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## Process characterization for tisane development using pomegranate waste: an herbal drink optimization strategy

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The present study aimed to utilize pomegranate waste in the form of its peel and seed for the development of an herbal extract called "tisane". A series of preliminary evaluations were performed where the dried pomegranate peel powder (PPP) and pomegranate seed powder (PSP) were studied for proximate analysis viz. moisture, crude protein, crude fat, ash, crude fiber and carbohydrate content. Notably, higher macromolecule percentages were recorded in both peel and seed powder, with peel containing 79.05% carbohydrate content and seed displaying 34% crude fiber content. The study then explored various blends of PPP and PSP viz. 9:1, 7:3, 6:4, and 5:5, resulting in an optimized blend, B-73 (7PPP:3PSP), exhibiting the highest antioxidant potential (98.7%), total phenolic content (46.926 mg GAE/100 g), and total flavonoid content (91 mg CE/100 g) along with a higher diffusivity rate (0.678 g min<sup>-1</sup>). The combination of PPP and PSP showed better results with respect to the antioxidant behavior when compared with the individual contribution of PPP and PSP. Furthermore, analysis of total solubility solids (TSS), pH and color of tisane prepared from different blends was carried out. In sensory evaluation, blend D-55 showed the highest overall acceptability score (8.7) surpassing other blends as well as market samples. Despite this, B-73 emerged as the best-formulated blend, boasting the highest concentration of bioactive constituents, suggesting its potential for promoting well-being when consumed as an herbal drink. This research underscores the innovative use of pomegranate by-products to develop a healthy beverage rich in bioactive components, offering a sustainable approach to utilizing pomegranate waste.

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### Sustainability spotlight

Food product development is considered as a cost-effective and scientifically proven intervention to address the issues of deficiencies related to micronutrients and in promoting the health aspects of mankind. It becomes a more sustainable option, environmentally as well as economically, when the peels and seeds, which are generally considered as food waste, can be reutilized for the development of healthy products. In the present study, tisane, a herbal drink has been developed by fortifying with pomegranate peel and seed waste. The proximate, chemical and sensory analysis showed that pomegranate waste is a vital source of phenols, flavonoids and antioxidants along with sensory acceptability for developed herbal tea. Therefore, pomegranate waste left after the extraction of juice could deliver nutritionally beneficial characteristics that can promote well-being.

## 1. Introduction

Pomegranate, scientifically named *Punica granatum* L., is an ancient mystical fruit that grows across the temperate zone, including the northern Himalayas and certain areas in the

United States.<sup>1</sup> The medicinal benefits of pomegranate make it a widely acceptable fruit among the majority of people. The processing of pomegranate into jams, juices, wine and other products results in a significant volume of waste. Every year, pomegranate production and processing reach approximately 3 million tons, leading to a global production of around 1.62 million tons of byproducts.<sup>2</sup> However, the nutrient rich profile of pomegranate necessitates appropriate handling techniques as the leftovers in the form of seed and peel are either discarded or used for manure conversion.

Pomegranate waste (seeds and peels) contains several valuable constituents including minerals, proteins, dietary fiber, etc. and is considered to have anti-inflammatory, anti-cancer and anti-microbial properties. Previous studies have asserted that

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pomegranate peel contains several minerals (calcium, magnesium, phosphorus, potassium and sodium), sugars (fructose, glucose, sucrose and maltose), fatty acids, crude fibers, proteins, organic acids and alkaloids.<sup>3</sup> 92% of the total antioxidant compounds found in pomegranates are attributed to water-soluble tannins present in the peel membrane.<sup>4</sup> It has been reported that pomegranate peel rich in ellagic acid is used in derma care to protect against UV rays and other related disorders *viz.* de-pigmentation, skin ageing and skin cancer issues.<sup>5</sup> Gallotannins, ellagitannins, anthocyanins, hydroxycinnamic acids, and hydroxybenzoic acids have also been studied to be present in the pomegranate peel.<sup>6</sup> Encapsulated pomegranate peel phenolics were utilized as bio-functional ingredients by Kaderides *et al.* in the formulation of cookies.<sup>7</sup> In a similar way, researchers have claimed that the pomegranate seed matrix contains lignins,<sup>8</sup> fusion products of cell wall components and hydroxybenzoic/cinnamic acids, isoflavones, and potentially antioxidant lignin derivatives.<sup>9</sup> Also, pomegranate seed oil is considered as an enriched source of steroidal as well as non-steroidal estrogens.<sup>10</sup> The pomegranate fruit, its peel and seed oil demonstrate anti-cancer properties and are considered to have the potential to effectively counteract the invasion of cancer cells.<sup>11</sup>

