and the central nervous system: Mechanisms of action, biochemical, metabolic, and psychostimulant effects, *Brain Res. Rev.*, 1992, **17**(2), 139–170.
- 30 P. F. Builders, B. B. Mohammed and Y. Z. Sule, Preparation and evaluation of the physicochemical and stability properties of three herbal tea blends derived from four native herbs, *J. Phytomed. Ther.*, 2020, **19**(2), 448–465.

