Para-Phenylenediamine (PPD) in commercially available Henna preparations in Bangladesh

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ABSTRACT: In Bangladesh Henna is one of the most popular cosmetic products. The leaves of Henna are dried to make powder on which oil or water is mixed to get a paste to stain the body or hair. Although the occurance of contact allergic dermatitis in natural Henna is not so common, but this risk is increased by adding *para*-Phenylenediamine (PPD), which is used to make the Henna color dark to deep black. According to Scientific Committee on Consumer Products (SCCP), *para*-Phenylenediamine (PPD) is treated as an allergen and is considered as a very strong potential skin sensitizer. The presence of PPD in the commercially used tube Henna increases the risk of allergic contact dermatitis and several cases have already been reported worldwide. For this study, about 10 Henna samples were randomly collected from 10 selected areas of Dhaka city. The presence of PPD in Henna samples was determined by using High Performance Liquid Chromatography (HPLC). The result showed the presence of PPD in all of the Henna samples at substantial concentrations, ranging between 79.12-204.77 mg/kg where the average range is 142.36 mg/kg, which is much higher than the permissible levels (<2% or 0.1 mg/m^3). The finding suggests that there should be a regulation and monitoring condition for the production and distribution of these adulterated Henna products in Bangladesh.

KEYWORDS: Henna, *para*-Phenylenediamine, contact allergic dermatitis, G6PD.

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Introduction

"Henna" (English) origin from "Hinna" (Arabic) is a flowering plant whose scientific name is *Lawsonia inermis*. This plant is found in tropical and subtropical region like Australia, Africa, Southern Asia (including India, Bangladesh) eastern Europe and also in middle east countries. Before 2100 BC the archaeological evidence of Henna was shown by Egyptian people. In Africa, India, Pakistan, Bangladesh and in many middle eastern countries Henna is used during wedding ceremony, birthday party, holiday and different types of occasion like Eid-ul-Fitar, Eid-ul-Adha, Sob-e-barat, Pohela Baishakh, thirty first night celebration, different types of Pooja, etc . Natural Henna contains a wine-red dye molecule 2-hydroxy-1,4naphthoquinone (Ayesha and Hafiz 2010). This molecule is able to bind with proteins and therefore is useful for coloring of hair, along with palms, fingers, and soles. (Cartwright, 2006; Hanna R et al. 1998). At 35 to 45°C Henna leaf is dried and grind to produce Henna powder. Henna paste is produced by adding oil or water to this powder (Sahar et al. 2016). When this Henna paste is applied to the outermost layer of the skin, it creates color; the longer the exposure, the more intense is the color.

It is evident that by applying natural Henna onto the skin, red color is formed within 4 to 12 hours, which has no or may be mild rate of allergic reaction. It is rare to find study for allergic contact dermatitis to natural Henna according to the literature in the countries where traditionally Henna is used as a popular cosmetic product. Therefore, it can be specified that, natural Henna can be considered as a weak allergen (Ayesha and Hafiz, 2010)

In the modern age, different business industry in different countries made packaged tube Henna which are popular cosmetic products and used as hair and body dye. To obtain a quick and darker color and to get more longevity of the color, *para*-Phenylenediamine (PPD) is commercially added with it. It is used to increase the intensity of the color in a short time. The other reason for adding PPD is to get an ebony color (black *para*-Phenylenediamine Henna) instead of the reddish color produced by natural Henna leaves (Ayesha and Hafiz, 2010).

para-Phenylenediamine (PPD) (molecular weight 108.15 g/mol) is a hydrocarbon which is aromatic amine in nature and its chemical formula is $C_6H_8N_2$. It appears as white to light purple powder which darkens upon air exposure (DHHS, 1993; OHEA, 1985; SCCP, 2006).

Because of its molecular nature, PPD can cause skin sensitization that may induce various clinically adverse

effects. By repeated exposures to PPD, allergic contact dermatitis (ACD), is one of the most common manifestations that can occur (Elisa et al. 2017).

