Determination of Para-Phenylenediamine (PPD) in Henna Samples Collected from Libyan Local Markets Using Spectrophotometric Method

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الملخص

تعتبر الحناء من أشهر مستحضرات التجميل في ليبيا. يتم استخدامه كمعجون يوضع على الجسم أو الشعر. تتم إضافة بارا فينيلينيديامين (PPD) إلى الحناء لجعل لونها أكثر قتامة. ومع ذلك، فإن استخدام PPD يمكن أن يزيد من خطر التهاب الجلد التماسي التحسسي وفقًا للجنة العلمية للمنتجات الاستهلاكية (SCCP). تم في هذا البحث تحديد تركيز PPD لثمانية أنواع من الحناء الطبيعية والتجارية. تم استخدام الطريقة الطيفية للأشعة فوق البنفسجية مع كاشف النينهيدرين في محلول ميثانول قلوي لقياس PPD للعينات التي تم تحليلها عند 430 نانومتر. وكانت نتائج العينات المختبرة خطية في نطاق التركيز 0.2–1.2 ميكروغرام/مل. وكانت معظم النتائج في حدود 2.6–4٪، وهي ضمن الحدود المسموح بها حسب SCCP، إلا أنه كان هناك استثناء لعينة الحناء (H5) (11٪ وزن/وزن) التي أظهرت أعلى نسبة PPD. ويجب الحذر من هذا النوع من الحناء لأنه قد يسبب حساسية جلدية. وتشير هذه النتائج إلى ضرورة توفر المتطلبات التنظيمية والإشرافية لإنتاج وتوزيع منتجات الحناء المغشوشة.

ABSTRACT

Henna is one of the most popular cosmetics in Libya. It is used as a paste applied to the body or the hair. Para-phenylenediamine (PPD) is added to henna to make it darker. However, PPD can increase the risk of allergic contact dermatitis according to the Scientific Committee on Consumer Products (SCCP). In this research, the concentration of PPD of eight types of natural and commercial henna was determined. The UV-vis spectrophotometric method with the ninhydrin reagent in an alkaline methanol solution was used to measure the PPD of analyzed samples at 430 nm. The results of tested samples were linear in the concentration range of 0.2–1.2 µg/ml. Most of the results were in the range of 2.6-4%, which is within the permissible limits according to the SCCP, however, there was the exception of the henna sample (H5) (11% w/w), which showed the highest percentage of PPD. Caution must be taken with this type of henna because it may cause skin allergies. These results indicate the necessity of regulatory and supervisory requirements for producing and distributing contaminated henna products.

Keywords: Henna, *p*-Phenylenediamine, Ninhydrin reagent, Spectrophotometric method.

Introduction

Currently, with increasing environmental consciousness, natural colorants have taken attention all over the world. Natural dyes have been a part of human life since time immemorial. The alchemy of colors started its use early **(Vankar, 2000).** Natural organic dyes exhibit better biodegradability and generally have a better compatibility with the environment. Moreover, they possess lower toxicity and allergic reactions than synthetic dyes **(Kumar & Bharti, 1998).** Lawsoniainermis L(henna) belongs to the Lythraceae family, generally

known as Henna. It is a tree species native to North Africa. Leaves of henna plants are entirely, opposite, sub-sessile, oval-shaped, and smooth **(Ashnagar & Shiri, 2011).** Leaves have a length of 2-3cm with a 1-2cm width **(Muhammad &Muhammad,2005)**. The henna shrub is highly branched and has grayish-brown barks grown up to 25 feet **(Rahmoun et al.,2010)**. In addition, leaves of henna are an ancient dye, evidence being the Egyptian mummies found in the tombs that had their nails dyed with henna. Currently, it is used in many countries for dyeing hair, eyebrows, and fingernails during religious festivals marriages, etc. The use of henna for dyeing the palms and fingernails is an auspicious ritual mostly in Asian countries **(Probu & Senthilkumar,1998)**. The powdered leaves of this plant (aqueous paste) are used as a cosmetic for staining hands, palms, hairs, and other body parts **(Bechtold,2009).**

