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ORIGINAL ARTICLE

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Detection of Paraphenylenediamine (PPD) in Henna Commercialized in the Constantine and Setif Markets, Algeria

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ABSTRACT

Introduction. Henna (Lawsonia inermis) is commonly used in Arab countries and India for skin embellishment and hair dyeing in the context of traditional social practices. Temporary henna tattoos, often supplemented with various additives, provide a practical alternative to permanent tattoos. However, the addition of paraphenylenediamine (PPD), a well-known allergen, increases the risk of allergic contact dermatitis among individuals using these henna mixtures. The objective of this study was to detect the presence of PPD in henna. Forty henna samples sold in the markets of Constantine and Setif in Algeria were analyzed. **Materials and Methods**. The experimental analysis employed a qualitative and semi-quantitative method, combining thin-layer chromatography (TLC) analysis with color reactions using vanillin and ferric chloride, as well as ultraviolet-visible and infrared analysis. **Results**. The results revealed that among the 40 products analyzed, 35% of them contained PPD, with 6 products not indicating its presence on the packaging. Conclusion. This lack of disclosure exposes users to the risk of sensitization, believing they are using natural henna.

ARTICLE HISTORY

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1. INTRODUCTION

Henna, an ancient tradition utilized for its natural coloring properties in Asia, the Middle East, and the broader Maghreb region, has become a popular beauty product in the Western world as a temporary tattoo, offering a practical alternative to permanent tattoos [1-4]. However, our study aims to reveal a concerning aspect of this practice : the presence of paraphenylenediamine (PPD), an aromatic amine used by women for cosmetic purposes, including black hair dye and as an additive for henna [1-6]. This substance, known as "TAKAOUT ROMIA" in Morocco and "ELHADJRA SOUDA" or "MERDOUMA" in Algeria [3,7], can cause allergic reactions such as contact eczema or asthmatic manifestations, especially in professional settings. In case of ingestion, it poses significant systemic toxicity, initially presenting with cervicofacial edema and macroglossia

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leading to rapidly fatal asphyxiation in the absence of medical intervention [1-6]. Its muscular toxicity results in massive myoglobin release, leading to renal impairment characterized by acute renal failure with necrosis and tubular obstruction [8-14].

The SCCNFP (Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers) issued very strict recommendations in 2001 regarding the concentration of PPD and similar substances used as ingredients in temporary tattoos and hair dyes (PPD <4%; combined with H2O2, PPD <2%). Health Canada requires concentrations <6% and completely prohibits the use of PPD in temporary tattoos [1, 3, 14]. Unfortunately, in Algeria, PPD continues to be freely sold by herbalists and in its pure form, exposing consumers or professionals to the sometimes-concealed risk of this molecule. In this context, our study aims to assess the presence of PPD in henna products marketed in the Algerian market.

2. MATERIELS ET MÉTHODES

This study employed a descriptive analytical bi-centric approach. For this study, 40 henna samples were randomly collected from the markets of Constantine and Setif.

Qualitative analysis was conducted following liquid-liquid extraction of PPD from 0.3 g of henna powder, to which 0.03 g of ascorbic acid and a few drops of 25% ammonia were added. The extraction was performed using 15 ml of ethanol. The extracts were then filtered, fractionated, and subjected to thin-layer chromatography (TLC) separation using a T1 migration system (Methanol: Ammonia (98.5:1.5 ml), with Bouchardat as the developer. The second fraction of the extract was used for colorimetric chemical reactions on strips, employing vanillin (a solution of 1 g vanillin in 15 ml isopropanol ; a positive test results in an orange color) and ferric chloride (5% aqueous solution of FeCl3 ; a positive test results in a green color). Simultaneously, in other extracts, UV-visible spectral analysis was performed using a PG Instrument Ltd T90+ UV/VIS spectrometer, and infrared analysis was carried out using a Shimadzu FTIR-8500S Fourier Transform spectrometer. For the infrared study, a homogeneous mixture of 3% of extractum dry residue and KBr powder was prepared. This mixture was finely ground and subjected to infrared analysis. Semi-quantitative analysis was conducted using UV-visible analysis to determine the absorbance of the ethanolic extracts at 240 nm, extrapolating from a pre-established calibration curve derived from a 3% standard solution of PPD.