PPD is primarily used as an ingredient of oxidative coloring products at a maximal concentration of 4.0%, If it is mixed with hydrogen peroxide in the ratio of 1:1, 2 % PPD will be applied to hair. Not only in hair dyes, PPD is also added in fur and textile dyes (SCCP, 2006). Occupationally individuals may be exposed to PPD during its exposure through inhalation, skin and/or eye contact, and ingestion (EPA, 1985; HSDB, 1993; SCCP. 2006). Acute effects like dermatitis, eye irritation, tearing, asthma, gastritis, renal failure, vertigo, tremors, convulsions, and even coma can cause by short-term exposure to high levels of PPD (>3%) and long-term use may result severe (chronic effect) eczematous contact dermatitis (Delco, 2006; EPA, 1985; HSDB, 1993; Lepoittevin, 2007; Zapolanski and Jacob, 2006;). Some reports say that the allergic reactions of PPD may not be happened for the first-time exposure, however, they can become "sensitized" and can have adverse reaction upon second time exposure (Schnuch et al. 2008).

Henna is being used not only in Bangladesh, but also in Indian sub-continent and Arabian world as well as a part of the traditional and social culture. Therefore, skin decoration by Henna is very popular among children, adolescents, young adults and even older aged women and it is considered to be an imperative part of the wedding programs as well as other social celebrations. Hence, for the first time, an attempt has been taken in this study to determine the presence PPD and also to quantify the amount of this allergen in Henna in most popular brands available in Bangladesh.

Materials and Methods

Samples

A total number of 10 commercially available Henna samples were randomly collected from 10 areas of the Dhaka city, Bangladesh for this study. Due to conflict of interest, we didn't mention the name of 10 Henna samples. Instead, we named them as S1, S2, S3,S4,S5,S6,S7, S8,S9, S10.

Standard Preparation

PPD standard (0.17mg per mL) was prepared by weighing PPD substances (LOVA Chemicals, India) and dissolving it in 50%. HPLC grade aqueous methanol solution. PPD standard was prepared into four levels of PPD concentrations, such as, 332ppm, 1250ppm, 2500ppm and 5000ppm.

Sample Preparation

One gram of each of the collected samples were weighed into 50mL volumetric flask and diluted with 50% aqueous methanol solution (50mL). After 15min this solution was filtered with 0.45 µm minimart filters (Germany). Finally, one mL of this solution was diluted to 5mL with 50% aqueous methanol solution and filtered with 0.45 µm minimart filters (Germany) again before putting into auto sampler tube and analyzed for PPD.

Standard Run Conditions

High Performance Liquid Chromatography (HPLC) was performed using as SHIMADZU HPLC equipped with Refractive Index Detector (RID) with wavelength 290mm and a spectrum range of 190 to 400mm. The HPLC conditions were mobile phase, 0.05M acetic acid-methanol (95/5) and adjusted to pH of 5.9 with ammonia; the temperature was 30°C; flow rate was 1.5mL/min; wavelength 290mm; pressure 174 bar; column: Altech Prevail C18 5µm, 250×4.6 mm reverse phase column as stationary phase.

Standard & Sample Data Collection

In order to ensure the identity of PPD, one mL of the standard was diluted to 5mL with 50% aqueous solution and analyzed before analyzing any samples to determine it's spectrum and it's retention time. The retention time calculated 4.6 min later.

Data Analysis

All data were analyzed using standard methods using Microsoft excel.

Result and Discussion

Determination of PPD in sample

A linear calibration curve of PPD standard was obtained to find the concentration of PPD in the collected samples. Figures (1,2,3,4,5,6,7,8,9,10) show the chromatograms of the PPD in Henna sample 1 to sample 10. The retention time for the PPD standard and Samples were found to be 4.64 minutes and 4.58 to 5.3 minutes respectively under the same analytical condition. Moreover, there was a good match of the spectrum of each of our samples with the standard, which indicated that the peak and the retention time of the PPD in samples was the same as that of the PPD in standard. PPD concentrations were monitored from HPLC readings.

As shown in Table1, the PPD was found in all the Henna samples (10 samples) with concentration ranging between 79.95 mg/kg to 204.77 mg/kg and average concentration was 142.36 gm/kg as shown in Table 1 and Figures (1,2,3,4,5,6,7,8,9,10). The PPD was found in all the Henna samples (10 samples) with concentration (percentage) ranging between 7.91% and 20.48% and average concentration in percentage is 14.195% (Table 2). The concentration of PPD was more than 10% in six Henna samples, more than 8% in eight samples, more than 2% in ten Henna samples.