Natural henna does not contain PPD. Currently, PPD is added to henna, and more than 1,000 hair dye formulations are marketed worldwide **(Stanley et al., 2005).** Epidemiological studies have shown that workers in the textile dyeing and rubber industries, hair dye users, and barbers are at high risk of developing bladder cancer, non-Hodgkin lymphoma, multiple myeloma, and carcinoma Hematopoietic cancers **(Thune et al., 2005).** Carcinogens typically cause damage to the genome to expose cells that may undergo either apoptosis or spread genome damage, potentially leading to Transformation in cancer cells **(Stiller, 1995).**

Para-phenylenediamine (PPD) is an aromatic amine compound; its molecular weight is 108.15 g/mol and its chemical formula is C6H8N2. It is a powder and has a white to light purple color, but when it is exposed to air, it oxidizes and turns red, brown, and finally black. It is also slightly soluble in water. Its main application is as a component in oxidative hair coloring solutions, where its maximum concentration is 4.0%. This concentration is 2% when the product is

applied to the hair because it has been mixed 1:1 with hydrogen peroxide before usage. PPD can also be present in textile or fur dyes and hair dyes **(SCCP, 2006).** In rubber compounds, PPD is also used as an antioxidant and a developing agent for photographs. Workers may contact PPD when manufactured or used; the exposure can happen by ingestion, skin or ocular contact, or inhalation **(HSDB, 1993). (HSDB, 1993).** Acute consequences of short-term high PPD exposure can include severe dermatitis, gastritis, asthma, renal failure, vertigo, tremors, convulsions, and coma in humans. In humans, prolonged exposure (chronic effect) can lead to eczematous contact dermatitis **(Lepoittevin & LeCoz, 2007)**. The European Standard Series for diagnostic patch testing for eczema patients includes PPD as a potent possible skin sensitizer, according to the Scientific Committee on Consumer Products **(SCCP, 2006)**. As an allergen, PPD can cause severe reactions when re-exposed, even in people who do not respond to it initially because they eventually grow "sensitized" to it **(Schnuch et al.,2008)**.

Furthermore, PPD induces cross-allergy, which results in allergic reactions to other substances containing para-substituted amino acids **(Zapolanski & Jacob, 2008)**. There is no information available regarding PPD's effects on human reproduction, development, or carcinogenesis **(EPA, 1985).** However, based on rat experimentation, SCCP reported that PPD and hydrogen peroxide may be carcinogenic **(SCCP, 2006).** Natural henna gives an orange/reddish hue; but, recently, PPD has been combined with it to provide an ebony color (black henna). In addition, adding PPD to natural henna will speed up (shorten) the tattooing process. While natural henna staining takes four to twelve hours, moreover, the addition of PPD can cut this time down to an hour or two, and the result will also persist longer. Henna painting has been identified as a new pattern of PPD

exposure that raises the possibility of experiencing negative PPD-related health outcomes **(Deleo, 2006)**.

The objectives of this study were to detect the presence and concentration of PPD in henna available in the Libyan market due to the increased risk of poisoning resulting from this ingredient. Many cases of dermatitis have been reported in the past few years in Libya, especially in Libya. City of Al-Khoms**.**

Samples collection

In this study, eight samples of natural and commercial henna were collected randomly from the markets of the city of Al-Khoms, Libya. They were given the names H1, H2, H3, H4, H5, H6, H7 and H8.

Chemicals and Reagents

P-Phenylenediamine, sodium hydroxide was purchased from (BDH Chemicals Ltd). Ninhydrin Extra pure AR was All other chemicals used were of analytical grade (AR grade).

Preparation of stock solutions.

Prepare a standard stock solution.

To obtain a practical standard concentration of approximately 1 mg/mL, 100 mg of a pure sample of PPD was accurately weighed and then dissolved in 100 mL of 0.1 N NaOH in a standard 100 mL beaker. To obtain 100 µg/mL and 10 µg/mL, successive dilutions of this solution were prepared.

Determination of the best wavelength.

The highest concentration of PPD was used, which is 7 ppm, and we measured the absorbance of PPD at wavelengths (350 to 460 nm), so the highest absorbance was at the wavelength of 340 nm as seen in Fig 1.