Taking into account the different challenges in the area of food-based industries, efforts must be made to optimize and standardize the processing technologies along with the alternative ways to utilize and hence, reduce the generated food leftovers. In this context, developing a healthy formulation by using food waste could offer solutions to various challenges concerning human health and the environment. Researchers have started focusing on developing herbal beverages and value-added functional drinks like tisane by utilizing fruit waste. For instance, Andika *et al.*<sup>12</sup> utilized red dragon fruit peel, and Trimedona *et al.*<sup>13</sup> and Al-Zughbi and Krayem<sup>14</sup> used ginger peel and quince fruit peel, respectively for the development of herbal drinks. Hence, in order to transform pomegranate leftovers into valuable products, the present work was conceptualized and designed with an aim to utilize the bioactive constituents of pomegranate seed and peel for the development of wellness drinks.<sup>15</sup> Tisane, often termed herbal tea, is a beverage created from the infusion or decoction of different spices, herbs, or any other plant material in hot water, usually without caffeine and is consumed for therapeutic purposes.<sup>16</sup>

Pomegranate fruit, along with its peel and seeds, is a rich source of various bioactive compounds *viz.* antioxidants and total polyphenolics.<sup>17</sup> Therefore, exploring such a material to be utilized by the food and pharma industries for propagation of novel products will somehow help to minimize the waste and also potentially beneficial for the well-being of mankind. The major aim of this research was to develop “tisane” by using pomegranate waste in the form of seed and peel through a series of standardization phases. The prepared blends of peel and seed powder were subjected to proximate and phytochemical analysis and the prepared tisane compositions were further analyzed and compared to that of the market samples.

## 2. Materials and methods

### 2.1. Raw material procurement and sample preparation

The pomegranate waste was collected from the regional local market of Hisar, Haryana, India. The variety “Phule Arakta” was selected based on the high yield of seeds and peel.<sup>11,18</sup> Empty filter tea bags were purchased from an online store (Flipkart). Manually, arils were separated from peels, further juice was extracted from seeds using a manual screw juicer to obtain the seeds without juice. The waste (peels and seeds) was thoroughly washed and dried in a hot air oven ( $60 \pm 5^\circ\text{C}$  for 42 h) (Fig. 1a), packed in suitable metallized airtight polyester pouches

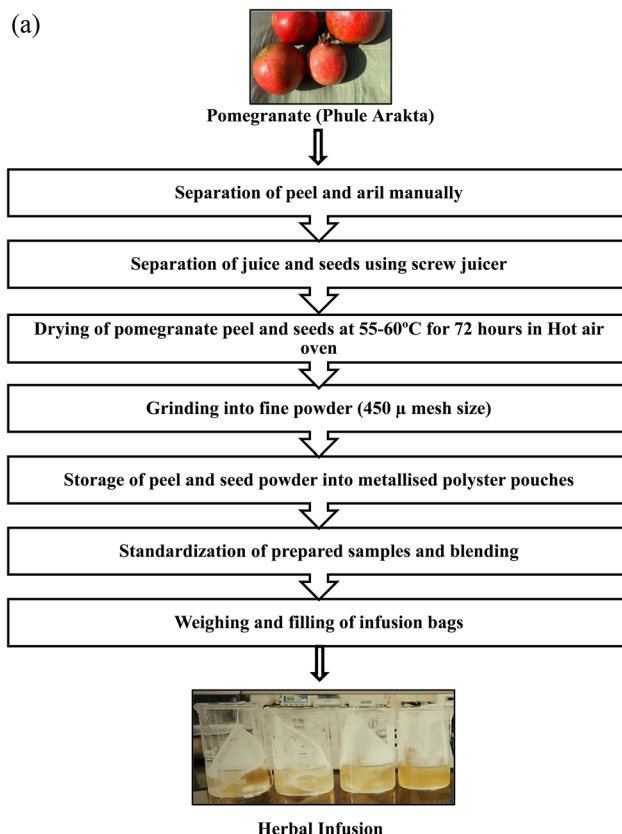


Fig. 1 (a) Schematic illustration to develop herb infusion formulation. (b) Pictorial view for the preparation of pomegranate peel and seed powder.



(Fig. 1b) and were stored in a deep freezer. The stored peels and seeds were brought to room temperature and then ground to a fine powder using a Sujata mixer-grinder with a pause of 5 seconds in between grinding cycles to avoid heat production and further 400 mesh size was used to sieve the powder in order to get the uniform particle size. The prepared powdered samples of pomegranate peels and seeds were stored in metallized pouches separately and stored in the dark for further analysis.

## 2.2. Proximate analysis of dried pomegranate peel and seed powder

**2.2.1. Moisture content.** The moisture content of dried pomegranate peel powder (PPP) and pomegranate seed powder (PSP) was determined using the AOAC<sup>19</sup> standard oven drying method where 2 g weighed sample was placed in a pre-weighed aluminum dish and dried for 8 h at 60 °C.

**2.2.2. Ash content.** Ash content of dried PPP and PSP was calculated using the AOAC method.<sup>20</sup> To obtain light grey ash, weighed samples (5 g each) were placed in crucibles, charred on a hot plate, and then heated inside a muffle furnace at 600 °C for 4 h.

