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Extraction and Antibacterial Activity of Pulicaria Gnaphalodes as a Natural Colorant: Characterization and Application on Wool Fibers

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ABSTRACT

Pulicaria gnaphalodes has various biological activities and is used as an herbal plant in traditional medicine. The major components of the aqueous extract of this plant are flavonoids, terpenoids and phenolic compounds which could be a good candidate for dyeing of wool fibers. In this study, the aerial part of *Pulicaria gnaphalodes* plant was extracted and used for the coloration of wool yarns. Firstly, the chemical components of the *Pulicaria gnaphalodes* extract were identified using a gas chromatography-mass spectrometer (GC-MS). After that, the effect of dyeing procedure parameters such as mordant concentration, pH of dyeing bath, dyeing duration, and dye concentration was assessed on the color characteristics of the dyed yarn samples. The results and observations indicate that the un-mordanted and alum mordanted dyed samples have a yellow color and their hue angle value is located between Hue_{max}= 89.11° and Hue_{min}=76.49°. The obtained yellow shade at the optimized dyeing condition presented a considerable good washing and light fastness. In addition, the aqueous extract of aerial parts of *Pulicaria gnaphalodes* presented an inhibitory effect against *S. aureus* and *E. coli* bacteria. *Prog. Color Colorants Coat. 12 (2019), 145-154© Institute for Color Science and Technology.*

1. Introduction

Natural dyes are obtained from various parts of different plants, insects, minerals and fungi. In recent years, there has been a renewed interest in the application of natural dyes and the optimization of natural dyeing processes due to several environmental and health-related concerns about synthetic dyes. Several synthetic dyes are non-biodegradable and able to split reductively to various banned aromatic amines in contact with human sweat [1-5].

Compared with synthetic dyes, natural dyes are environmentally safe and can produce various shades on textile substrates depending on the type of mordant and the dyeing procedure conditions [6-11]. Furthermore, some natural dyes show functional properties such as antibacterial, antioxidant, insect repellent and UV protection, when applied to textile fibers [7, 12-15]. The coloring matters identified in natural dyes include several classes of compounds such as tannins, alkaloids, anthraquinones, naphthoquinones, carotenoids, etc. Due to the relatively low exhaustion of natural dyes, mordants are usually employed to improve the color strength and fastness properties and obtain multiple shades [10, 16-18].

Exploring new sources of natural dyes and the optimization of their extraction and application procedures using various technologies has gained great attention of the researchers in recent years. Ultrasonic energy has been used to improve the extraction and dyeing efficiency of nylon, cotton, wool, and silk with

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different natural dyes [8, 9, 19, 21]. Microwave radiation has improved the color strength of cotton fabric dyed with milkweed, cochineal, and harmal seed [22, 23]. The use of UV and gamma radiations as pretreatments has improved the color strength of cotton fibers dyed with different natural dyes [24-26]. Plasma treatment has shown a great ability for enhancement of dyeability of natural and synthetic fibers with natural dyes [4, 27-30]. Furthermore, pretreatment of fibers with different compounds, including cationising agents [6], silver nanoparticles [31], bentonite [18], chitosan [32], β-cyclodextrin [27, 33], dendrimer [28], and tannin [34] are other methods which have been investigated for improvement of natural dyeing of textiles.

Pulicaria gnaphalodes is a medicinal plant known under the name of "Kak Kosh" in Khorasan, Iran. The major components of the water extract of this plant are flavonoids, terpenoids and phenolic compounds [35, 36]. The antioxidant and medicinal properties of the extract of this plant have been well studied [35, 37]. The flavonoids and phenolic compounds present in the aqueous extract of *Pulicaria gnaphalodes* can be used as a candidate for dyeing of textile fibers. However, there is no report on the application of this plant for dyeing of textile fibers.

In this study, *Pulicaria gnaphalodes* aerial parts have been used as a new source of natural dye for coloration of wool fibers. The chemical constitution of the aqueous extract of the plant was analyzed by chromatography-mass spectrometer (GC-MS). The effect of dyeing conditions on the color strength of the dyed samples was studied. The fastness properties of the dyed samples were evaluated and the optimum conditions for obtaining the highest color strength and fastness ratings were estimated. The antibacterial activity and minimum inhibitory concentration of the aqueous extract and the dyed sample under optimal conditions was evaluated.

