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Natural extracts as an Eco-friendly additive to Henna paste for safely skin usage In Jazan market

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Abstract

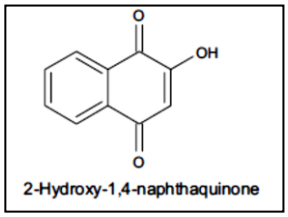
The objectives of this study was to identify the additives present in commercial Henna pastes in Jazan market, and to investigate if it contain harmful materials as paraphenylenediamine (PPD). The pH value of henna samples was found comparable except for some of the pastes that may prove harmful effect on use. Henna samples were found to contain both alkaloids, resins, flavonoids, and tannins . It does not contain saponines. The FTIR spectral analysis of Henna samples demonstrated characteristic absorptions of OH, C = O, C-H, and C = C, while NH peak was detected only for Jazan sample. This sample was found to contain 19% PPD content. The GC-MS analysis revealed also the difference of this sample on comparing with pure Henna powder. A natural substituent is suggested that give henna pastes dark colour, and safe for use.
Keywords: Henna, PPD, Tattoos, GC-Ms.

Introduction

Lawsonia inermis L., commonly known as 'Henna' or Hina is a flowering plant, shrub or small tree native to tropical and subtropical regions of Africa, and Southern Asia. It is frequently cultivated in India, Pakistan, Egypt, Yemen, Iran and Afghanistan. Powdered leaves of this plant (aqueous paste) are used as a cosmetic for staining hands and hairs. [1, 2] It contains Lawsone a red–orange pigment (the molecule of which is also known as hennotannic acid is the chief constituent of henna leaves). [1-5]

The dyeing property as well as the UV absorption, antibacterial, antispasmodic, corrosion inhibitor were attributed to the presence of lawsone; 2-hydroxy-1,4-naphthaquinone in Henna leaves. Industrial classifications also describe Lawsone as Natural Orange 6 and CI 75480. [1-3, 6, 7]

The aqueous extract of henna leaf found to inhibit the growth of both Gram-positive and Gram-negative bacteria. In addition, henna leaf extracts are capable of inhibiting the growth of microorganisms that are involved in causing burn wound infections. Use of henna in management of burns may offset the complications that arise in the use of conventional wound dressings such as silver nitrate, which imparts stains, and is time consuming apart from being able to cause hyponatraemia or hypokalaemia. [1, 2]



Schrme 1: chemical structure henna leaf

This dye molecule has the ability to bond with proteins, and consequently has been widely used in body art to dye skin, hair and fingernails, and to dye silk, leather, and wool. Henna body art is made by applying henna paste to the skin. Henna paste is prepared by drying the henna leaves and grinding them to powder, and then this powder is mixed with oil or water to form the paste. When this henna paste is applied to the skin the dye (lawsone) migrates from the paste to the outermost layer of the skin; more lawsone will migrate if the paste is left

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on the skin for a longer time, thus creating a red-brown stain. [1-5]

Despite the wide spread use of natural henna, specially, in countries where henna art is traditionally practiced, reports of allergic contact dermatitis to natural henna are very rare in the literature. It can therefore be assumed that natural henna is a very weak skin allergen. [1, 2]

Example of the Henna additives include para-phenylenediamine (PPD), which has been mixed with natural henna to give an ebony color (black henna) instead of the orange/reddish color given by natural henna. The other reason for adding PPD to the natural henna is to speed up (shorten the time) of the tattooing process, while natural henna staining takes 4 to 12 hours, addition of PPD can reduce this time to an hour or two and also there will be a longer lasting effect as well. 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. [8-13]

Acute allergic contact dermatitis, eczema, chemical burn, acute renal failure, acute and severe angioneurotic edema, abdominal pain and vomiting as adverse health effects associated with the use of henna containing PPD (black henna) are well documented in the literature. [10-13] In addition, cases of persons being sensitized from use of black henna (containing PPD) followed by cross reaction to oxidative hair dyes and to clothing dyes have also been described in the literature. [14-17]

In the Jazan, the use of henna is part of the tradition and culture. Women of all ages use henna for skin decoration, and it is considered an essential part of the wedding ceremonies and other social celebrations. Thus the objectives of the study were to identify the additives present in commercial Henna pastes in Jazan market.

Experimental

Materials

Henna powder and Henna pastes were obtained from different local markets in Jazan. Potassium Iodide, Iodine, Potassium hydroxide, Ferric Chloride, Sosium hydroxide, Hydrochloric acid , Sulfuric acid (H_2SO_4, 98%) , p- phenylenediamine, Ethyl alcohol, Methyl alcohol, Wagner indicator , Folin reagent, and Chloroform, were of laboratory grade chemicals.