**2.2.3. Crude fat content.** Fat content was determined by following the approved method of AOAC.<sup>21</sup> 3 g sample was taken in a thimble and transferred into the extraction chamber of Soxhlet apparatus. Extraction was done using petroleum ether at 70 °C for 6 h. Then, the solvent was collected in a pre-weighed beaker and kept in a hot air oven (70 °C) in order to remove any residual solvent.

**2.2.4. Crude protein content.** The total nitrogen of the samples (PPP and PSP) was measured using the Kjeldahl technique,<sup>21</sup> with minor modifications utilizing the kjel-plus digestion system and distillation equipment. 0.5 g of catalyst mix (1 : 4 copper sulphate and potassium sulphate) and 15 mL of nitrogen-free concentrated sulphuric acid (conc. H<sub>2</sub>SO<sub>4</sub>) were added. The tubes were then moved to the digestion unit where the content was digested for 90 min at 420 °C. Then, the digested content was allowed to cool to room temperature, and each tube was loaded into Kjeldahl distillation apparatus, and 45 mL of 40% NaOH was automatically added for neutralization. The ammonia that had been freed was concentrated and collected in a 50 mL saturated boric acid solution containing a mixed indicator. A reagent blank was immediately run after the distillate was titrated against 0.02 N H<sub>2</sub>SO<sub>4</sub>.

Crude protein content was calculated as % nitrogen × 6.25.

**2.2.5. Crude fiber content.** For the determination of crude fiber of PPP and PSP, the AOAC method<sup>21</sup> was followed. 2 g of defatted solid sample was weighed and refluxed with 200 mL conc. H<sub>2</sub>SO<sub>4</sub> (1.25%) in a flask connected with a condenser. The content was filtered and washed with distilled water to remove acid. 200 mL of sodium hydroxide solution (1.25%) was boiled and refluxed with the content for 30 min followed by filtering and washing with distilled water. Then, the content was placed in a hot air oven (105 °C for 12 h). The content was reduced to ash at 550 °C for 3 h and weighed after cooling.

**2.2.6. Carbohydrate content.** Total carbohydrate content was determined from the difference using the formula (eqn (1)) as follows:

$$\text{Total carbohydrate (\%)} = 100 - (\text{moisture\%} + \text{protein\%} + \text{fat\%} + \text{ash\%} + \text{crude fibre\%})(1)$$

## 2.3. Chemical analysis

**2.3.1. Total flavonoid content (TFC) (mg CE/100 g).** TFC was determined by following the aluminum chloride colorimetric assay<sup>22</sup> with minor modifications. 2.25 mL of distilled water (DW) and 0.15 mL of 5% NaNO<sub>2</sub> solution were added to 0.5 mL of crude extract and incubated for 6 min. Then, 0.3 mL of 10% AlCl<sub>3</sub> solution was added and incubated for 5 min at room temperature. With further addition of 1.0 mL (1 M) NaOH, the sample was mixed vigorously using a vortex mixer and absorbance was recorded at 510 nm. The results were presented in mg of catechin equivalent (CE)/100 g of sample.

**2.3.2. Total polyphenol content determination (TPC) (mg GAE/100 g).** A standard procedure utilizing the Folin–Ciocalteu technique was followed to evaluate the TPC content of PPP and PSP samples.<sup>23</sup> 0.5 mL of crude extract was taken, 7.5 mL distilled water and 0.5 mL of the Folin–Ciocalteu (FC) reagent were added and the content was mixed for 4 min, followed by the addition of 2 mL Na<sub>2</sub>CO<sub>3</sub> solution to it. The reaction mixture was then incubated at room temperature by keeping undisturbed for around 2 h. The color intensity was measured at 750 nm using a UV-VIS spectrophotometer. Blank was prepared using 0.1 mL of ethanol, 0.5 mL of FC reagent, 2 mL of sodium carbonate solution and 7.9 mL of distilled water. Gallic acid was used as a standard and total phenolics were expressed as mg gallic acid equivalents (GAE) per g.

**2.3.3. Antioxidant activity (AOA).** The antioxidant activity of PPP and PSP was determined by using the method given by Brand-Williams *et al.*<sup>24</sup> with slight modifications. 1 g of dried sample was macerated in 20 mL methanol and the mixture was vortexed for 10 min. Also, 0.06 mM solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was prepared in 100 mL methanol. Furthermore, 100 µL of sample was diluted in 3.9 mL of DPPH solution and absorbance was recorded at 515 nm after the incubation period of 60 min. % DPPH inhibition was calculated and AOA was expressed in inhibition %.

**2.3.4. Water holding capacity.** The water holding capacity of pomegranate peel and seed powder was calculated using Robertson's formula, with slight modifications. 250 mg of dried sample was diluted in 25 mL distilled water, stirred properly and incubated for 1 h at room temperature. Samples were then centrifuged, residue was weighed, and water holding capacity was calculated as one gram of water retained per gram of material.

