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EVALUATION OF *RHEUM PALMATUM* RHIZOMES AVAILABLE IN JORDANIAN MARKETS AND INVESTIGATION OF ITS POTENTIAL USE AS HAIR DYE COSMETICS

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Context: *Dyeing hair with natural components derived from plant material is a common practice since ancient time. Variable colour shadows obtained from different plant extracts. The folkloric practices need optimization to find alternatives for the chemical dyes, which carry loads of health hazards. For decades, people use Rhubarb roots extract in treating chronic constipation and other minor health disorders. The pharmacological effects refer to their active constituents; anthraquinone glycosides. Hair dyeing properties of Rhubarb roots extracts need further scientific investigations.* ***Aims:*** *To evaluate the hair-dyeing properties of rhubarb's aqueous extract as an eco-friendly alternative to the commercially available hair dyes and to measure the fastness of the produced colour in different conditions.* ***Methods:*** *Identification of the plant material was done according to international specifications. Wool pieces dyed by aqueous extracts of rhubarb's roots and rhizomes under different conditions. Variables that might be affecting the dyeing process such as concentration, pH, using colour mordant, swelling agent, and dyeing time were studied. Spectroscopic methods used to record colourfastness measurements.* ***Results and Conclusion:*** *The extract of rhubarb roots and rhizomes show good dyeing properties. The obtained colours were pale yellow, intense yellow and pink. Colour fastness is enhanced and optimized using 2 mg/ml concentration, 2 h dyeing time, acidic pH, adding mordant and pretreating the fibres with a swelling agent.*

Keywords: *cosmetics, hair coloring, natural products, Rheum palmatum, rhubarb.*

INTRODUCTION

The increased awareness of environmental and health hazards of using synthetic compounds in all life aspects arises the impact and importance of going back to nature to minimize the negative impact of these chemicals on the ecosystem and human health. Individuals seek the help of natural compounds from their local environment for alleviating health problems and providing an alternative to cosmetic and beautifying products at lower cost and with fewer undesirable effects.[1]

The subterranean parts of the rhubarb plant, *Rheum palmatum* L. Polygonaceae are well known since ancient times for their purgative properties. This biological action refers to their anthraquinones content include chrysophanol, aloe-emodin, emodin, rhein and physcion.[2] The astringent activities of the plant extract, due to its glucogallin, gallotannins and epicatechin gallate contents. Tannins abundancy clarifies its traditional use in treating burns, jaundice, tonic, and in cleansing teeth. Moreover, the active constituents isolated from rhubarb have been investigated for their anticancer, antimicrobial, anti-inflammatory,

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cardio-protective and hepatic-protective activities.[3&4] Rhubarb is endogenous to South East Asia and not grown or cultivated in Joran, accordingly, it has been imported and traded in herbalist markets for their laxative and, sometimes, claimed slimming activities.

The use of natural compounds in cosmetics was introduced early in ancient times, starting from making hygienic products such as soaps or for aesthetic purposes to prolong youth appearance.[5&6] Although hair care products and hair dyes have existed for decades, sadly they were greatly replaced with synthetics for their stability, wide range of colour shades, and their ease to use and to formulate into different products. There is an increased concern for health and environmental hazards associated with the use of synthetic hair dyes. In particular, the oxidative hair dyes and their component.[5] These include sensitization reactions, carcinogenicity, and genotoxicity.[7-9] On the other hand, there are negligible toxicities of the natural hair, food, and textile dyes that have been used traditionally since ancient times. Examples of natural colouring compounds include: Lawsone in Henna leaves *Lawsonia inermis*, carotenoids from Saffron flowers *Crocus sativus*, juglone from walnut *Juglans regia*, anthocyanin from flowers and calyxes of roselle *Hibiscus sabdariffa*, anthocyanins from blackcurrant *Ribes nigrum*.[10-15] Using rhubarb for its colouring properties has modest scientific research confined to textile dyes either by itself or with the addition of colour mordant.[16-18] Regarding rhubarb safety profile, low to negligible toxicities were reported with the use of the oral extract. Serious effects confined to extensive and prolonged use include nephrotoxicity.[19-21]

With the recent increase in demands on cosmetic and beautifying products, greater emphasis needs to be put on scientific research to find out an eco- and health-friendly replacement of the chemical substances currently in use.