3. RESULTATS

Qualitative Results

For the qualitative analysis on TLC plates (Figure 1) and colorimetric tests (Figure 2), PPD was identified in 14 samples, accounting for 35% of all analyzed brands. Additionally, UV-visible spectral analysis confirmed these results. However, the infrared study was inconclusive (Table 1). Among these 14 products, 6 samples did not initially indicate the presence of PPD as a component of henna and were sold as natural henna products.

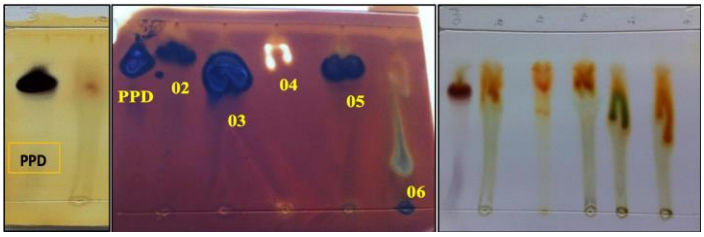

Figure 1. Identification of PPD on TLC. From left to right, black spot of PPD, spots of samples E2, E3, E4, E5, and E6 after revelation with BOUCHARDAT, and finally a TLC plate without the addition of BOUCHARDAT.

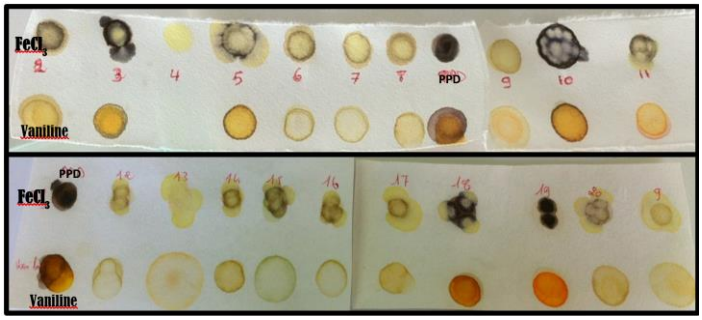

Figure 2. Identification of PPD through Colorimetric Tests (Example of results for 20 henna products. At the top, in the first row, the reaction developed with FeCl3 is shown, and in the second row, at the bottom, the reaction obtained with vanillin is displayed).

For all the obtained results, the UV-visible spectra of the positive samples exhibit a spectral signature similar to that of PPD, characterized by the presence of a distinct band at a maximum wavelength (λmax) of 240 nm (Figure 3).

In the infrared spectrum of PPD, two absorption bands are observed at approximately 3300.31-3375.54 cm-1, which are characteristic of the stretching vibration of the primary amine. The band at 1627.97 cm-1 corresponds to a deformation vibration of the N-H bond of the amine. Another band corresponding to the stretching vibration of the aromatic C-N bond is detected at 1257.63 cm-1. The characteristic absorption due to the stretching vibration of the C=C bond in the aromatic ring is observed at 1514.17 cm-1 in the infrared spectrum. Furthermore, the band at 831.35 cm-1 is associated with the deformation vibration of the Csp2-H bond in a para-disubstituted aromatic nucleus (Figure 4).

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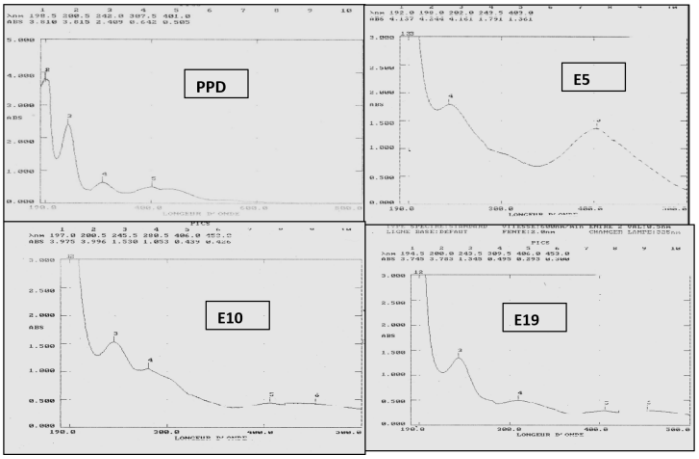

Figure 3. Identification of PPD by UV-VISIBLE Spectroscopy. From left to right, the UV-visible spectra of PPD in ethanol, samples E5, E10, and E19.