## 2.4. Standardization of “tisane”

Pomegranate peel and seed powder were blended into four different proportions as mentioned in Table 1.





**Table 1** Standardization of tisane with codes and proportions of pomegranate peel powder and seed powder

Sample code	Pomegranate peel powder (PPP) (%)	Pomegranate seed powder (PSP) (%)
A-91	90	10
B-73	70	30
C-64	60	40
D-55	50	50

## 2.5. Preparation of blends in tea bags

Dried and powdered samples (PPP and PSP) were blended using a Sujata mixer grinder as per the prepared proportions (Table 1), and packed in tea bags with a total weight of 3 g per bag.

## 2.6. "Tisane" preparation and analysis

The formulation was standardized using a teacup in which the volume was measured and fixed as 125 mL. Based on this, 125 mL of distilled water was taken and heated using a pan till it reached the boiling point, then the prepared teabag was added to it and allowed to boil for 3 min. After boiling, the infusion was allowed to steep for 2 min for the extraction of the essence and was then transferred to a cup. All samples were prepared in a similar manner. The prepared tisane samples along with market samples have been shown in Fig. 2.

**2.6.1. Diffusion rate of tisane.** Each tea bag was soaked for 5 min in 125 mL of preheated (100 °C) water before being removed and dried in a hot air oven until no further drying occurred. Each bag was then weighed. Eqn (2) was used to compute the diffusion rate.

$$W_0 - W_1 = DR \quad (2)$$

where  $W_0$  is the initial weight of each bag (g),  $W_1$  is the final weight of the bag (g), and DR is the diffusion rate.

**2.6.2. TSS and pH.** The total soluble solids (TSS) expressed as degree brix were analyzed using a hand refractometer at room temperature by following the standard AOAC protocol.<sup>20</sup> The pH was measured by using a pH meter (Eutech) after calibrating with buffers of pH 4, 7 and 10 following the standard procedure of AOAC.<sup>20</sup>

**2.6.3. Color analysis.** Color values ( $L^*$ ,  $a^*$  and  $b^*$ ) of formulated infusions were measured using colorimetric system CIELab\* space (Chroma Meter; Make-Konica Minolta, USA; CR-400 employing spectraMagic NX2 software). A glass cell containing the sample was placed above the light source, covered with a white plate and  $L^*$ ,  $a^*$  and  $b^*$  values were recorded. The instrument (45°/0° geometry, 10° observer) was calibrated against a standard red colored reference tile ( $L_s = 25.54$ ,  $a_s = 28.89$ ,  $b_s = 12.03$ ). The  $L^*$  value gives the lightness (0–100 gives dark to light). The  $a^*$  value signifies the degree of change of red to green color, with a +ve  $a^*$  value indicating redness and –ve value indicating greenness. The  $b^*$  value gives the degree of yellow to blue color, with +ve  $b^*$  value designating yellowness and –ve value implying blueness.

**2.6.4. Sensory evaluation.** Sensory evaluation of the prepared herbal infusion (tisane) was done following the method of Lu *et al.*<sup>25</sup> with minor modifications. In brief, 20 semi-trained panelists including staff members and students from the Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar were invited for sensory evaluation. 9-Point hedonic scale (from 1 = extremely dislike to 9 = extremely like) was used. The panelists evaluated the product's appearance, flavor, taste and overall acceptability.

## 2.7. Comparative evaluation of formulated tisane with market available herbal teas

In order to understand the sensory aspects and acceptability of pomegranate waste formulated tea, different parameters were tested and compared with market available herbal teas (labelled as Brand A, B and C). The selection criteria for off the shelf herbal teas were their composition *i.e.*, the teas composed of fruit and flower extracts were randomly selected for study and compared with formulated tisane. These brands were formulated with synthetic colors and additives to provide fruity flavor. Parameters like pH, TSS, diffusion rate, color and sensory evaluation were determined for comparison.

## 2.8. Statistical analysis

The data reported are average of triplicate readings and otherwise stated. The data given in tables were subjected to one-way



**Fig. 2** Formulated herbal infusions along with market samples (sample sequence from left starts with A-91, B-73, C-64, D-55, Brand-A, Brand-B, Brand-C).



analysis of variance (ANOVA) to identify the significant differences among the samples and means were compared by the least significant difference (LSD) test at  $P < 0.05$ . All statistical analyses were done using Statistical Package for the Social Sciences (SPSS) 19.0.