MATERIAL AND METHODS

Plant material

Roots and rhizomes were purchased from Kabatilo/ a local herbalist shop in Amman – Jordan. The roots were ground into fine powder. Powdered roots were identified using the British Pharmacopeia (2009) and Trease, and Evans's descriptive specification.[22] Voucher specimen have been kept at Mutah University Herbarium.

Identification test

Powdered rhubarb was identified microscopically according to the descriptive references. The plant materials tested to be genuine and not mixed with Rhapontic Rhubarb according to Trease and Evans specifications.[22] Powdered drug 0.5 g was mixed with ethanol (45%, 10 ml) for 20 min with continuous shaking. Then the filtrate was examined under UV light before and after exposure to ammonia vapour.

Plant extraction

Powdered plant material was suspended in distilled water for 16 h at 25 ℃ with continuous shaking using mmemert shaker waterbath®. Filtered twice, then the extract was used for dyeing wool pieces under variable conditions.

Anthraquinones content

Following Bornträger's test, powdered extract was macerated with immiscible organic solvent (ether), filtered, then aq. Ammonia solution was added. Change in colour in the aq. layer to pink, red, or violet indicates positive results.[22]

Sheep's wool

White sheep's wool pieces, used for modelling human hair, are obtained from local farmers in Al-Karak-South Jordan. These pieces were bleached, washed thoroughly, dried and dyed with rhubarb extracts under different conditions.

All chemicals were obtained from Sigma-Aldrich, GmbH.

Color analysis

Colour fastness measurements recorded by Double Beam SPUV-26 spectrophotometer, Lab-World (Advanced Technologies), Germany.

Dyeing process

Three pieces of wool (each of 0.25 g) were immersed in the aqueous extracts with continuous shaking. Then, squeezed and allowed to dry at 25 ℃ for 24 hrs. Each dyed piece was washed with 40 ml distilled water with continuous stirring at 25 ℃. One millilitre was taken from the resulted solution, filtered and placed in the scanning spectrophotometer cell. The absorbance was measured λ=520 nm.

Variables affecting the dyeing process
Concentration

Powdered rhubarb roots and rhizomes were macerated overnight in distilled water in three different concentrations (1, 2, and 4 mg/ml). The extracts were filtered twice and used for dyeing the wool pieces.

Dyeing time

The wool pieces were immersed with continuous shaking in previously prepared aq. rhubarb extracts for 30, 60, 120 min and overnight.

The pH of the dyeing solution

A series of dyeing solutions were prepared at different pH values (pH= 3, 5, 7, and 9). The pH was adjusted using 1M HCl and 1M NaOH aq. solutions.

Pre-treatment of the wool pieces by an alkaline solution

The wool pieces were pretreated by a swelling agent (1M aq. NaOH) before being immersed in the dyeing solutions at different pH values.

Adding a color mordant: Anhydrous sodium sulphate

Anhydrous sodium sulphate salt was added in a concentration of 1 mg/100 ml to the dyeing solution at different pH values.
Each experiment was performed in triplicate.

Statistical analysis

The current project aims to evaluate the different variables affecting the dyeing process with rhubarb aqueous extract. Statistical analysis and analysis of variance of the findings was performed through one way ANOVA, 2016. *P*-values equal or less than 0.05 are considered significant.

RESULTS AND DISCUSSION

Plant material identification

Powdered rhubarb has an orange- brown colour. The macro- and microscopical characteristics were found to comply with the requirements of BP (2009). No blue colour fluorescence was observed with and without exposure to ammonia vapour In addition to, the rose-red colour obtained with Borntrager's test confirming the anthraquinone content. This finding excludes the admixture with rhapontic rhubarb.[22]

Variables affecting the dyeing process and color substantivity
Concentration

Wool pieces were well-stained by the used concentrations of the colouring solutions (1, 2 and 4 mg/ml). The yellowish colour was obtained and persists after washing. The visible intensity of the colour was a bit weaker at the lowest concentration, and it was almost the same as the other two concentrations (Fig. 1).