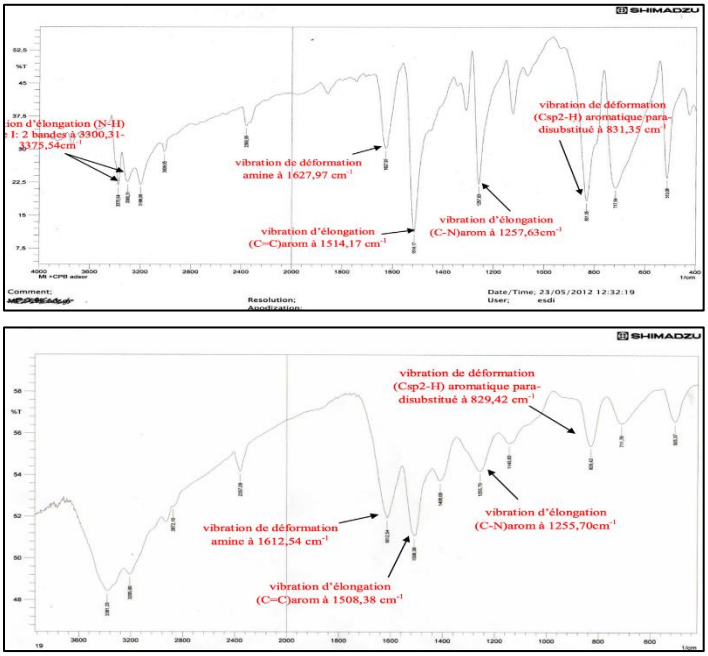

Figure 4. Identification of PPD through Infrared Analysis. From top to bottom, the IR spectrum of PPD powder, and the IR spectrum of sample E19.

Semi-Quantitative Analysis

Regarding the semi-quantitative analysis, the concentration of PPD is significantly higher than what is indicated in the composition of the positive samples. What is concerning in our results is that some samples, for which the presence of PPD is not even mentioned on the packaging, show very high levels, exceeding 6% for sample E18 and 45% for sample E19 (table 2).

Table 1. Qualitative Analysis Results of Henna Products

Sample number	PPD MENTIONED	RETENTION FACTOR (R_f)	Bouchardat test	Test de $FeCl_3$	Vanillin Test	Uv-visible	IR
PPD	/	0,64	(+)	(+)	(+)	(+)	(+)
E1	Témoin (-)	/	(-)	(-)	(-)	(-)	(-)
E2	NM*	0,70	(+)	(+)	(+)	(+)	(+)
E3	M**	0,64	(+)	(+)	(+)	(+)	(+)
E4	NM*	/	(-)	(-)	(-)	(-)	(-)
E5	NM*	0,67	(+)	(+)	(+)	(+)	(+)
E6	NM*	/	(-)	(-)	(-)	(-)	(-)
E7	NM*	/	(-)	(-)	(-)	(-)	(-)
E8	NM*	/	(-)	(-)	(-)	(-)	(-)
E9	NM*	/	(-)	(-)	(-)	(-)	(-)
E10	M**	0,62	(+)	(+)	(+)	(+)	(-)
E11	M**	0,65	(+)	(+)	(+)	(+)	(+)
E12	NM*	/	(-)	(-)	(-)	(-)	(-)
E13	NM*	/	(-)	(-)	(-)	(-)	(-)
E14	NM*	/	(-)	(-)	(-)	(-)	(-)
E15	NM*	/	(-)	(-)	(-)	(-)	(-)
E16	NM*	/	(-)	(-)	(-)	(-)	(-)
E17	NM*	/	(-)	(-)	(-)	(-)	(-)
E18	M*	0,65	(+)	(+)	(+)	(+)	(-)
E19	NM*	0,64	(+)	(+)	(+)	(+)	(+)
E20	NM*	/	(-)	(-)	(-)	(-)	(-)
E21	NM*	/	(-)	(-)	(-)	(-)	(-)
E22	NM*	/	(-)	(-)	(-)	(-)	(-)
E23	NM*	/	(-)	(-)	(-)	(-)	(-)
E24	NM*	0,67	(+)	(+)	(+)	(+)	(+)
E25	NM*	/	(-)	(-)	(-)	(-)	(-)
E26	NM*	/	(-)	(-)	(-)	(-)	(-)
E27	M*	0,68	(+)	(+)	(+)	(+)	(+)
E28	NM*	/	(-)	(-)	(-)	(-)	(-)
E29	NM*	/	(-)	(-)	(-)	(-)	(-)
E30	NM*	/	(-)	(-)	(-)	(-)	(-)
E31	M**	0,70	(+)	(+)	(+)	(+)	(+)
E32	NM*	/	(-)	(-)	(-)	(-)	(-)
E33	NM*	/	(-)	(-)	(-)	(-)	(-)
E34	NM*	0,68	(+)	(+)	(+)	(+)	(+)