### 3. Results and discussion

#### 3.1. Proximate composition of pomegranate peel powder (PPP) and pomegranate seed powder (PSP)

The proximate composition of PPP and PSP has been given in Table 2. The moisture, protein, ash and fat content of PPP were observed to be as 12.26%, 3.08%, 3.56%, and 1.52%, respectively (Table 2) which was in proximity with the results obtained by Akhtar *et al.*<sup>6</sup> The carbohydrate content of PPP was 79.05% which was consistent with the results of Chaudhary *et al.*<sup>26</sup> claiming the total carbohydrate content of 79.6% in pomegranate peel. Carbohydrates are naturally occurring organic substances essential for the sustenance and nourishment of both plant and animal life, and they serve as fundamental raw materials in numerous industries.<sup>27</sup> Pomegranate peel constituting 43% w/w of the fruit is a rich source of bioactive compounds, including complex carbohydrates, minerals and dietary fiber.<sup>2</sup> The moisture, fat, protein, and ash content of PSP were 6.87%, 32.87%, 13.67%, and 1.49%, respectively which were consistent with the results reported by Jalal *et al.*<sup>28</sup> The pomegranate seeds from different cultivars were found to be a rich source of calcium, magnesium and sodium, while iron, manganese and zinc were present in trace amounts.<sup>29</sup> A previous study has claimed that the fat in PSP comprises 45 identified different fatty acids where the major type is punicic

acid followed by linoleic and oleic acids.<sup>2,30</sup> Several studies have revealed the health benefits of punicic acid in reducing the risk of skin cancer, type-II diabetes and, anti-obesity, anti-inflammatory and anti-oxidant properties.<sup>2</sup> A significant amount of crude fiber content was recorded in PPP and PSP where crude fiber in PSP (34%) was observed to be higher than that of PPP (10.25%). PPP is considered a good source of ash, crude fiber and carbohydrates and PSP is considered a good source of crude proteins crude fat and crude fiber.<sup>28</sup>

#### 3.2. Phytochemical analysis comprising total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity

The results of the phytochemical analysis of raw materials, *i.e.*, pomegranate peel and seed powder along with prepared blends for standardization of tisane are given in Table 3. The plant material used in the present study was evaluated for its phytochemical properties and the results showed the presence of secondary metabolites. The findings indicated that PPP had much greater TPC and TFC values than that of PSP. Pomegranate peel was observed to be rich in tannins, such as gallic and ellagic acid.<sup>31</sup> Furthermore, blends had greater TPC and TFC levels than native crude extracts of peel and seeds. Phenols encompass a broad spectrum of complex organic chemical compounds, the quantity and quality of which vary based on growth phases, environmental conditions, extraction conditions, extraction solutions employed, and other variables.<sup>32</sup> The phenolic compounds present in pomegranate seeds and pericarp have been documented to display potent antioxidant activity.<sup>33</sup> The results revealed that the TPC of blend B-73 was the highest, followed by the TPC of blends D-55, C-64, and A-91,

Table 2 Proximate composition of pomegranate peel and seed powder<sup>a</sup>

Parameters	Pomegranate peel powder (PPP) (%)	Pomegranate seed powder (PSP) (%)
Moisture content (%)	12.26 <sup>b</sup> ± 0.02	6.87 <sup>a</sup> ± 0.01
Ash content (%)	3.56 <sup>b</sup> ± 0.02	1.49 <sup>a</sup> ± 0.02
Crude fat content (%)	1.52 <sup>a</sup> ± 0.04	32.87 <sup>b</sup> ± 0.06
Crude protein content (%)	3.08 <sup>a</sup> ± 0.02	13.67 <sup>b</sup> ± 0.05
Crude fiber content (%)	10.25 <sup>a</sup> ± 0.03	34.00 <sup>b</sup> ± 0.07
Total carbohydrate (%)	79.05 <sup>b</sup> ± 0.01	12.12 <sup>a</sup> ± 0.02

<sup>a</sup> Data represented as mean value ± standard deviation. Values with different superscripts in a column differ significantly ( $P \leq 0.05$ ).

Table 3 Antioxidant activity (AOA), total phenolic content (TPC), total flavonoid content (TFC) antioxidant activity of PPP and PSP standardized blends<sup>a</sup>

Samples	TPC (mg GAE/100 g)	TFC (mg CE/100 g)	AOA (% inhibition)
PPP (crude)	34.11 <sup>b</sup> ± 0.02	56.22 <sup>b</sup> ± 0.03	40.12 <sup>a</sup> ± 0.02
PSP (crude)	15.05 <sup>a</sup> ± 0.04	47.21 <sup>a</sup> ± 0.01	87.33 <sup>c</sup> ± 0.04
A-91	45.17 <sup>c</sup> ± 0.03	72.11 <sup>d</sup> ± 0.06	80.41 <sup>b</sup> ± 0.01
B-73	46.93 <sup>c</sup> ± 0.01	91.14 <sup>f</sup> ± 0.05	98.72 <sup>f</sup> ± 0.02
C-64	45.92 <sup>d</sup> ± 0.02	75.05 <sup>e</sup> ± 0.04	89.84 <sup>d</sup> ± 0.03
D-55	46.07 <sup>c</sup> ± 0.02	68.55 <sup>c</sup> ± 0.05	97.71 <sup>e</sup> ± 0.06

<sup>a</sup> Data represented as mean value ± standard deviation. Values with different superscripts in a column differ significantly ( $P \leq 0.05$ ).



respectively. Similarly, blend B-73 had the highest TFC (91.14 mg CE/100 g) followed by C-64, A-91 and D-55. The TPC and TFC results showed that standardization with 70% peel powder and 30% seed powder had the best results in the composition of herbal tisane.