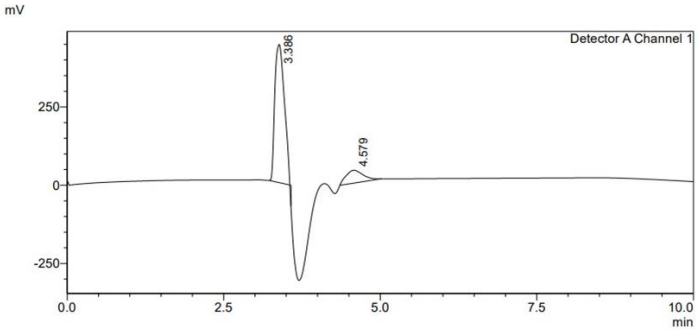
Table 1. The Concentration of para-Phenylenediamine in the ten Henna Samples.

Sample	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Conc (mg/kg)	204.77	79.12	85.35	117.18	79.95	82.05	147.03	119.83	112.93	116.61
%PPD	20.48	7.91	8.53	11.72	8.00	8.20	14.70	11.98	11.29	11.66

Table 2. Average range in concentration and in percentage of PPD present in 10 Henna samples

Range of Conc.	Avg Conc.(mg/kg)	Range of PPD (%)	Avg PPD (%)	PPD Conc		
				>10%	>8%	>2%
79.12-204.77	142.36	7.91-20.48	14.195	60%	80%	100%

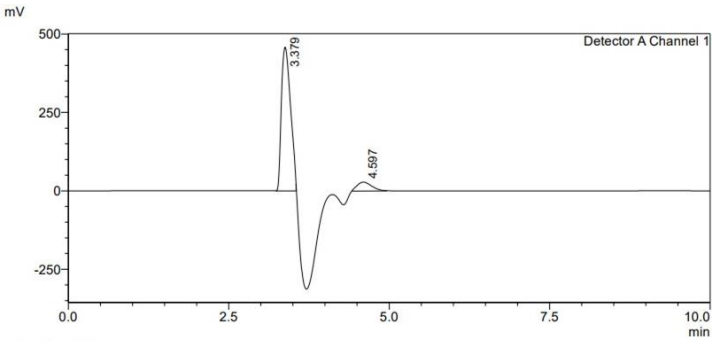
By the chromatographic spectrum and retention time, HPLC determines the concentration of PPD. For this, it is free from any risk of false identification. Thus, HPLC accurately quantify the PPD concentration.



<Peak Table>
Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	3.386	4653242	440990	85.425		M	
2	4.579	793930	41212	14.575		M	
Total		5447172	482202				

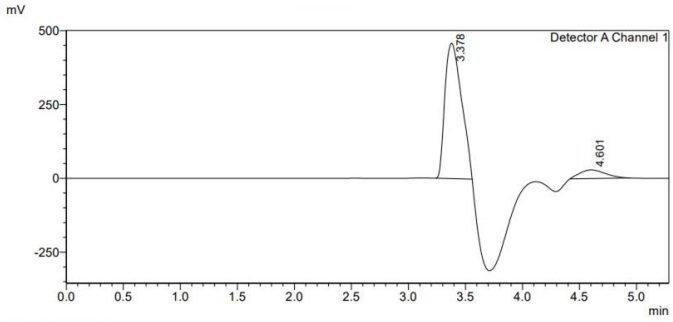
Figure 1. Chromatogram of PPD in Henna sample 1.



<Peak Table>
Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	3.379	4578196	457901	90.962		M	
2	4.597	454864	28676	9.038		M	
Total		5033060	486576				

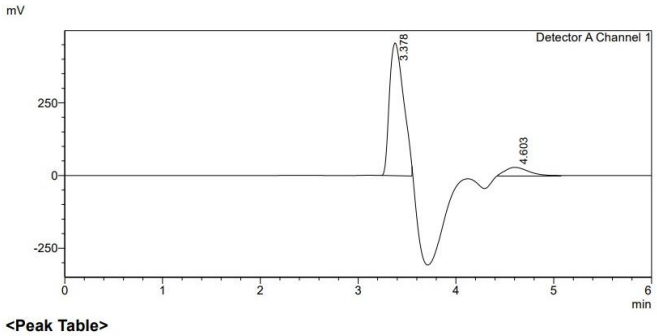
Figure 2. Chromatogram of PPD in Henna sample 2.