2. Materials and Methods

2.1. Materials

In order to remove the impurity, the wool yarn (200tex) were scoured before the dyeing procedure by 1% Triton X100 at 50 °C for 30min. The Triton X100, acetic acid, sulfuric acid, and ammonia were purchased from Merck Company, Germany. The commercial alum (Aluminum potassium sulfate, $KAl(SO_4)_2$) was used as a mordant. The aerial parts of *Pulicaria gnaphalodes* (Figure 1) were collected from Birjand suburbs in South Khorasan province, Iran, at July 2017. The plant material was dried naturally on laboratory benches at room temperature (23– 27 °C).

2.2. Methods

2.2.1. Gas chromatography-mass spectrometry analysis

The composition of the *Pulicaria gnaphalodes* extract was identified using gas chromatograph-mass spectrometer (GC-MS, HP and Agilent Technology) and an HP-5MS capillary column (60 m × 0.25 mm; film thickness 0.25 µm). The carrier gas was helium with a flow rate of 1 mL/min, and the oven temperature was kept at 50 °C for 5 min then it was increased at a rate of 4 °C /min until the temperature reached 280 °C.

Figure 1: The aerial parts of *Pulicaria gnaphalodes* plant which were collected from Birjand suburbs in South Khorasan province.

2.2.2. Wool yarn treatment with metal mordant before dyeing

According to the preliminary experimental and literature [5, 38] the pre-mordanting method was chosen. The scoured wool sample was pretreated with different alum concentrations (Table 1). The wool yarn was dipped into the proposed mordant bath solution (fibers: liquor ratio = 1:40) at 40 °C and the temperature gradually was increased with of 3 $°Cmin^{-1}$ to the boiling point (95 °C at the laboratory condition) and continued for 30 min. After that, the wool fibers were rinsed with distilled water and dried at the room condition.

2.2.3. Aqueous extraction of colorant

The dye was extracted from leaves, stems, and flowers of P. gnaphalodes as follows. The well-milled powder was boiled in water for 60 minutes under stirring. Then, the extract solution was cooled and passed through the filter and its concentration was adjusted to 10% wt by distilled water.

2.2.4. Dyeing process

The experimental runs was determined by using Design-Expert DOE 10.0.7 Software and central composite design (CCD) method according to the dyeing variables (Table 1). The four independent numerical variables were selected which leaded to determine 30 runs. For each run (Table 2), the temperature of the dyeing bath was increased gradually to 50 °C. Then, a wool sample yarn was dipped into the dye bath solution and the temperature was increased with the rate of 3 $°C.min^{-1}$ to the boiling temperature. The dyeing procedure was continued for the proposed time and then the sample was rinsed thoroughly with distilled water and dried at room condition.

2.2.5. Evaluation of the color characteristics of dyed samples

The spectral reflectance of dyed wool yarns was measured using a Ci60, X-Rite spectrophotometer in the visible region. The CIE color-coordinates namely, L*, a*, b* and hue angle were measured under illuminate D_{65} and 10º standard observer. The values of a* and b* are related to the red-green (+ve= red, -ve = green) and yellow-blue (+ve = yellow, -ve= blue) coordinates, respectively.

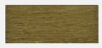
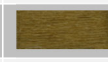
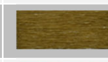
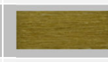
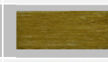
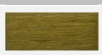
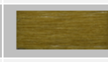
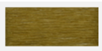
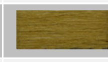
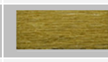
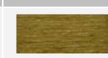
2.2.6. Antibacterial studies and minimum inhibitory concentration

The antibacterial efficiency of P. gnaphalodes natural dye extract was determined against S.aureus ATCC 6538 as a Gram-positive and *E. coli* ATCC 8739 as Gram-negative bacteria using microplate systems. The phosphate buffer solution (pH 6) was used as a diluent for standard and preparation of sample solutions. The Mueller-Hinton Broth was employed as a culture medium. A volume of 100 µL of culture medium was added to the sterile 96 well plate after that 100 µL of extract was added to the first row. The wells with different extract concentration from 0.09 up to 50 µL were prepared. Finally, the 100µL bacterial suspensions (10^8 CFU/mL) were added to each well. The plates were prepared in triplicate and placed in an incubator set at 37 °C for 24 h. The lowest concentration with inhibition of bacterial growth was taken as the MIC value.