Due to the presence of colored pigments and other bioactive compounds, fruits and vegetables are considered a principal source of natural antioxidant compounds.<sup>34</sup> PSP showed a higher antioxidant activity than that of PPP (Table 3). *Punica granatum* fruit waste extracts, i.e., peel and seed powder exhibited enhanced phenolic content and antioxidant capabilities, indicating that they can be employed as a natural source of antioxidants.<sup>35</sup> Pomegranate pericarp exhibits antioxidant behavior due to its polyphenolic elements (majorly, punicalagin and ellagic acid) which helps in the inhibition of the oxidation process, even in minute quantities and hence, display a positive influence on the human body.<sup>36</sup> Blend B-73 was found to have the highest antioxidant activity, followed by D-55, C-64 and A-91. PSP and PPP extracts were observed to be potent free radical scavengers and antioxidants that bind with free radicals. A similar study of antioxidant extraction from pomegranate peel and seed by using water, acetone and methanol has been reported previously.<sup>37,38</sup>

### 3.3. Water holding capacity (WHC) of PSP and PPP

WHC refers to a moist object's retaining water under the influence of an external centrifugal/gravitational pull or tension.<sup>39</sup> Water is retained in dietary fiber *via* absorption and adsorption mechanisms, with some water remaining outside the fiber matrix (free water).<sup>40</sup> As per the observations, PPP and PSP showed water holding capacities of 4.24 and 4.47 times their own weight, respectively. These findings were in accordance with the results obtained by Jalal *et al.*<sup>28</sup> who reported that PPP and PSP exhibited a water holding capacity of 4.84 and 4.45 times its own weight. For other fibrous residues like, sugarcane,<sup>41</sup> pear,<sup>42</sup> similar results were reported by researchers in previous studies. Hence, our results indicate that WHC is a critical parameter in terms of water retention and formulation affecting hydration with dried PPP and PSP blends, implying good water absorption during the infusion.

### 3.4. Comparative analysis of pomegranate peel-seed-based herbal tisane with commercially available herbal teas

**3.4.1. Diffusion rate.** Diffusion is described as the transfer of chemicals from a high concentration area to a low concentration area. There are several molecules within the herbal infusion bags which cannot flow through the bags, but smaller particles comprising essence, color, and taste may diffuse through the membrane of the bags. Heat from boiling water enables the molecules in the infused substance to move significantly quicker than they would at a normal temperature.<sup>43</sup> The diffusion rate of formulated as well as commercial samples ranged between 0.593 and 0.692 g min<sup>-1</sup> (Table 4). Blend B-73, among all formulated blends, showed the highest diffusivity rate followed by A-91. However, Brand-B exhibited the highest diffusivity rate followed by Brand-C and Brand-A. The diffusion rate is one of the vital factors for the market acceptability of formulated tea.<sup>44</sup> Longer infusion periods are due to polyphenols that take longer to disperse (highly polymerized catechins) and give tea a disagreeable stewed flavor when boiled for too long.<sup>45</sup> This is justified by the results of the present study that the blend D-55 with a low diffusion rate secured a high sensory score, as discussed in the Sensory evaluation section.

**3.4.2. pH and total soluble solids (TSS).** The result showed that sample C-64 had the highest pH, followed by samples D-55 and A-91 (Table 4). Blend B-73 had a highly acidic pH value which was equivalent to that of commercially available herbal teas. The pH values for aril powder/bagasse mixture and whole fruit were observed to be 4.4 and 4.5, respectively.<sup>46</sup> The possible reason for the low pH values of pomegranate peel and seed powder might be the presence of polyphenols and other bioactive compounds.<sup>47</sup>

Blend C-64 indicated the maximum TSS, while blend A-91 was observed to have a minimum brix. Increasing the amount of PSP increased the brix value till sample C-64 which might be due to the high concentration of compounds like crude fat and crude protein in pomegranate seed. However, blend D-55 did not show a further increase in brix with an increasing proportion of PSP which indicated the addition of an optimized amount of seed powder for a high brix value. Brix values of B-73 and D-55 blends were close to that of most commercially available herbal teas (Table 4).