<Peak Table>
Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	3.378	4626060	458295	90.995		M	
2	4.601	457791	29131	9.005		M	
Total		5083851	487426				

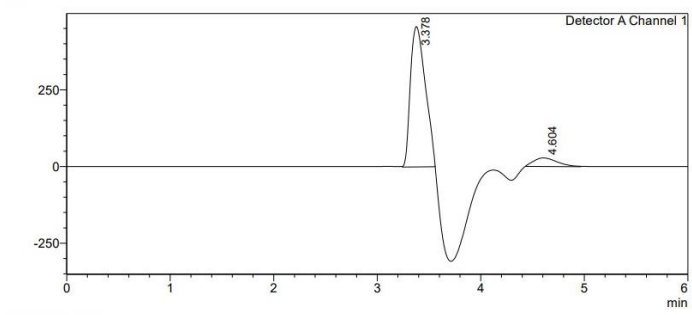
Figure 3. Chromatogram of PPD in Henna sample 3.



<Peak Table>
Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	3.378	4572099	456093	90.046		M	
2	4.603	505422	29655	9.954		M	
Total		5077521	485748				

Figure 4. Chromatogram of PPD in Henna sample 4.

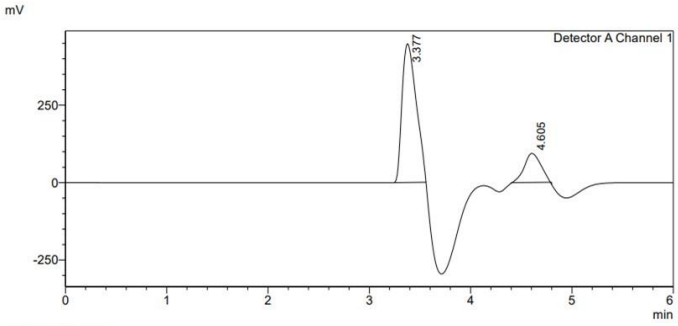


<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	3.378	4596054	457267	91.198		M	
2	4.604	443607	27920	8.802		M	
Total		5039661	485187				

Figure 5. Chromatogram of PPD in Henna sample 5.



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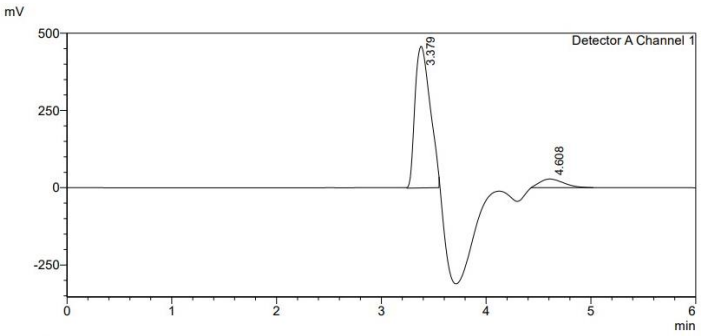
Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	3.377	4380605	447855	80.501		M	
2	4.605	1061062	93843	19.499		M	
Total		5441667	541698				

Figure 6. Chromatogram of PPD in Henna sample 6.

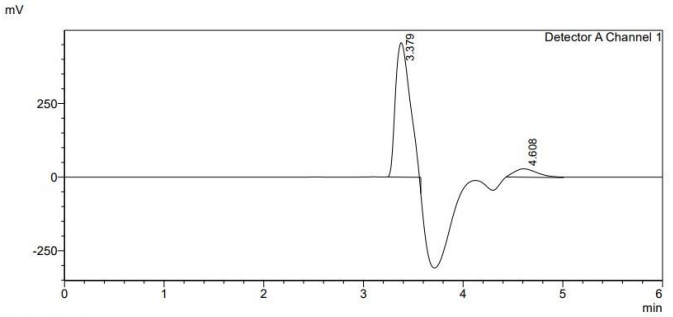


<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	3.379	4600634	457673	91.395		M	
2	4.608	433148	27760	8.605		M	
Total		5033782	485433				

Figure 7. Chromatogram of PPD in Henna sample 7.



