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Optimization of Natural Colorant Extraction from the Fruit Peels of Wild Pomegranate

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Abstract

Punica granatum var. daru, commonly referred to as wild pomegranate, is widely distributed across Himalayan region and is characterized by its sour flavoured arils (anardana). A large quantity of fruit peels generates during the anardana production, which are traditionally used for various herbal and tanning purposes. These biowaste peels are a rich in polyphenolics and other bioactive constituents. Despite of its phytochemical richness and traditional values, limited research has focused on the valorisation of its fruit peel waste, especially for natural dye extraction. To validate its traditional claim, present study aimed at the valorisation of underutilized fruit peels of wild pomegranate through protocol optimization for extraction of natural colorant. The influence of three critical parameters including material to liquor Ratio (MLR), pH, and time on the optical density were systematically examined. Additionally, biochemical parameters such as total tannin and flavonoid content were assessed. The optimum extraction conditions were determined to be an MLR of 3 g/100 mL, pH 3, and time of 15 minutes, under which a reddish-brown dye with optical density 1.41 was obtained with the yield of 18.33%. Biochemical parameters including total tannin content (TTC), and total flavonoid content (TFC) were also found 0.28%, and 0.14%, respectively. The extracted dye imparted various shades of yellowish-brown when applied to different textile substrate. This is first time report that highlights a sustainable and eco-friendly approach for the valorisation of such biowaste, with promising implications for its integration into large scale sectors such as textiles, nutraceuticals and pharmaceuticals.

Keywords: Optimization, natural dye, bioactive compounds, flavonoids, tannins, wild pomegranate.

1. Introduction

Excessive use of synthetic dyes results in significant environmental constraints as well as various health hazards, including carcinogenicity, organ damage, and potentially life-threatening health complications[1]. To overcome these obstacles, it is necessary to find better natural coloring alternatives with sustainable extraction processes for colorant that optimize energy, time, and chemical consumption, providing the colorant with high absorbance and yield[2, 3, 4]. The advantages of natural dye extraction are cost effective, renewable, and non-carcinogenic and have no allergic reaction on skin.

Wild pomegranate (family Punicaeae), also known as daru, dadim, or darmu (Figure 1). It is a deciduous thorny shrub or tree reaching heights of 8 to 10 meters native to Central Asia, Iran, and Turkmenistan to northern India at 900 to 1800 masl[5]. It is widely distributed across mid-hill regions of Himachal Pradesh, Jammu and Kashmir, and Uttarakhand states in India[6]. This variety of pomegranate is distinguished from its cultivars due to its sour flavored arils known as anardana. During the processing of anardana, a large quantity of biowaste fruit peels is generated that remains underutilized. Fruit peels have traditionally been utilized in herbal applications and tanning to obtain yellow, brown, or darker shades due to their coloring properties. They were also used in dyeing leather and preparing ink for writing on takhti by school children[7, 8, 9]. The coloring ability of daru is due to the presence of an abundant quantity of chemical compounds, including phenolics (mostly hydrolyzable tannins, flavonoids, anthocyanins). These metabolites, notable polyphenols, are of particular interest from a nutraceutical perspective because of their antioxidant potential and resultant health advantages such as preventing various ailments, such as inflammation, cancer, diabetes, cardiovascular disease, and neurodegeneration[10].

Figure 1: **a**. Shrub, **b**. fruits of *Punica granatum* L. (wild pomegranate)

Despite the presence of bioactive compounds and traditional use of wild pomegranate peels, there is an evident research gap in scientific research focused on developing an efficient and optimized extraction protocol for natural colorant from biowaste fruit peels of daru variety. Moreover, empirical validation of traditional claims regarding their dying properties is limited. Therefore, the present study aims to extract a natural colorant from the peels of wild pomegranate using a classical extraction method, optimizing parameters such as MLR, pH, and extraction time to achieve maximum optical density. Additionally, biochemical parameters, including total tannin and flavonoid contents responsible for coloration, are also evaluated. These underutilized fruit peels can be a potential alternative for synthetic colorant that can be used in various nutraceutical and pharmaceutical industries.

2. Materials and Methods

2.1 Plant material and Chemicals

The wild pomegranate fruit peels (biowaste) were procured from the Himalayan Forest Research Institute (HFRI), Shimla, sourced from the Kariyali region of Himanchal Pradesh. The peels were thoroughly washed, lyophilized and pulverized into fine powder, which was subsequently stored into airtight containers at -20°C until further analysis. Analytical grade reagents including aluminium trichloride, citric acid, anhydrous sodium carbonate, ferrous chloride, Folin–Ciocalteu reagent, gallic acid, quercetin, potassium sodium tartrate tetrahydrate were obtained from Merck (India).

