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A Non-Destructive Technique for the Determination of Paraphenylenediamine (PPD) in Commercial Henna Samples

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Abstract

Henna is very popular in India as it is a part of various cultures and traditions. In India, henna is mainly used on skin and hair for special occasions. Allergy to natural henna is quite rare but adding para-phenylenediamine (PPD) to natural henna increases the risk of allergies. Recently PPD is being mixed with natural henna to give an ebony color (black henna) instead of the usual orange/reddish color given by natural henna. One more reason for adding PPD to the natural henna is to speed up (shorten the time) the tattooing process. Thus, a new pattern of exposure to PPD has been recognized through henna art which increases the risk of developing adverse health effects related to PPD.

The main objective of the study was to determine the presence of PPD in dyes and henna samples available in Bangalore, India. This study also aimed to give a comparative analysis of various henna samples using Thin Layer Chromatography followed by a UV-Visible Spectrophotometer. Out of the 10 samples that were collected, 7 were extracted successfully and further analysed by Thin Layer Chromatography and UV-Visible Spectrophotometry. PPD was found in all the samples. The presence of PPD in henna and dyes increases the risk of allergic contact dermatitis among frequent users. Additionally, it is also being used for committing suicides and is an emerging household poison because of its easy availability in the market. Since PPD was found in all the extracted samples collected from different parts of Bengaluru city, it is evident that its presence is not being monitored.

Keywords: Henna; Para-Phenylenediamine; UV-Vis Spectrophotometer; Allergic Contact Dermatitis; Household Poison; Hair Dye Poisoning

Abbreviations: PPD: Para-Phenylene Diamine; TLC: Thin Layer Chromatography; DCM: Dichloromethane

Introduction

In Hindu and Islamic cultures Henna which is a dried and powdered leaf extract of Lawsonia inermis of the family Lynthraceae is mostly used as a dye for hairs, tattoos on skin, and as an expression of body art [1]. This plant grows in an acrid climate, which includes countries like North Africa, South Asia, Saudi Arabia, Iran, Sri Lanka, India, Egypt, and Sudan [1]. Henna also contains a burgundy dye molecule, lawsone (2-hydroxy-1,4-naphthoquinone) [2], which is an active drying agent also known as naphthoquinone [3]. Red-orange color is produced by this dye molecule when it interacts with keratin in the skin, nails, and hair [4]. The color of this stain may vary from pale orange to dark brown/black depending upon the ingredients of henna and how well one's skin takes it.

Henna paste is made by mixing henna powder with oil or water and this paste is used for body art [2]. This paste then stains the topmost layer of the skin, the Epidermis. The greenish paste after application slowly dries and flakes off revealing an orange or brown colour within 1-3 days. Allergy to natural henna is quite rare, but the addition of a compound called Paraphenylenediamine (PPD) to henna mixtures increases the risk of allergies on human skin [2]. It is primarily used as an ingredient in oxidative hair collaring products [2]. The lethal dose of PPD is not known; estimates vary from 7-10 grams [5]. The systemic toxicity of PPD has serious consequences which may eventually lead to death [6]. Nowadays, PPD is being used in henna mixtures to give an ebony black color instead of the usual orange/reddish color given

by natural henna. It's also being added to accelerate the staining process.

Natural henna staining takes around 4-12 hours; with PPD this time is reduced to an hour or two [2]. Acute exposure to PPD may cause allergic contact dermatitis [7], eye irritation, and tearing, asthma, gastritis, renal failure, vertigo, tremors, convulsions, and coma in humans [2]. Ingestion of PPD produces rapid developments of edema of face, neck, pharynx, tongue, and larynx with respiratory distress which often needs tracheostomy [5]. PPD may get oxidized on the mucosal surface and may transform into quinondiamine which causes intense local irritation and induces severe edema of face and neck structures [8]. In the later stages, Rhabdomyolysis (muscle death) and acute tubular necrosis with acute renal failure and hepatic failure develops [5]. It also has potential toxicity which includes acute toxicity such as allergic contact dermatitis and sub-acute toxicity i.e., the apoptotic effects. PPD poisoning is normally indicated by chocolate brown colour of the urine of victim [5].

Apart from being used in Henna, it is increasingly used for committing suicides and is an emerging household poison since it is easily available in the market at low cost [5]. Accidental poisoning may also occur in case the children ingest the dye accidentally [9]. Several case studies have also been reported with respect to PPD poisoning. Hence, studies on PPD can prove to be helpful in the forensic investigation of such suicidal cases. Gude, D., et.al presented a case report of a 23-year-old schoolteacher who was brought to an emergency department of a hospital in Hyderabad, India after consumption of hair dye (200 ml of 4% PPD-based emulsion type) because of suicidal ideation. She was having difficulty in opening her mouth, swallowing and cramps in hands along with body pain. She also had trismus, calf tenderness, abdominal tenderness, guarding and rigidity. The quantity of PPD ingested was calculated to be 8 gm, which is toxic. She was treated accordingly and was counselled by a psychiatrist [10]. To date, various chromatographic techniques for the detection of PPD have been developed.