On the other hand, the fastness of the colour after washing was considered as a reflection of the absorbance value of the washing solution and measured at λ= 520 nm. The average absorbance of the washing solutions were (0.134, 0.213, and 0.156 for the 0.2, 0.4 and 0.8 mg/ml respectively). A marginally significant decrease (P= 0.1) in the mean absorbance values of the lowest tested concentrations compared to the highest one. No significant differences (P> 0.05) were measured between the internal variables (Table 1).

These findings matched the expectations, as the strength of the colour intensity would be higher with the increase in the number of dyeing particles that interact with the wool fibres. Besides this, the increased amount of rhein (cassic acid) extracted from the highest strength dyeing solution would enhance the dyeing properties by acidifying the solution. Which, in turn, would help to protonate more particles that facilitate the interaction with the negatively charged keratin in the wool fibres. At a pH of around 6, the species of keratin are 50% positively charged and 50% negatively charged.[23] The pH dropped slightly with the increase in the concentration, it was measured to be 5.4, 5.8, and 6 for the 0.8, 0.4, and 0.2 mg/ml respectively.

The decision was made to process with a concentration of 2 mg/ml.

Table 1: The effect of different concentrations on the dyeing process

Groups	*Count*	*Sum*	*Average*	*Variance*		
0.2 mg/ml	3	0.467	0.155667	0.000342		
0.4 mg/ml	3	0.64	0.213333	0.003436		
0.8 mg/ml	3	0.401	0.133667	0.000212		
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	0.010156	2	0.005078	3.817172	0.085222	5.143253
Within Groups	0.007982	6	0.00133			
Total	0.018138	8				

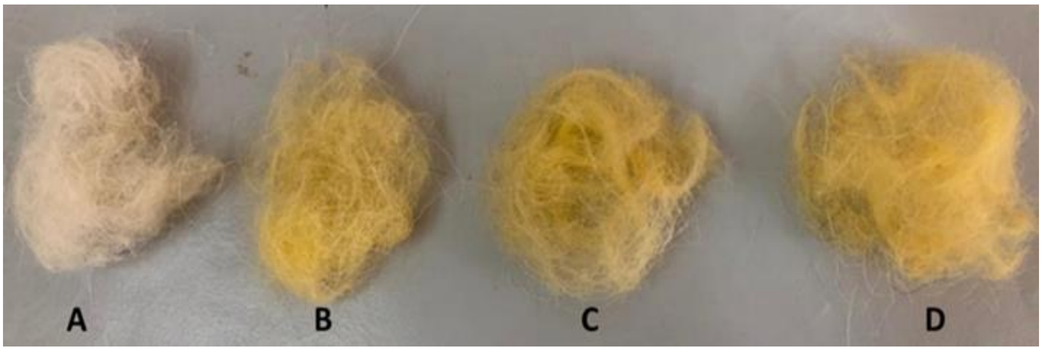

Fig. 1: Effect of different concentrations of the dyeing solutions Control (A), 1 mg/ml (B), 2 mg/ml (C), 4 mg/ml (D)

Dyeing time

The intensity of the colour was noticed to be stronger by extending the dyeing time. Prolonged exposure of the wool fibres to the dyeing solution enhances its uptake of the dyeing material. By measuring the absorbance of the washing solutions at λ= 520 nm; there were no significant differences between the uptakes after 30, 60, and 2 hrs dyeing times (*P* value> 0.0.5). This is due to the good interaction between the dye molecules and the wool fibres. Prolonged dyeing for 24 hrs shows a visible increase in the colour intensity compared to the 2 hrs dyeing time, but a significant increase in the absorbance after washing (P < 0.05). In this case, prolonged dyeing accumulated the excess dye molecules over the fibres, which made them easier to be washed and less sustainable. The effect of dyeing times on the dyeing process was analyzed as shown in Table 2.