2.2 Extraction of the colorant

Optimization of natural dye extraction method in cultivated pomegranate has been previously reported in Sinha et al.[11], and Lei et al.[12]. In contrast, the present study focused on sustainable extraction of dye from wild grown pomegranate. Based on these previous reported studies, an optimized sustainable protocol was developed and applied for extraction process.

In this method, three independent variables including material to liquid ratio (MLR), pH and extraction time were assessed. Optimization was carried out by varying one parameter at a time while keeping the others constant to determine its effect on the optical density (OD) of the extract. Fruit peel samples (1, 2, 3, 4, 5 and 6 g) were extracted with boiled with 100 g distilled water for a period varied from 15-90 minutes at 15 minutes interval under different pH conditions (3,7 and 9). Citric acid (0.01%), and sodium carbonate (Na_2CO_3, 0.01%) were used to adjust the pH. The boiling temperature was maintained at 90^0 C by simmering the burner after reaching boiling water to avoid complete evaporation. The resulting extracts were filtered using Whatman No. 1 filter paper and stored in amber bottles at 4 °C for further analysis. The optical density of the dye solution was measured at 535 nm using UV-Visible Spectrophotometer. Optical density was calculated using Beer-Lambert law:

$$A=\varepsilon.l.c$$

The yield of the extracted dye was also calculated in percentage (%).

2.3 Determination of bioactive constituents

2.3.1 Total tannin content (TTC)

Total tannin content was quantified using Folin–Ciocalteu method with some modifications as described by Lahare et al.[13]. In this method, 1 mL of dye extract was mixed with 5 mL of Folin–Ciocalteu reagent, followed by the addition of 10 mL of sodium carbonate solution. The mixture was incubated for 1.5 hr at room temperature, after which the absorbance was recorded at 740 nm using a UV–Visible spectrophotometer.

2.3.2 Total flavonoid content (TFC)

The total flavonoid content was estimated using aluminium chloride colorimetric method, as previously reported by Lahare et al.[13]. 1 mL of extract was mixed with 4 mL of distilled water and 0.1 mL of 5% $NaNO_2$. After 5 minutes, 0.1 mL of 10% $AlCl_3$ solution was added, followed by 2 mL of 1 M NaOH after another 6 minutes. The final volume was adjusted to 10 mL with distilled water, and the absorbance was measured at 510 nm.

The results were expressed in percentage (%). Standard calibration curve of quercetin and tannin acid are displayed in Figure 2.

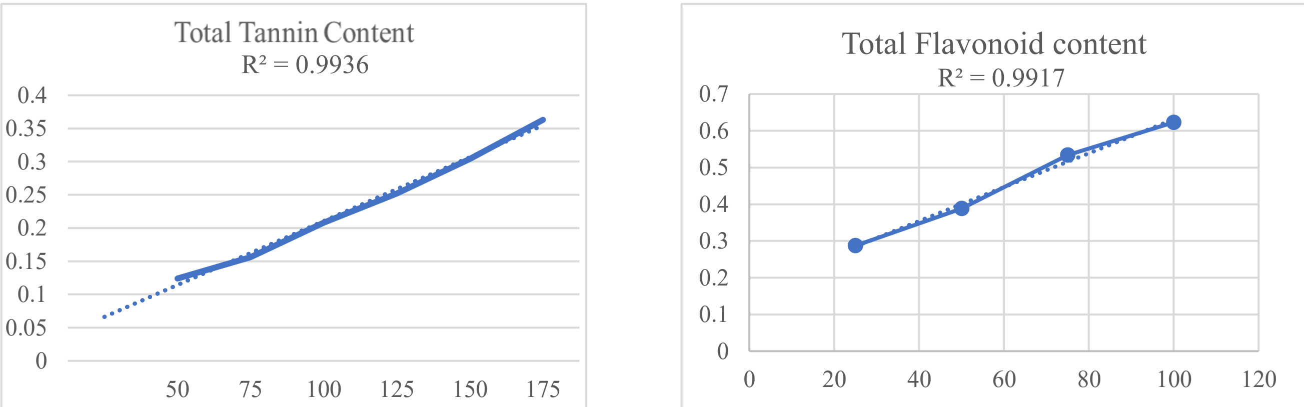

Figure 2. Standard Calibration Curve of TTC and TFC of Extracted Colorant

3. Results and Discussion

The experimental data generated during optimization for MLR, pH and time is presented in Table 1a and Table 1b, respectively. The optimized natural dye extraction protocol yielded a colorant with enhanced optical density, yield and extraction efficiency compared to previously reported methods. This indicates a significant improvement in both methodology and dye quality, areas that were underexplored in prior studies.