These include HPLC (High-Performance Liquid Chromatography) [2,11,7,12], HPTLC (High-Performance Thin Layer Chromatography) [13], GC-MS (Gas Chromatography-Mass spectrometry) [14], etc. Apart from these, studies have been conducted using spectroscopic methods too [15-17]. Commercially available henna samples have been analysed in Korea [11] UAE [2], Turkey [18] and Egypt [12,16], and they were found to contain PPD in different quantities which suggests that the addition of PPD in cosmetic preparations is not regulated at the global level. Temporary black tattoos also have been found to contain PPD in it which has later given rise to allergies [7,19,20]. Hence, this research aimed to identify the presence of para-phenylenediamine (PPD) in commercially sold henna dye samples available in local markets in Bengaluru, India. Conventional methods of PPD detection are costly, time–consuming and need manpower to perform analysis, therefore the authors have attempted to devise a systematic and inexpensive approach for examining PPD using Thin-Layer Chromatography followed by UV- Visible spectroscopy. A comparison of various samples of henna dyes with respect to the presence of PPD was also done by noting their absorbance values.

Materials and methods

Chemicals and Reagents: The standard of p-Phenylene-diamine was purchased from NICE Chemicals Pvt. Ltd. Ethanol (99.9% purity) was obtained from Changshu Hong sheng Fine Chemical Co., Ltd. Chloroform (99.8% purity), Methanol (99.5% purity), Dichloromethane (99.0% purity) and Acetone (99.0% purity) were purchased from SD Fine-Chem Limited. Ethyl Acetate (99.0% purity) was procured from Isocheims Laboratories. Triple distilled water was used throughout the experiment.

General considerations: EI UV – Visible Spectrophotometer (Model No. 2373) was used for the analysis of PPD which comprises of the wavelength range of 190 – 1100 nm and the spectral bandwidth was 4 nm. There was a single beam optical system in the instrument and the grating was 1200 lines/mm. The detector used was Silicon Photodiode. Tungsten and Deuterium were used as light sources. The sample compartment contains a Standard 10 mm path length cuvette and accommodates 100 mm path length cuvette with an optional holder. REMI Centrifuge (Model: R-8C) was used to centrifuge all the samples in the range of 1000- 4000 rpm. UV Cabinet (Model No. R/340/OC) from Super fit India was used to visualize the spots obtained in TLC plates for all the samples.

Sample Collection: A total of ten henna dye samples were collected from different regions of Bangalore, India.

Sample Extraction: 1 gm of the henna sample was taken in a clean and dry test tube and 10 ml ethanol was added to it. This solution was centrifuged for 5-6 minutes at 1000-4000 RPM. The supernatant obtained was filtered using Whatman Filter Paper Grade 1 to filter out impurities. This filtrate was taken into a separating funnel to which 10 ml of Chloroform and 10 ml of distilled water was added. This mixture was vigorously shaken and then kept undisturbed in the separating funnel for around 15 minutes to allow the separation of organic and aqueous layers. (Liquid-liquid extraction). The organic layer, thus formed, was taken out and this extraction process was repeated three times. The resulting extract was kept overnight to dry completely. The dried extract was then scraped out and dissolved in 10 ml of Ethanol to obtain the stock solution of the sample. (Working Procedure Manual on Toxicology. Directorate of Forensic science, Ministry of Home Affairs, Govt. of India.)

Analysis: Seven out of ten samples were extracted successfully. Thin-layer chromatography (TLC) was performed as a preliminary analysis to determine the presence of PPD in all the

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samples and Rf values were obtained and compared with those of the standard. Following this, UV- Visible spectroscopy was conducted to perform the characterization of the samples where the absorbance values were noted at different wavelengths [15].

Results and Discussion

All the ten samples collected were given codes as S1, S2, S3, S4, S5, S6, S7, S8, S9, S10. Out of these samples S1, S2, S5, S6, S7, S8, S9 were successfully extracted and further analysed using TLC and UV-Visible Spectrophotometry. TLC was performed for the standard PPD as well as for all the extracted henna sample solutions. Three solvent systems consisting of Ethyl Acetate and Dichloromethane (DCM) were taken in the ratio of 2:8, 3:7 and 4:6 [16]. TLC was carried out on standard solutions of PPD dissolved in Ethanol of different concentrations ranging from 1 ppm, 10 ppm and 100 ppm. Out of these, 4:6 ratio of ethyl acetate and dichloromethane was selected as the solvent system since maximum separation of constituents was obtained. The retardation factor (Rf value) of TLC for standard PPD solution of different concentrations has been summarized in (Table 1).