Table 2: The effect of dyeing time process

Groups	*Count*	*Sum*	*Average*	*Variance*		
30 min	3	0.31	0.103333	0.002226		
60 min	3	0.194	0.064667	0.000534		
120 min	3	0.193	0.064333	0.000745		
24 h	3	0.217	0.072333	0.002422		
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	0.003075	3	0.001025	0.691594	0.582385	4.066181
Within Groups	0.011857	8	0.001482			
Total	0.014932	11				

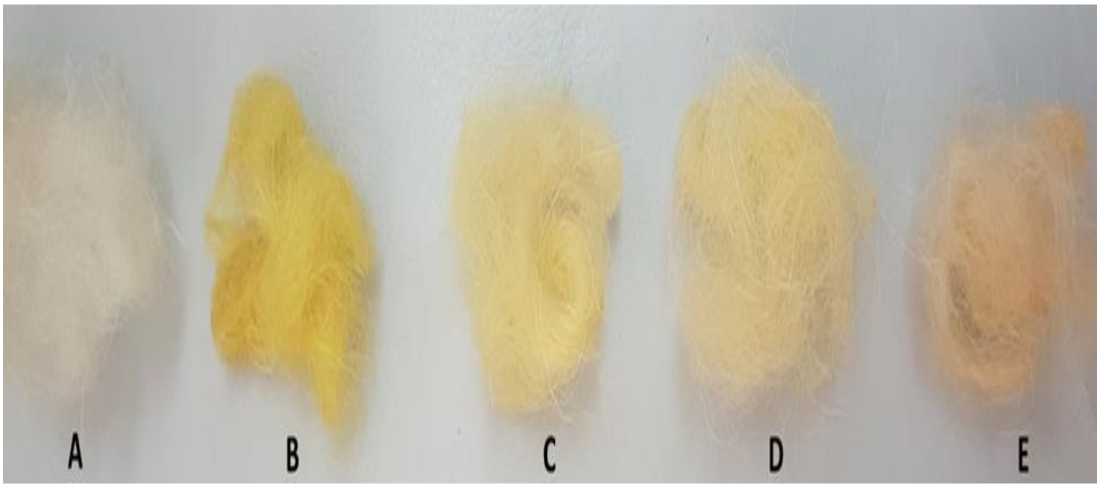

Fig. 2: The effect of a range of pH values on the dyeing process Control (A), pH=3(B), pH=4 (C), pH=5 (D), pH=9 (E)

pH of the dyeing solution

Different shades of yellowish-pinkish colours were obtained by different acidity of the dyeing solutions. The visible colours were strong yellow at pH= 3, to faint yellow at pH = 5 and 7, to faint pinkish colour at pH = 9 (Fig. 2).

The calorimetric findings confirmed that the fastness of the colour was highly affected by the acidity of the solutions. At pH= 3, the strongest colour was accompanied by the highest measured absorbance at λ= 520 nm in an average of 0.255. By increasing the pH to 5, a significant decrease in the loss of dyeing particles was found compared to pH= 3 (*P*> 0.05). Fewer particles to be lost is due to the lower number of dyeing molecules interact with the fibres, and this explains the faint stain. There were no significant differences in the measured readings between pH= 5 and pH= 7. Also, there were no visible changes in the colour intensity. This is mainly due to the buffer capacity of the keratin molecules.[24] On the other hand, by increasing the pH, the visible colour changed to light pink with the least measured absorbance at λ= 520 nm in an average of 0.141. There was a significant decrease in the uptake compared to the neutral pH (P< 0.05). The explanation of the observation is that at the basic pH of the anthocyanins, the chief dyeing molecules in *R. palmatum* is unstable, therefore fading in observed colour has occurred.[25] The effect of the dyeing solution pH was analyzed as shown in Table 3.

Table 3: The effect of pH

Groups	*Count*	*Sum*	*Average*	*Variance*		
pH =3	3	0.423	0.141	0.000139		
pH=5	3	0.664	0.221333	0.002404		
pH= 7	3	0.656	0.218667	0.000576		
pH= 9	3	0.765	0.255	0.002191		
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	0.020957	3	0.006986	5.261528	0.02691	4.066181
Within Groups	0.010621	8	0.001328			
Total	0.031578	11				

Effect of fibers pretreatment with strong alkaline solution

Theoretically, pretreating the wool fibres with a strong alkaline solution would help the fibres to swell. This, in turn, facilitates the penetration of the dyeing particles in between the cuticle layers. At pH = 9, pH = 7, and pH = 3, the obtained results denied the expectations; as no visible changes in the colour were noticed and no significant differences in the absorbance values of the washing solution were measured (P> 0.05) in the pretreated fibres compared to the untreated ones. The results confirmed that there is no need for pre-soaking with an alkaline solution in the cases of dyeing at alkaline, neutral, and acidic media. On the other side, dyeing fibres with no adjustment of the pH (the natural pH= 5.5) had benefited from the pretreatment step where it was found that a significant decrease (P< 0.05) in the missed particle after washing the dyed wool pieces, which indicated an increase in the colour substantively.