NM*: Not mentioned on the packaging . M*: Mentioned on the packaging

4. DISCUSSION

PPD poisoning has become a serious public health issue in Algeria, with 42 registered cases from 1993 to 2005 at the Algerian Poison Control Center. This increasing trend is alarming, especially among young adults aged 21 to 40 years [3]. Similarly, Morocco has also experienced a high prevalence of PPD poisoning, mainly among women aged 15 to 25 years, with 289 registered cases between 2000 and 2005 at the Marrakech Poison Control Center. This situation highlights the urgent need for intervention to prevent future cases of poisoning [15].

Table 2. Results of Quantitative Analysis of Henna Products.

N°	E2	E10	E18	E19	E4	E7	E11
Found Content %	2,30 %	8 %	6,6 %	45,5 %	2.4 %	8.3 %	6.77 %
Declared Content on the Packaging %	NM*	<2%	NM*	NM*	NM*	NM*	2%
Origin	**Pakistan**	**Inde**	**Inde**	**UAE**	**Pakistan**	**Inde**	**Inde**

This study underscores the necessity of rigorous monitoring of the henna industry. The concealment of paraphenylenediamine (PPD) in henna products can trigger severe allergic reactions, endangering the lives of susceptible individuals. This risk is particularly high for people with glucose-6-phosphate dehydrogenase (G6PD) deficiency or those who have previously shown sensitivity to paraphenylenediamine (PPD) [4]. Allergic

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reactions due to PPD can lead to polysensitization (rubber, clothing dyes), thus limiting career choices, especially in the hairstyling profession. Once sensitized to PPD, individuals become allergic to most hair dyes and other similar chemicals containing an amine group (azo dyes, aminophenol, aminobenzene...), chemicals used in photography or photocopying, sunscreens, medications such as sulfamides, dapsone, benzocaine, local anesthetics, antiseptics, etc. This can have a significant impact on their professional and daily lives [5, 2, 16].

5. CONCLUSION

In conclusion, this study reveals the presence of PPD in henna samples at high concentrations; in some samples, the presence of PPD is not even mentioned on the packaging, thereby increasing the risk of allergic reactions. The availability of pure PPD is concerning, emphasizing the need to educate the public, professionals, and authorities about the dangers of henna products containing this substance. It is crucial to inquire about the practitioner's application time for the product because natural henna tattoos take longer to develop compared to preparations containing PPD, which can be ready in as little as 30 minutes. It is also recommended to avoid temporary tattoos that last more than one or two weeks to reduce the risks associated with prolonged exposure to potentially harmful substances. It is imperative to avoid any contact with suspected henna products. Before applying any dye, it is strongly advised to check the product's composition to ensure it does not contain PPD or other non-natural substances.

6. DECLARATION D'INTERETS

Les auteurs ne déclarent pas de conflits d'intérêt en rapport avec cet article.

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