Table 1: The range of dyeing variables at the dyeing procedure of wool fibers with aqueous extract of *Pulicaria gnaphalodes* aerial parts plant.

Dyeing variables	Values of levels	
	Low	High
Aluminum potassium sulfate (%, owf),	0	10
pH of dyeing bath solution	1	8
Dyeing concentration (%, owf)	50	200
Dyeing time (minute),	20	90

Table 2: The proposed experimental design of dyeing procedure and related color strength of the dyed sample.

Run	Mordant (%, owf)	pH	Dye concentration (%,wf)	Dyeing time (minute)	K/S	Sample
1	0	1	200	90	15.2	
2	0	8	50	20	4	
3	5	4.5	125	55	4.8	
4	10	1	50	90	11.5	
5	5	1	125	55	4.3	
6	5	4.5	200	55	5.4	
7	10	1	200	20	10.2	
8	0	8	200	20	12.8	
9	0	8	50	90	3.6	
10	5	4.5	125	20	1.6	
11	10	1	200	90	4.5	
12	5	4.5	50	55	2.5	
13	5	8	125	55	6.5	
14	0	1	50	20	9.2	
15	5	4.5	125	55	9.1	
16	0	4.5	125	55	0.9	
17	10	4.5	125	55	13.9	
18	10	8	50	90	15.5	
19	0	1	50	90	12.8	
20	5	4.5	125	55	9.7	
21	5	4.5	125	55	6.8	
22	0	1	200	20	10.5	
23	10	8	50	20	11.1	
24	10	1	50	20	11	
25	0	8	200	90	5.1	
26	5	4.5	125	55	9.9	
27	10	8	200	20	10.8	
28	5	4.5	125	90	12	
29	10	8	200	90	11.8	
30	5	4.5	125	55	11.5	

3. Results and Discussions

3.1. The chemical composition of the extract

The major constituent of the P. gnaphalodes aerial parts were found to be β-citronellol (18.05%), 1,8-cineole (9.28%), α-pinene (8.9%), terpinen-4-ol (2.96%), geraniol (3.67%), α-terpineol (6.51%), citronellyl acetate (1.35%), myrtenol (2.28%) and chrysanthenone (15.98%). The yellow color obtained from the extract of aerial parts is due to the phenolic and flavonoids compounds found in the extract. The P. gnaphalodes aerial parts used in this study contain a phenolic and flavonoids component percentage of about 27.2 and 25.12 mg/g, respectively [39]. However, the major components of P.gnaphalodes obtained from individual parts of Iran are completely different [40]. The sites of P. gnaphalodes collection with different geographical locations might influence the major component of its extract.

3.2. Effects of dyeing parameters on the color strength

The wool fabric samples were dyed according to the proposed recipe of experimental design with the extract of P.gnaphalodes aerial parts. The obtained data (dyeing variables and response value) were fitted to various models by Design-Expert DOE Software. According to the ANOVA results (F= 3.33, $p < 0.0954$) the 2FI model (Sequential sum of squares for the two-factor interaction terms) was chosen.

The CIELAB color space position of the dyed samples is presented in Figure 2. The results and observations indicate that the un-mordanted and mordanted dyed samples have a yellow color and their hue angles value is between $Hue_{max}= 89.11^{\circ}$ and $Hue_{min}=76.49^{\circ}$.

3.2.1. Effect of dyebath pH and Mordant concentration

The effect of pH on the color strength of dyed samples is presented in Figure 3. It is seen that the pH of dyeing bath solution has a significant effect on the color strength. Increasing the dyeing bath pH from 1 to 8 has resulted in a decrease of the color strength value of the dyed samples. The effect of pH on color strength value can be attributed to the interaction of wool fibers and dye molecules [41]. The wool fiber gains more positive charges at acidic pH [42] and the P.gnaphalodes extract which possess negative charge due to its phenolic and flavonoids compounds, can be better absorbed on the positively charged wool fibers at this condition. Therefore, at the acidic pH, the color strength value of the dyed fabric was greater compared to the alkali and neutral conditions.