Effect of adding color mordant: Anhydrous sodium sulphate

Adding sulphate salt, as a mordant agent, to the dying solution after filtration aims, theoretically, to increase dye substantivity and decrease the loss of dyeing particles after the washing step. Our findings match the expectations; adding the mordant salt decreases the loss of the rhubarb particles significantly. This was concluded by the measured decrease in the absorption values of the washing solution at λ= 520 nm. At pH= 3 and at pH= 7 the intensity of the yellow colour didn't visibly change and the amount of the lost particles was significantly decreased (P< 0.05) compared to dyeing the fibres at the same conditions without adding the mordant salt. At the acidic solution, the absorbance of the washing solution (average of triplicate) was found to be 0.255 in the first case compared to 0.102 (average of triplicate) in the second case. At pH = 7, the absorbance of the washing solution (average of triplicate) was found to be 0.221 in the first case compared to 0.193 (average of triplicate) in the second case. A marginally significant decrease in the absorbance (P= 0.1) was recorded at pH= 5. No visible changes in the colour (pale-yellow) were noticed. The case was not the same at pH= 9. Although the visible intensity of the colour did not change after washing, there were no significant differences in the readings of the washing solutions in both cases (P> 0.05).

Conclusion

After the latest revolution in cosmetics and beautifying products, the health and ecosystem hazards warning arise to alleviate the negative impact of recurrent and excessive use of chemicals in such products. Back to nature is the optimum solution to find eco-friendly alternatives to cosmetic agents. Modest research was performed to investigate and develop a natural hair colourant agent with low or negligible undesirable effects. The harmful effects of excessive oxidative dyes were well known and reported. The output of the current research project highlights the cosmetic activity of *R. palmatum* as a natural hair dyeing agent, whereas its use was confined only to its laxative activity. After studying different variables affecting the dyeing process, a range of colours and substantivity could be achievable. Further investigations are encouraged to examine the safety of its topical application.

Conflict of Interest

The authors declared that there is no conflict of interest with the data contained in the manuscript.

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نشرة العلوم الصيدليـــة
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تقييم ريزومة الراوند المتوفرة في الأسواق الأردنية و فحص إمكانية استخدامها في مستحضرات التجميل

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يعد صبغ الشعر بالمنتجات الطبيعية ممارسة شائعة. يتم الحصول على ظلال الألوان مختلفة من العديد من المستخلصات النباتية في الممارسات الفولكلورية. لعقود من الزمن ، تستخدم جذور الراوند في علاج الإمساك الحاد والاضطرابات الصحية الأخرى؛ ويرجع ذلك أساسا إلى محتواها من جليكوسيدات الأنثراكينون. تحتاج خصائص صبغ الشعر بجذور الراوند إلى مزيد من التحقيقات لتقييم مستخلص الراوند المائي كبديل صديق للبيئة لصبغات الشعر المتاحة تجاريا.

استخدمت قطع صوف لصباغة بمستخلصات مائية من جذور الراوند تحت ظروف مختلفة. تمت دراسة المتغيرات التي قد تؤثر على عملية الصباغة ، مثل التركيز ، ودرجة الحموضة ، واستخدام مثبت اللون، وعامل التمدد، ووقت الصباغة. استخدمت الطرق الطيفية لتسجيل قياسات ثبات اللون. و أظهر مستخلص جذور الراوند خصائص صباغة جيدة. كانت الظلال التي تم الحصول عليها صفراء شاحبة وأصفر حاد و وردي. يتم تعزيز ثبات اللون وتحسينه باستخدام تركيز ٢ مجم / مل، ووقت صباغة ساعتان، فى بيئة حمضية، وإضافة مثبت اللون و معالجة الألياف بمعامل التمدد.