Table 1: Rf values obtained for standard solutions of different concentrations.

Spots	R_f values		
	1 ppm	10 ppm	100 ppm
1	0.06	0.2	0.24
2	0.18	-	0.31
3	-	0.41	0.4
4	-	0.85	0.85

PPM: Parts Per Million

Whereas for Sample 01, the Rf values were found to be 0.07 & 0.19 for spots 1 and 2 respectively. No spots were developed for Samples 02, 03, 04 and 05. For Sample 06, the Rf value was found to be 0.15. Likewise, for Sample 07, the Rf value was 0.86. (Figure 1,2) similarly, Ultraviolet-Visible Spectrophotometry (UV-Vis) was also performed on standard PPD as well as for the extracted sample solutions. It was carried out for different concentrations of standard solution viz., 1ppm, 10 ppm and 100 ppm. It is summarized in (Table 2). Further, UV-Visible spectrophotometer was run for all the extracted sample solutions at different wavelengths from 300 nm to 565 nm which can be seen in (Figure 3) [15]. From (Figure 3) it can be clearly seen that the highest absorbance is shown at 292 nm for extracted samples i.e., S2, S5, S6, S7, S8 and S9 except for S1. From this analysis we observed that as the wavelength increases absorbance decreases which may have happened due to decrease in the concentration of the sample as stated by Beer's law. Absorbance values for sample S1, S2, S5, S6, S7, S8 and S9 have been compiled in (Figure 3), (Table 3).

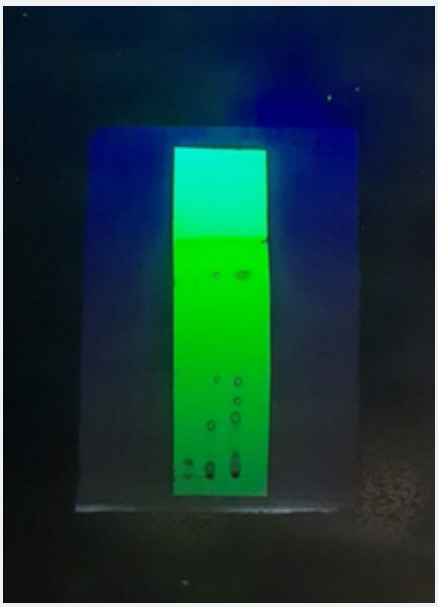

Figure 1: TLC Plate Under UV Showing Standard PPD Solutions at 1ppm, 10ppm and 100ppm.

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Table 2: Absorbance values for the standard solution of different concentrations at 535 nm.

Concentration of standard PPD solution	Absorbance (a)
1 ppm	4.1805
10 ppm	4.4685
100 ppm	4.4949

Table 3: Absorbance values for samples at different wavelengths.

Sample No.	1	2	5	6	7	8	9
Wavelength (nm)							
292	3.6364	3.4685	3.7011	3.9172	4.665	5	4.1135
416	3.757	3.4078	3.3766	3.58	3.7399	4.4437	3.8356
513	4.3875	1.2979	0.8771	1.3666	1.8311	2.5844	0.9089
523	4.301	0.9821	0.6831	1.0641	1.3921	2.3109	0.8123
535	5	1.2644	0.839	1.2909	1.767	2.0122	0.9499
564	4.3768	0.6194	0.4338	0.6641	0.8799	1.967	0.9242

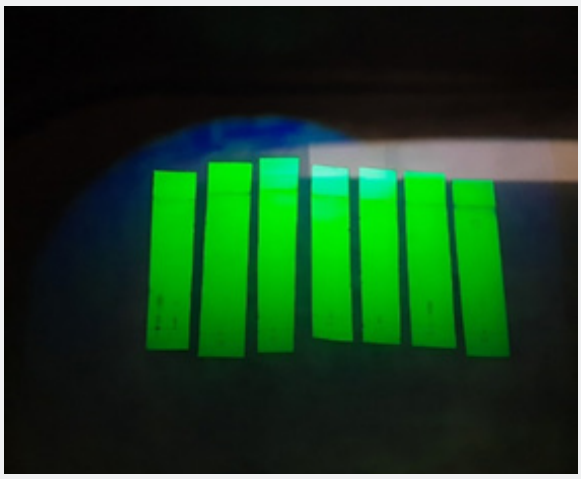

Figure 2: TLC of Sample Solutions Under UV.