**Table 4** Diffusion rate, TSS (total soluble solids), pH, sensory evaluation and color analysis of various infusions formulated from blends and the market available brands<sup>a</sup>

Samples	Rate of diffusion <sup>b</sup> (g min <sup>-1</sup> )	pH	TSS (°brix)	Color analysis		
				<i>L</i> <sup>*</sup>	<i>a</i> <sup>*</sup>	<i>b</i> <sup>*</sup>
A-91	0.667 <sup>b</sup>	3.76 <sup>c</sup> ± 0.15	1.97 <sup>a</sup> ± 0.15	41.45 <sup>b</sup> ± 1.12	0.42 <sup>a</sup> ± 0.12	10.74 <sup>b</sup> ± 1.48
B-73	0.678 <sup>b</sup>	3.30 <sup>ab</sup> ± 0.10	2.30 <sup>bc</sup> ± 0.15	46.98 <sup>d</sup> ± 1.04	0.53 <sup>a</sup> ± 0.01	8.76 <sup>a</sup> ± 0.02
C-64	0.603 <sup>a</sup>	4.06 <sup>d</sup> ± 0.05	3.00 <sup>d</sup> ± 0.20	38.71 <sup>a</sup> ± 0.13	0.71 <sup>a</sup> ± 0.03	12.75 <sup>c</sup> ± 0.51
D-55	0.593 <sup>a</sup>	3.90 <sup>b</sup> ± 0.10	2.16 <sup>ab</sup> ± 0.15	37.48 <sup>a</sup> ± 0.91	1.65 <sup>b</sup> ± 0.07	20.23 <sup>d</sup> ± 0.50
Brand-A	0.668 <sup>b</sup>	3.34 <sup>b</sup> ± 0.05	2.45 <sup>c</sup> ± 0.12	44.92 <sup>c</sup> ± 1.05	3.87 <sup>c</sup> ± 0.03	23.08 <sup>c</sup> ± 0.67
Brand-B	0.692 <sup>b</sup>	3.39 <sup>b</sup> ± 0.10	2.20 <sup>b</sup> ± 1.10	40.63 <sup>b</sup> ± 0.32	3.99 <sup>c</sup> ± 0.21	25.37 <sup>f</sup> ± 1.23
Brand-C	0.687 <sup>b</sup>	3.23 <sup>a</sup> ± 0.05	2.95 <sup>d</sup> ± 0.16	50.66 <sup>e</sup> ± 0.96	1.5 <sup>b</sup> ± 0.43	11.33 <sup>bc</sup> ± 1.58

<sup>a</sup> Data represented as mean value ± standard deviation. Values with different superscripts in a column differ significantly ( $P \leq 0.05$ ). <sup>b</sup> Except for rate of diffusion.



Table 5 Sensory characteristics of various infusions formulated from blends and the market available brands<sup>a</sup>

Sample	Appearance	Flavor	Taste	Overall acceptability
A-91	7.1 <sup>a</sup> ± 0.43	6.0 <sup>a</sup> ± 0.37	7.02 <sup>b</sup> ± 0.27	6.7 <sup>ab</sup> ± 0.35
B-73	7.0 <sup>a</sup> ± 0.24	7.02 <sup>b</sup> ± 0.25	6.99 <sup>b</sup> ± 0.27	7.0 <sup>b</sup> ± 0.25
C-64	8.04 <sup>b</sup> ± 0.30	6.1 <sup>a</sup> ± 0.23	7.0 <sup>b</sup> ± 0.19	7.04 <sup>bc</sup> ± 0.24
D-55	8.98 <sup>c</sup> ± 0.60	9.05 <sup>c</sup> ± 0.23	7.99 <sup>c</sup> ± 0.23	8.67 <sup>d</sup> ± 0.35
Brand-A	8.04 <sup>b</sup> ± 0.36	7.0 <sup>b</sup> ± 0.19	7.0 <sup>b</sup> ± 0.19	7.35 <sup>c</sup> ± 0.24
Brand-B	8.02 <sup>b</sup> ± 0.26	7.02 <sup>b</sup> ± 0.24	7.06 <sup>b</sup> ± 0.19	7.36 <sup>c</sup> ± 0.23
Brand-C	6.99 <sup>a</sup> ± 0.26	6.03 <sup>a</sup> ± 0.13	6.06 <sup>a</sup> ± 0.24	6.33 <sup>a</sup> ± 0.21

<sup>a</sup> Data represented as mean value ± standard deviation. Values with different superscripts in a column differ significantly ( $P \leq 0.05$ ).

**3.4.3. Color analysis.** Numerous variables affect the color of the product, including fruit type and age, but mainly the pulp process conditions show the major impact on color. During pulp dehydration, high temperatures are utilized, which generates enzymatic and non-enzymatic browning (Maillard reactions) that darken the final product.<sup>48</sup>