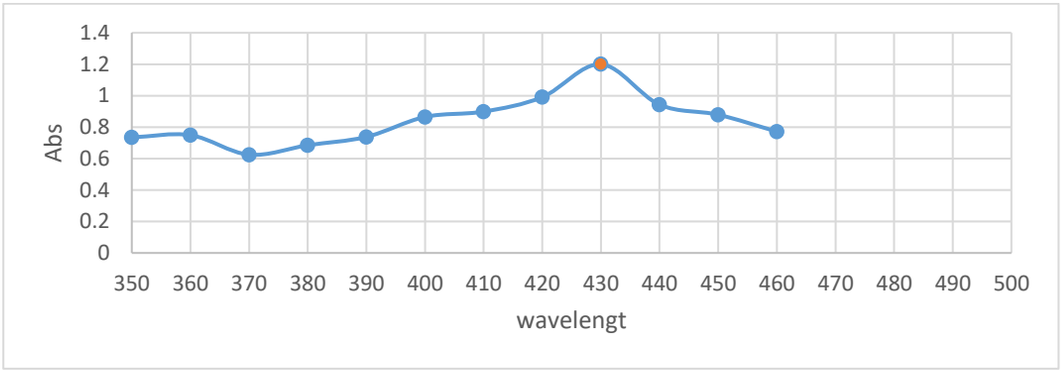

Fig.1 shows the best wavelength at 430 nm.

Calibration curves

A series of 10 ml volumetric vials were filled with standard PPD solution of the following aliquots: 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 ml of 100 µg/ml. Next, 1.0 mL of anhydride reagent and 1 mL of sodium hydroxide solution were added. The absorbance of each solution was measured at 430 nm against the reagent blank as shown in Fig 2. , which was made in the same way but without the analytic.

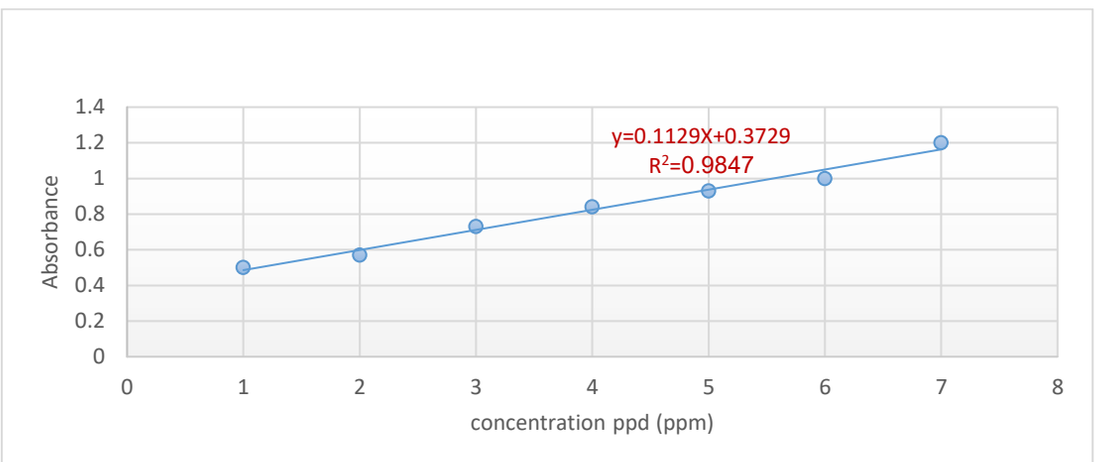

Fig.2. Calibration curve for standard solutions of PPD at 430 nm with ninhydrin reagent.

Sample preparation (PPD extraction procedure).

The extraction procedures were carried out with some modification and development,

to obtain a concentration of approximately 1 mg/ml, 0.833 g of natural and commercial henna formulation was carefully weighed and dissolved in 10 ml of a 0.1 N NaOH solution. Centrifugation was used and, the solution was sediment, 8 ml of it was taken, and 1 ml of ninhydride was added and measured. The absorbance of henna samples was measured at different dilution factors. It differed from sample to sample, at a wavelength of 430 nm, as seen in Table 1 and Fig 3.

Table. 1 shows that the absorbance of the samples is 430 nm at different dilutions.