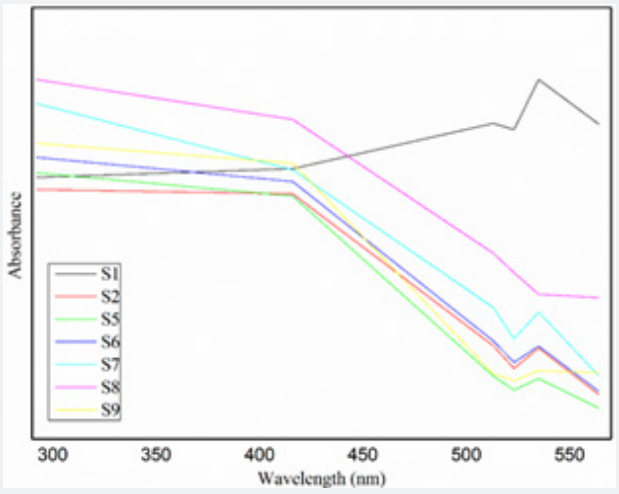

Figure 3: Absorbance of PPD in Commercial Henna Samples at Different Wavelengths.

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Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD and LOQ were determined by using standard deviation of the response and slope approach as defined in ICH guidelines. The following formula was used.

where, σ = standard deviation (SD) of the response (intercept)

S = Slope of the calibration curve.

The calculated values of LOD and LOQ were found to be 0.431 uM and 1.307 uM respectively (Figure 4).

Out of the 10 samples that were collected, 7 were extracted successfully and further analysed by Thin Layer Chromatography and UV-Visible Spectrophotometry. PPD was found in all the samples.

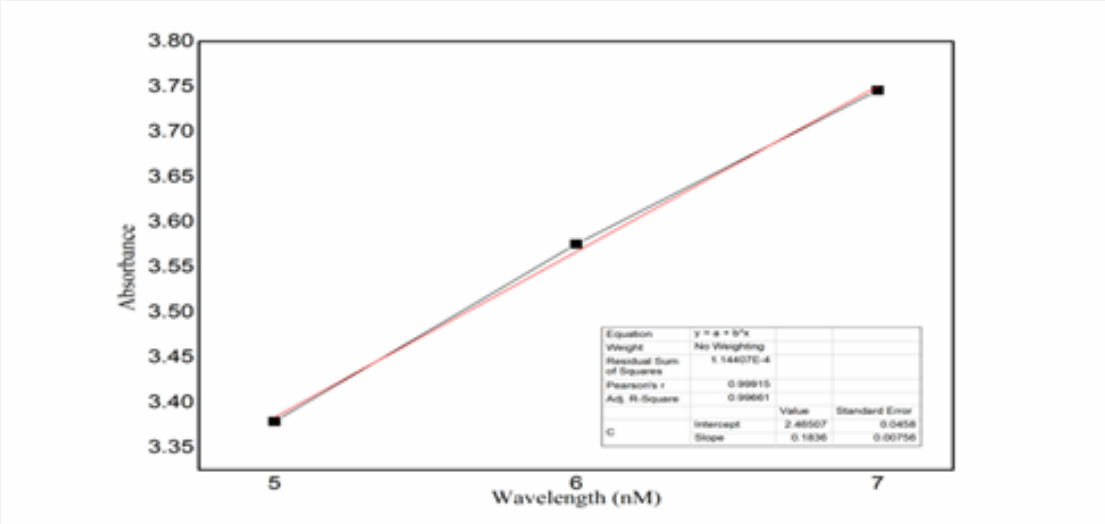

Figure 4: Calibration Curve (Slope = 0.1836).

Conclusion

Since PPD was found in all the extracted samples collected from different parts of Bengaluru city, it is evident that its presence is not being monitored. The public needs to be aware of this and use commercial henna cautiously. Toxic chemicals in hair dyes are frequently added in commercial brands and it is of utmost importance to check for the presence of such harmful compounds time and again on a regular basis so that human health is protected from the adverse effects of such substances. To avoid this, the monitoring organizations of the government need to impose stringent laws on the addition and concentration of such chemicals in products which are routinely used by public.

Laws need to be imposed on all the brands regarding the regulation of amount of PPD in dyes. The above study is non-destructive and can effectively screen many samples. It can serve as a first-hand reference for forensic medical examiners in cases of accidental/suicidal poisoning of PPD. However, this article does not comprise of concentration studies as well as interference studies. In future, studies must also be conducted to determine the fatal dose of PPD as the exact amounts are yet unknown. This piece of work will further be useful for carrying out various examinations on different types of compounds present in cosmetic products, skin & hair care products and the safety index of such products.

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