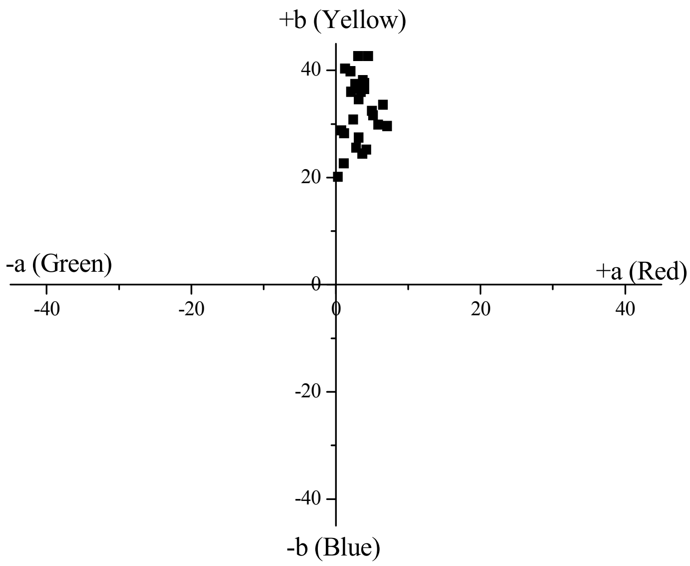


Figure 2: The CIELAB color space position of dyed samples at different dyeing condition with aerial parts of P. gnaphalodes.

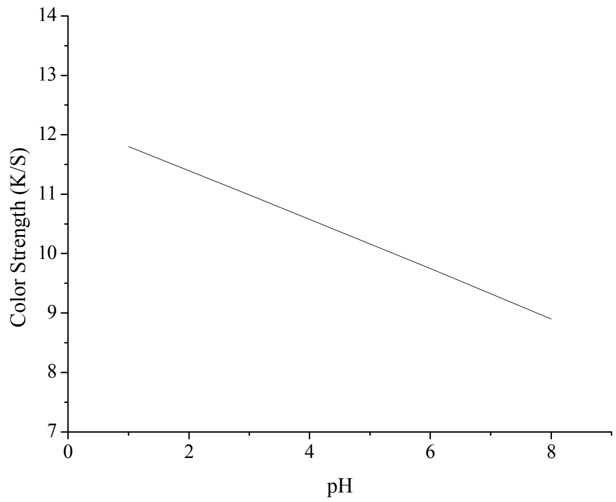


Figure 3: The effect of pH on the color strength of dyed samples with aqueous extract of aerial parts of *P. gnaphalodes* (Dye concentration= 200%, Dyeing time=60 minute, and Alum concentration=0%).

The effect of mordant concentration on the color strength of dyed wool yarns is presented in Figure 4. It is confirmed that the addition of aluminum potassium sulfate as a mordant and increasing its concentration resulted in an increase of the color strength value of the dyed wool yarns and the dye exhaustion were greater at the higher mordant concentration. It means that the dye uptake of the mordanted samples has been higher than the non-mordanted sample. The presence of mordant increases the interaction between the hydroxyl groups of phenolic and flavonoid compounds as the main colorant molecules and amine groups of wool fibers [41, 43]. Transition metal ions with a strong coordinating power have been used as mordant in the textile substrate. It can act as bridging material to create substantivity of natural dyes into a textile material. However, the mordant concentration has a lower effect at the week acidic condition of dyeing bath (Figure 4 b), which is the isoelectric point of wool fiber and is around pH = 4-5 [44]. The isoelectric point is the pH of an aqueous solution of a peptide at which the molecules on average have no net charge.