The absorbance of the samples is 340nm	
Samples	Absorbance
H1	0.963
H2	0.968
H3	0.770
H4	0.852
H5	0.870
H6	0.955
H7	0.985
H8	ND

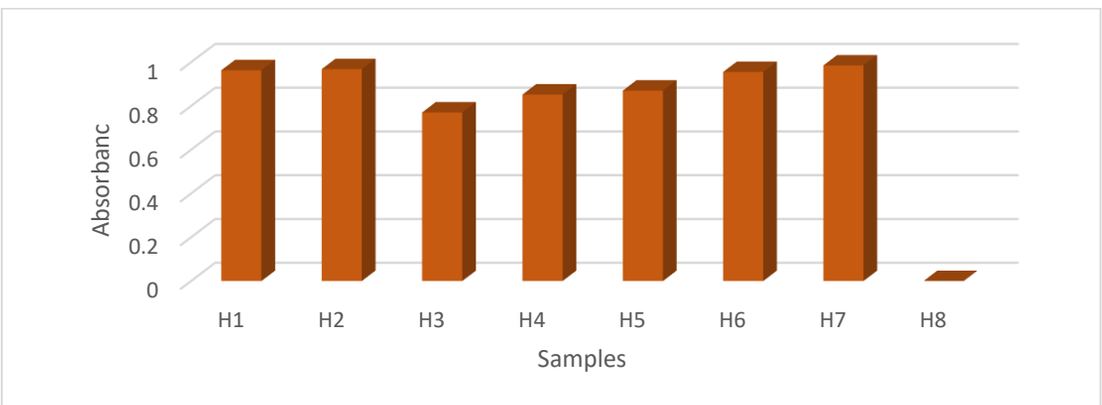

Fig shows the absorbance of the samples is 430 nm at different dilutions.

Results and Discussion

In this work, we have used a spectroscopic approach that is a clear, direct, sensitive, fast, reproducible, accurate, and specific method as previously reported by Gurupadayya et al (**Gurupadayya et al., 2014**). PPD analysis with ninhydrin reagent was utilized. This reagent is widely used in laboratories and is particularly cost-effective compared to other reagents. Ninhydrin reagent, one of the important reagents for the detection of amino acids, has been traditionally used to detect their microgram amounts. Amino acids that have free alpha-amino groups react with an excess amount of ninhydrin and yield a purple-colored product as seen in Fig 4