3.1 Optimization of Extraction Parameters

A total of two protocols were evaluated namely, Sinha et al.[11], Lei et al.[12] and the optimized method developed in the present study. The previously reported protocols failed to simultaneously achieve both maximized optical density and dye yield. However, the protocol optimized in this study utilized energy efficient and sustainable reagents and solvents to extract a reddish-brown dye with high optical density and yield. The optimal extraction conditions were determined to be MLR of 3 g/100 mL, pH 3 and extraction time of 15 minutes, resulting in the highest recorded optical density of 1.41 and yield of 18.33%. The yield found superior to reported literature 0.64%[11] and 17.4%[12]. The enhanced performance under these optimized conditions may be attributed to both individual effect and synergistic interaction among the three independent variables. A linear increase in MLR may be attributed to an increment in optical density, consistent with previous findings by Tabaraki et al.[14], which suggest enhanced mass transfer kinetics during the process.

In contrast, both pH and time showed a distinct behavior when compared to the trends observed in the MLR model. Initially, the optical density increased with time and decreasing pH, followed by a decline. This can be explained by the pH sensitive nature of natural colorants, high alkalinity often leads to colorant degradation, while extreme acidic conditions may hinder tannin hydrolyzation, both of which results in reduced optical density[15]. This behavior observed in extraction time and pH may be attributed to the attainment of a saturation point, beyond which the diffusion of colorant compounds reaches to equilibrium, resulting in no significant increase in optical density, even when the extraction is continued at a constant temperature of 90 °C and after a time duration of 15 minutes.

Overall, lower MLR and shorter extraction time were chosen, making the optimized method not only energy-efficient but also cost-effective, eco-friendly, and scalable. These attributes make it a promising sustainable alternative for natural dye applications in large scale industries including textiles, nutraceuticals and pharmaceuticals.

Table 1a. Experimental Data of Optical Density

MLR and pH

MLR	**pH**		
	3	**7**	**9**
1g	0.536	0.482	0.697
2g	0.847	0.841	1.030
3g	1.410	1.291	1.266
4g	1.411	1.231	1.234
5g	1.412	1.244	1.244
6g	1.413	1.211	1.290

Table 1b. Extraction Time

Time	**Optical density**
15	0.211
30	0.211
45	0.214
60	0.217
75	0.217
90	0.218

3.2 Functional performance of Extracted Dye

The qualitative functional performance of the extracted dye was assessed through its application on textile dying. The evaluation parameters included appearance, color strength (K/S) and absorbance. The dying procedure was performed sequentially as mentioned below:

3.2.1 Scouring of Fabric

Scouring was performed to remove impurities and enhance dye absorption. Cotton fabric was treated with a solution containing 0.5 g/L Sodium carbonate and 2 g/L nonionic detergent at 50 °C for 25 minutes, maintaining a MLR of 1:50. Post treatment, the fabric was thoroughly rinsed with tap water and air-dried at room temperature. Before dying and mordanting, the scoured fabric was soaked in clean water for 30 minutes prior to ensure uniform dye uptake.

3.2.2 Dyeing and mordanting

The natural dye extract (0.5%) was applied to silk, cotton, and wool fabrics using alum as a mordant (Figure 3). Three different mordanting techniques were evaluated through pre-mordanting, post-mordanting and simultaneous mordanting. Simultaneous mordanting resulted in dye precipitation and was therefore not recommended for further use. Conversely, pre- and post-mordanting approaches yielded better outcomes. Among these, post mordanting is highly recommended for textile application with the highest color strength (K/S= 12.68). The dye exhibited excellent affinity for silk and wool, likely due to their high protein content which promotes stronger interaction and enhanced dye fixation, resulting in deep and uniform color shades. Nevertheless, cotton, a cellulose fabric, exhibited comparatively lighter shades, but mordanting significantly improved color adherence. The mordant facilitated effective chelation with the dye molecules, producing intense brownish hues on the fabric.

The promising results indicate that the extracted dye is particularly suitable for proteinaceous fibers, while cellulosic fibers like cotton can also be effectively dyed with the aid of mordants. Further studies should include color fastness evaluation to future optimize dyeing applications and expand shade diversity from this natural colorant.

Figure 3. Mordant alum **a.** Wool, **b.** Silk, **c.** Cotton

3.3 Chemical constituents of extracted dye

The fruit peels of wild grown pomegranate are known to be rich in various bioactive constituents, particularly poly-phenolics such as tannins, flavonoids, and phenolic acids[10]. These polyphenolics (tannins and flavonoids) are primarily responsible for the color imparting properties of the extracted dye. In this study, TTC and TFC of the dye extract were assessed to evaluate its pigmenting potential. Phytochemical screening confirmed a substantial presence quantity of these color-imparting constituents, suggesting a high coloring potential and strong affinity for textile fibers, especially when used with mordants. Chemical constituents such as ellagic acid, gallic acid, quercetin and kaempferol are known not only for their contribution to the color stability but also for their antioxidant properties, which enhances the functional profile of the dye.