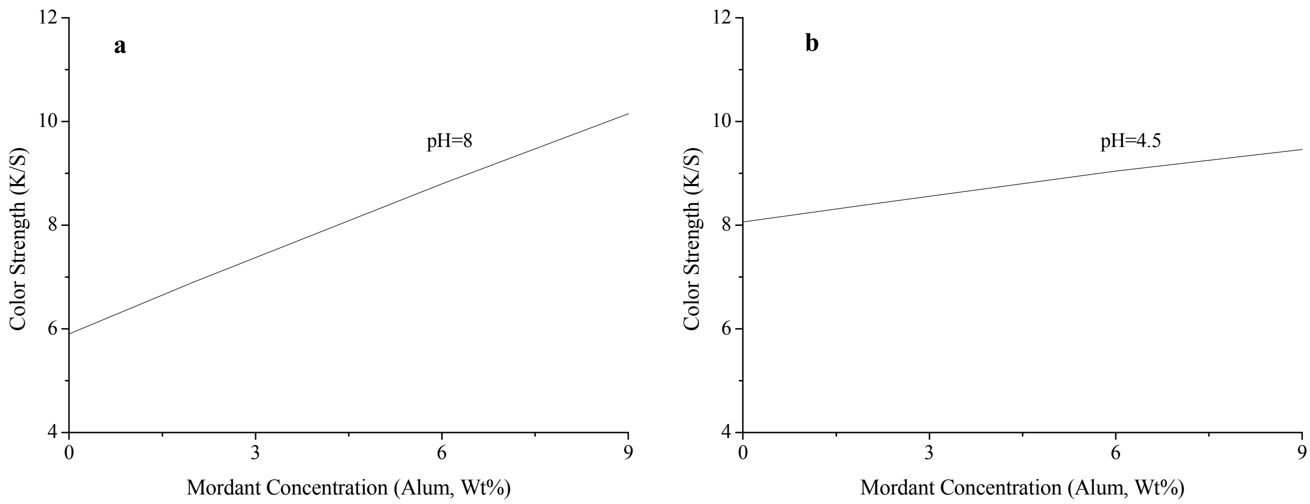


Figure 4: The effect of mordant concentration on the color strength of dyed samples at a) pH=8 and, b) pH=4.5 of dyeing bath solution (Dye concentration= 125%, Dyeing time=60 minute).

3.2.2. Effect of dye concentration and dyeing time

The effect of dye concentration on the color strength of dyed samples was evaluated and the results are presented in Figure 5. It is clear that the color strength increased with an increase in the dye concentration in the dyeing bath solution. Increasing the dye concentration from 50% to 200% caused an increase in the color strength of the dyed samples. In addition, the effect of dyeing time on the color strength of dyed wool fiber is presented in Figure 6. It is clear that the color strength increases with an increase in the dyeing time because diffusion of the dye molecules into the fiber structure depends on the time [5, 41].

3.3. Optimization of dyeing condition

The dyeing condition of wool fibers with P. gnaphalodes extract was optimized. The dyeing condition was chosen in the proposed range and the color strength as the response was adjusted in the highest amount. The proposed optimized dyeing condition of wool fiber with P. gnaphalodes extract is presented in Table 3.

3.4. Fastness properties of dyed samples

The color fastness to washing and light of the dyed sample with P. gnaphalodes extract at optimized condition were assessed and is represented in Table 4. Washing fastness results of these samples were assessed according to grayscale, and the results of light fastness were assessed according to the blue scale. Presence of alum mordant in the dyeing recipe results in comparable values of washing fastness. The light fastness result of the dyed sample indicates that the use of alum as a mordant was advantageous for the dyed wool fibers with P. gnaphalodes extract. The presence of mordant in the wool fiber increased the color strength of the dyed sample due to higher dye uptake as well as enhancing their fastness properties.

3.5. Antibacterial activity

P. gnaphalodes can be found in different areas of Iran and is a traditional herbal plant which is used as a flavoring agent in food. Some species present various biological activities such as antibacterial, antifungal and insecticidal properties [39]. The MIC of aqueous extract of aerial parts of P. gnaphalodes against studied bacteria is presented in Table 5. The aqueous extract of aerial parts of P. gnaphalodes presented a good inhibition effect against S.aureus and *E. coli*. This biological activity of P. gnaphalodes aerial parts could be due to the presence of different components such as 1,8-cineole, borneol, alpha-pinene, p-cymene, geraniol and thymol [40]. This component is more effective at the lower concentration on S.aureus compared to *E. coli*.