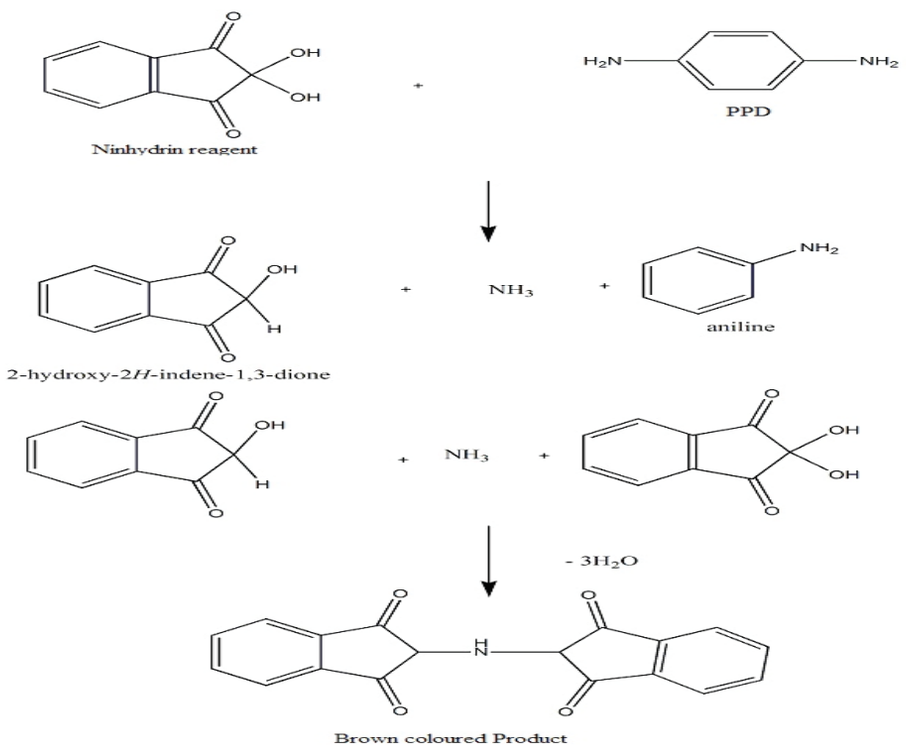

Fig. 4 shows the chemical reaction of PPD and the ninhydrin reagent

Through suitable situations, the intensity of the color generated is proportional to the concentration of the amino acid. Ninhydrin reagents have been widely used in the identification of pharmaceutical compounds and kinetic studies **(Nafisur et al., 2001; Nafisur & Mohammad,2003)**. The modified extraction method used in this study offers several advantages, including inexpensiveness and simplicity. The results of this study indicated that PPD levels in the henna samples were in the range of 2.6% w/w to 11% w/w. These results are shown in Table 2 and Fig 5.

Table 2. Shows the concentration (w/w %) of PPD in different samples.

Percentage % of PPD in henna samples	
Samples	PPD%
H1	3.8
H2	3.9
H3	2.6
H4	3.1
H5	11
H6	3.8
H7	4
H8	ND

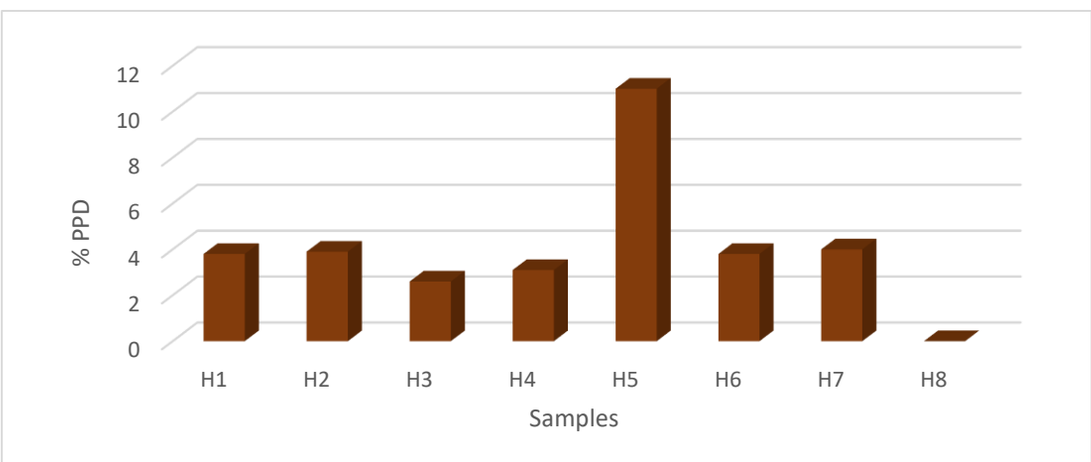

Fig 5. Analysis of the concentration (w/w %) of PPD in different samples**.**

The minimum PPD level was observed in the sample Henna (H3) (2.6% w/w), while the highest level of PPD was observed in the Henna (H5) sample (11% w/w), as shown in Table 2 and Fig 4. While one of these samples was free of PPD (not detected), this result was for natural henna. In addition, the PPD concentrations were comparable in the remaining samples. The PPD content in the henna sample (H5) was higher than the recommended concentration of 4–6% **(Al-Suwaidi and Ahmed, 2010; SCCP, 2006).** The use of henna, which contains a high percentage of more than 4% of PPD, causes harmful health effects, including acute allergic contact dermatitis, eczema, chemical burns, acute renal failure, acute and severe angioneurotic edema, abdominal pain, and vomiting **(Jovanovic& Slavkovic, 2009).**

Conclusion

The study analyzed the PPD content in commercial henna samples using visible spectral differences. Most samples were below the FDA and Scientific Committee limits, while the H5 samples had higher levels. Extra caution is advised when using black dyes of unknown origin. Direct contact of PPD on the skin, eyelashes, or eyebrows is strictly prohibited in the European Union and the Food and Drug Administration (FDA) has not permitted the use of PPD directly on the skin. Nowadays, raising public awareness about the side effects of PPD has become essential. Therefore, based on the results it can be concluded that adulteration of natural henna by adding PPD should be closely monitored in Libya because PPD poses a toxic health risk. However, more research work and public awareness are needed to prevent adulteration of henna with PPD in Libya.

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