Total tannin and flavonoid content of colorant extracted under the optimized conditions were 0.28% and 0.14%, respectively. These values are consistent with, and in some cases higher than, those reported for cultivated pomegranate variety, such as tannin content of 0.100 ± 2.49%[16] and flavonoid content of 0.57 ± 0.3%[17]. The higher tannin and flavonoid content observed in wild pomegranate peels underscores their phytochemical richness and supports their potential use as a natural source of dye. Moreover, the presence of these bioactive compounds highlights dye's applicability not only in textile coloration but also in bifunctional formulations, including food products, cosmetics, and pharmaceuticals, where natural antioxidant non-toxic pigments are increasingly in demand.

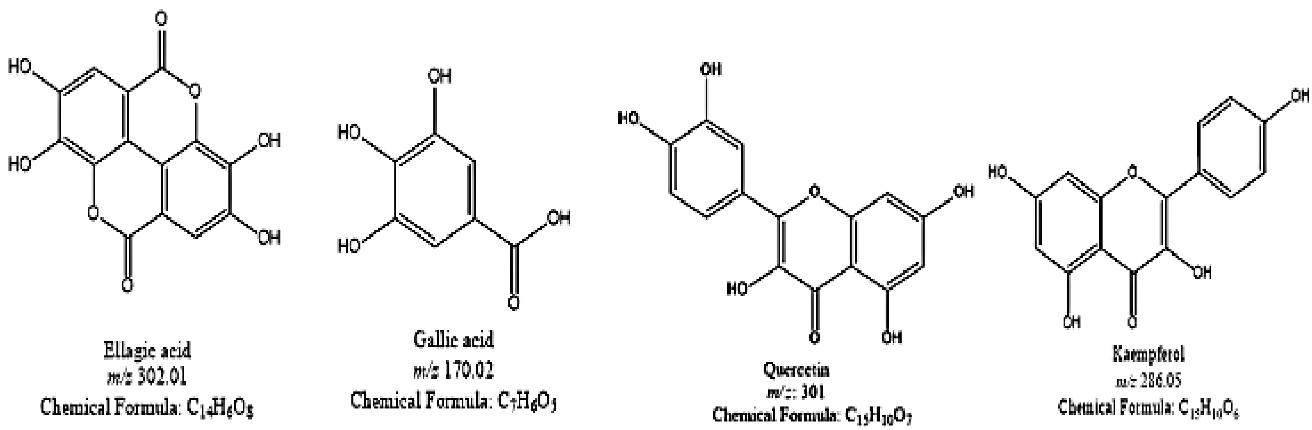

Figure 4. Characteristic unit of major color imparting chemical constituents

4. Conclusion

This study presents the first report on the extraction of a natural colorant from biowaste fruit peels of wild pomegranate using an eco-friendly, non-toxic and energy-efficient heat-assisted classical method. The extraction process was systematically optimized by evaluating the effects of three independent variables including MLR, pH, and extraction time on dye yield and optical density. The optimized conditions, i.e. MLR of 3 g/100 mL, pH 3, and extraction time of 15 minutes, yielded a reddish-brown dye obtained with elevated optical density (1.41) and an appreciable yield (18.33%). Phytochemical evaluation confirmed the presence of significant levels of TTC (0.28%) and TFC (0.14%), which are key contributors to the dye's coloring properties and antioxidant potential. Textile applications revealed strong dye-fiber affinity, especially in protein-based fabrics such as silk and wool. Post mordanting with alum resulted in improved color strength and uniformity, further supporting the dye's suitability for textile applications. However, optimization using statistical methods can provide more robust and valuable insights. Application of dye with different mordants for textiles (including fastness properties evaluation) and food products can also be explored in the near future. Overall, this study highlights the potential of wild pomegranate peel-derived dye as a natural, sustainable alternative to synthetic colorants with promising applications in textiles, nutraceuticals, and pharmaceuticals.

Author contributions

AB: Investigations, Data curation, Formal analysis, conceptualization, manuscript writing; **VJ**: Sample collection; **YCT**: Supervision and guidance; **VKV**: Funding acquisition, Project administration, Resources, Overall Supervision and guidance, Writing - review & editing.

Conflict of Interest Statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data Availability

All data generated or analyzed during this study are included in this published article.

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