Table 3: The proposed optimized dyeing condition of wool fiber dyeing with *P. gnaphalodes* extract.

Alum Mordant (%, owf)	pH of dyeing bath solution	Dyeing concentration (%, owf)	Dyeing time (minute)
10	8	200	60

Table 4: Fastness properties of the dyed sample with the proposed optimized condition.

Fastness property	Alum Mordant concentration (%, owf)	
	10%	0%
Washing	4-5	4
Staining on wool fibers	5	5
Light	7-8	5-6

Table 5: The MIC of aqueous extract of aerial parts of *Pulicaria gnaphalodes* against studied bacteria.

MIC (% w/v)	
S. aureus	*E. coli*
0.6	0.9

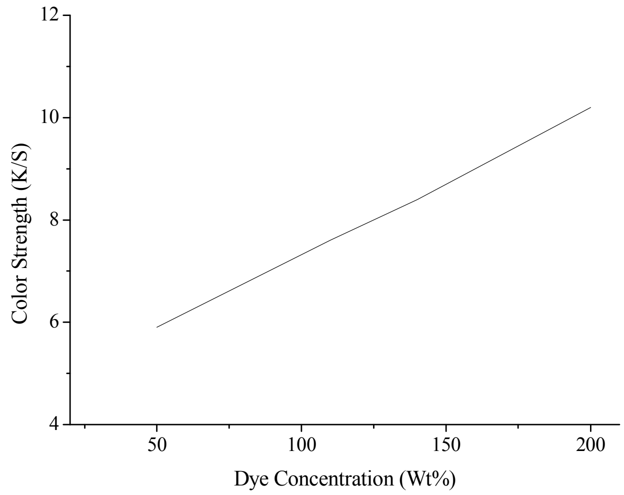


Figure 5: The effect of dye concentration on the color strength of dyed samples (pH= 5, Dyeing time=60 minute, Mordant Concentration=0%).

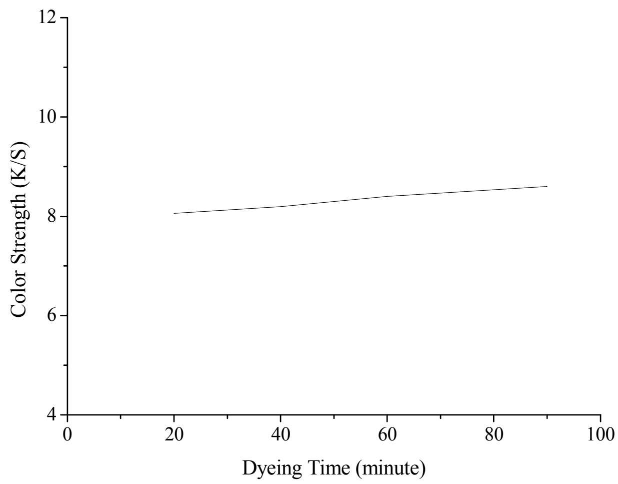


Figure 6: The effect of dyeing time on the color strength of dyed samples (Alum mordant concentration= 0%, pH= 5, Dye concentration =125%).

4. Conclusion

Pulicaria gnaphalodes has been used as an herbal plant in traditional medicine. The aqueous extract of the aerial part of *Pulicaria gnaphalodes* plant was used for the coloration of wool yarns. The major constituent of the P. gnaphalodes aerial parts was found to be β-citronellol, 1,8-cineole, α-pinene, terpinen-4-ol, geraniol, α-terpineol, citronellyl acetate, myrtenol, and chrysanthenone. The yellow color obtained from the extract of aerial parts is due to the phenolic and flavonoids compounds found in the extract. The results indicated that the un-mordanted and mordanted dyed samples have a yellow color. The pH of dyeing bath and mordant concentration presented a significant effect on the color strength of dyed samples. Increasing the dyeing bath pH from 1 to 8 has resulted in a decrease of the color strength of the dyed samples. Moreover, the addition of alum mordant and increasing its concentration resulted in an increase in the color strength of the dyed samples and the dye exhaustion were greater at the higher mordant concentrations. The aqueous extract of aerial parts of *Pulicaria gnaphalodes* presented a good inhibition effect against S.aureus and *E. coli* bacteria.

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