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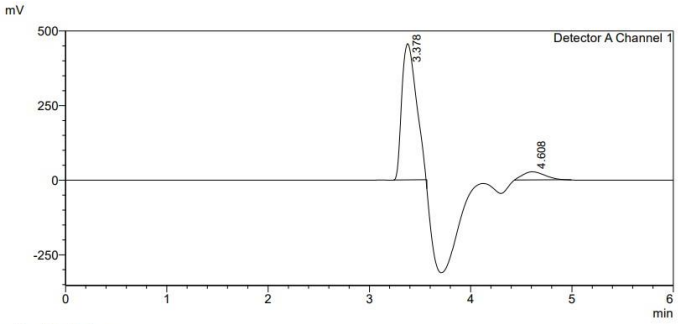
Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	3.379	4544838	455887	90.752		M	
2	4.608	463142	28226	9.248		M	
Total		5007980	484113				

Figure 8. Chromatogram of PPD in Henna sample 8.

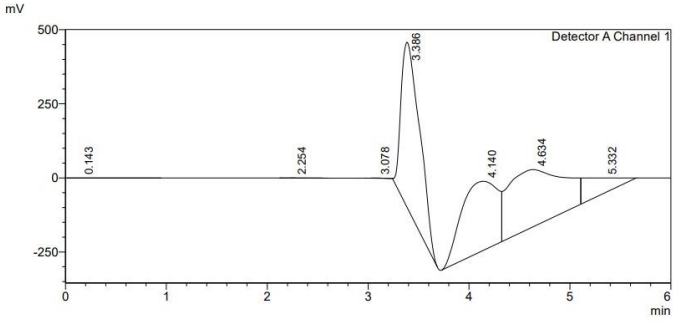


<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	3.378	4562107	456317	91.700		M	
2	4.608	412942	27369	8.300		M	
Total		4975050	483686				

Figure 9. Chromatogram of PPD in Henna sample 9.



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.143	4128	159	0.000			
2	2.254	3953	398	0.000			
3	3.078	8826	1183	0.000			
4	3.386	7229270	556772	0.000			
5	4.140	5963781	233425	0.000			
6	4.634	7386859	193273	0.000		V	
7	5.332	1463830	52636	0.000		V	
Total		22060647	1037847				

Figure 10. Chromatogram of PPD in Henna sample 10.

The concentrations of PPD in ten Henna samples reported in this study were much higher than the permitted concentration of PPD in hair dye products established by the European Union and specified by SCCP (Scientific Committee on Consumer Products).

It is reported that, if patch test done on the patient with a PPD concentration more than the recommended range, the patient may suffer from vomiting and abdominal pain which results from poisoning due to Henna (Suwaidi. 2009). If the patient also has glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency, which is a genetic disease, the condition may turn even worse (Zapolanski and Jacob, 2008). There is evidence that many PPD arises cross-allergy, causing people hyper sensitized to other substances which contain para-substituted amino compounds. There are number of reports which indicates that PPD is one of the potentially harmful substances to people with G6PD deficiency and this can cause acute hemolysis (Zapolanski and Jacob, 2008). Though no data of adverse effect is available for human subject of PPD on reproductive, developmental, or carcinogenic effects (EPA, 1985), according to experimental studies with rats, SCCP reported that PPD may have carcinogenic effect when applied together with hydrogen peroxide (SCCP, 2006).

Conclusions

The study ends up with a result of high concentration of PPD present in almost all of the Henna samples brought from different stores of Bangladesh, which indicates an alarming situation of high risk among the users. An Australian study found that, 44 patients experienced a significant life style hazards who were diagnosed with PPD allergy (Krishna et al. 2017). However, direct contact of PPD on the skin, eyelashes or eyebrows, is strictly prohibited in European Union and Food and Drug Administration (FDA) has not permitted to use PPD directly on the skin. Moreover, PPD sensitized people are vulnerable to have cross contamination with oxidative hair dye and textile dye. Nowadays it becomes a time's demand to raise public awareness about the side effects of PPD. Therefore, based on the findings it can be concluded that natural Henna adulteration by adding PPD should be closely monitored in Bangladesh since PPD poses toxicological health hazard. However, more research work and public awareness is needed to prevent Henna adulteration using PPD in Bangladesh.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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