The  $L^*$  values varied greatly among different formulated infusions from blends (Table 4). Blend D-55 had the lowest  $L^*$  value, indicating the lighter color of the formulation, followed by blends C-64 and A-91. Blend B-73 showed the highest  $L^*$  value, indicating the dark colored formulation. The pale color of blend A-91 might be due to the high peel content and low seed percentage. In all samples, there was a significant lightening in the color of the formulation with increasing PPP content. A positive  $a^*$  (indicating redness) was recorded in all samples (Table 4). Among the blends, D-55 had the highest and A-91 had the lowest  $a^*$  values (Table 4). The high  $a^*$  for D-55 indicated that the infusion was bright and red which also reflects the results of sensory evaluation.<sup>49</sup> This variation in red hue can be attributed to different pomegranate peel contents in different blends. The highest  $b^*$  value (indicating yellowness) was observed in blend D-55, while the lowest  $b^*$  was recorded for blend B-73. The possible reason behind this variation might be the infusion of carotenoids and lipophilic chlorophylls present in seeds and peels of pomegranate.<sup>50</sup>

Green and yellow color in fruits is due to carotenoids and lipophilic chlorophylls present in plastids, whereas red coloration is due to hydrophilic anthocyanins and carotenoids present in the vacuole.<sup>51</sup> On ripening, pomegranate (*Punica granatum* L.) cultivars yield fruit that ranges in color from green to scarlet. Anthocyanins found in peels contribute to the nutritional value of pomegranate juice. Various research studies reported a great number of polyphenols including hydrolysable tannins, flavonoids, and anthocyanins in peels.<sup>38,52</sup> While present in lower quantities compared to hydrolysable tannins, anthocyanins are the main molecules accountable for the bioactivity observed in pomegranate peel extract.<sup>53</sup> The seeds are considered unsuitable for anthocyanin study due to the lack of enticing red colors and it has been reported previously that no anthocyanins were discovered in seeds.<sup>54</sup> The significantly higher color values ( $L^*$ ,  $a^*$  and  $b^*$ ) for infusions prepared from market samples might be due to the inclusion of synthetic color and additives in order to make final formulation appealing to the consumers.

**3.4.4. Sensory evaluation.** Processing might result in a pleasant and delectable tisane with excellent nutritional content along with acceptable sensory properties. The right infusion process improves the color, flavor, and nutrition of tisane. Color greatly influences the sensory attributes of a product, affecting its taste perception and playing a crucial role in its market acceptance.<sup>55</sup> Sensory evaluation results (Table 5) showed that the panelist expressed the highest overall acceptability for blend D-55. The sensory score for appearance and flavor indicated great acceptance for blend D-55 with a score of 8.98 and 9.05, respectively, however, the lowest was observed for A-91 with a score of 7.1 and 6.0, respectively. The sensory score for flavor was observed in the order of D-55 > B-73 = Brand-B > Brand-A > C-64 > Brand-C > A-91 (from highest to lowest). In terms of flavor, there existed a very minute difference between blend A-91 and blend C-64. Infusion resulted in the development of a delightful and delicious taste of tisane with enhanced sensory features and nutritive value.<sup>56</sup> All tisane samples were developed by infusion of pomegranate peel and seed powder blend. The inclusion of pomegranate peel in pomegranate wine increased its nutritional value in terms of phenolic content, antioxidant potential as well as flavor enhancement.<sup>57</sup> The PPP addition in the development of various bakery products up to a limit of 10% or less was reported to enhance the flavor and overall acceptability.<sup>58</sup> However, Ayoubi *et al.*<sup>58</sup> developed a fortified cake with PSP where sensory scores decreased when PSP increased above 5%. Thus, a balanced proportion of PPP and PSP (5 : 5) was required to achieve the acceptable sensory characteristics of developed tisane.

## 4. Conclusion

Pomegranate waste (peel and seed) was utilized to formulate herbal infusion "tisane" providing a rich blend of antioxidants and phenolics. Pomegranate peel powder was observed with high carbohydrate and low-fat content, while pomegranate seed powder had high protein, crude fat and fiber content. Antioxidant activity was exceptionally high in both peel and seed extracts along with high TPC and TFC values. The comparison between PPP and PSP indicated that PPP had more TPC and TFC than PSP. Blending of PSP and PPP in the right proportion boosted their antioxidant behavior by more than two-fold. Among all blends, blend B-73 showed the highest diffusion rate and antioxidant activity as well as the maximum TPC and





TFC, thus, making it the best blend with respect to health benefits. However, in terms of sensory evaluation, the highest scores for appearance, flavor and taste were recorded for blend D-55 among all the samples. Despite the fact that the market available brands were formulated with synthetic flavor and color, these secured low sensory scores in comparison with blend D-55. Thus, pomegranate waste when utilized wisely in the form of herbal extract may not only provide beneficial phytonutrients and bioactive compounds but also protect consumer health from products developed using synthetic additives.

## Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Author contributions

Aastha Dewan: conceptualization, supervision, statistical analysis; Sanya Dawra: writing draft, experimentation, statistical analysis; Nita Kaushik: editing, reviewing; Ajay Singh: data verification and correction; Sheetal Thakur: reviewing, plagiarism detection, manuscript preparation and revision; Sandeep Kaur: reviewing and correction; Janifer Raj Xavier: revision and correction.

## Conflicts of interest

The authors declare no conflict of interest.

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