Methods

pH-Measurement

Acidity of Henna aqueous solution have been determined using pH meter.

Test for Alkaloids [18]

2.5 g of powder alcoholic extract was heated with 25 ml of a solution acidified with 4% hydrochloric acid to boiling, then the mixture was filtered and allowed to cool. Then 5 ml of the filtrate is placed on an hour bottle, and a few drops of Wagner reagent are added to it. This reagent is prepared by dissolving (2 g) of potassium iodide in 5 ml of distilled water, adding to it 1.3 g of iodine, then mixing until dissolution and completing the volume to (100 ml) of distilled water, the appearance of a brown color is evidence of the presence of alkaloids.

Test for Resins [18]

50 ml of ethyl alcohol was added to 2.5 g of alcoholic extract powder and the mixture was heated on a water bath until boiling for two minutes, then the solution was filtered and 50 ml of distilled water acidified with HCl, if the turbidity of the solution indicates the presence of resinous substances.

Test for Flavonoids [18]

A mixture of solution (A) was prepared by placing 2.5 g of the alcoholic extract powder in methyl alcohol, then filtered, and solution (B) was prepared by adding 10 ml of 50% ethyl alcohol to 10 ml of 50% potassium hydroxide solution. When equal amounts of (A) and (B) are mixed, the appearance of yellow color indicates the presence of flavones.

Test for Tanins [18]

2.5 g of the alcoholic extract powder was heated in 25 ml of distilled water until boiling for several minutes, then filtered and a 1% solution of ferric chloride was added to it. The appearance of a bluish green color indicates the presence of tannins.

Test for Saponine [18]

When the aqueous solution of the alcoholic extract was vigorously shaken inside a test tube, a thick foam appeared indicating the presence of soap.

TLC Analysis

TLC paper were cut 10 cm long and 8 cm wide, then prepared the mobile phase from ethanol and distilled water (80:20 ml) and determined the height of the start and end line 1 cm, put dots of dye by the capillary stem, then put them in the beaker with a capacity of 400 ml, put 10 ml of the mobile phase in the beaker and put a TLC sheet and then cover it with tin until the colors are separated and the mobile phase reaches the end line

Determination of p-Phenylenediamine (PPD) content [19]

The analysis of PPD was performed spectrophotometrically using alkaline solution of Folin's reagent in methanol at 453nm using (UV-OPTIMA SP-300 spectrophotometer).

Preparation of standard stock solution

About 0.1 g of pure sample of PPD was accurately weighed and dissolved in 100 mL of 0.1 N NaOH in a 100 mL standard flask to get a working standard concentration of about 1 mg/mL.

Preparation of assay solution

0.833 g of marketed Henna dye formulation (Henna sample VII) was weighed accurately and dissolved in 25 mL of 0.1 N NaOH solution, followed by the addition of 1.0 mL of Folin's reagent to get a concentration of about 1 mg/mL. The solution is filtered using Whatman filter paper.

Calibration curves

Standard solutions of PPD different aliquots 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 mL of 100 µg/mL were transferred into a series of 10 mL volumetric flasks, followed by the addition of 1.0 mL of Folin's reagent and 1 mL sodium hydroxide. The volume of this solution was diluted up to the mark with water and absorbance of each solution was measured at 453 nm.

I.R. analysis

The Henna samples have been analyzed using FTIR spectrophotometer, Model, Nicolet iS10, USA, using KBr pellets

GC-MS analysis

GC-MS analysis have been done at the Center for Environmental Research and Studies, Jazan University according to the following procedure.

Sample Preparation

Take 5mL of sample solution add 3g solid Na2SO4 to remove water content in the solution, filter the supernatant solution by syringe filter, use this solution to inject in GC-MS.

GC-MS perimeter

Gas chromatography coupled to a mass spectrometer (GC - MS) equipped with TG-5MS SIL fused-silica capillary column ('Resets') (30m x 0.25 mm internal diameter, 0.25 m film thickness). Helium (1.0 ml/min) was used as a carrier gas. Samples were injected in the splitless mode. The injector was kept at 230^{o}C and the transfer line at 250^{o}C. The column was maintained at 50^{o}C for 2 min and then programmed to 280^{o} C at 7^{o} C /min and hold for 10 min. The MS was operated in the EI mode at 70 eV, in m/z range 40-500.

Results and Discussion

Measurement of pH

The pH values of aqueous solutions of Henna formulations are shown in Table (1).

The results show that sample (VII) has the lowest pH value. This means it is more acidic, and can cause harm to the skin.

Table (1): pH measurement of Henna samples

No.	sample	PH
(I)	Abu Hajar	4.17
(II)	Ahd almsarh	2.74
(III)	Al Tiwal	4.00
(IV)	Al ardih	2.79
(V)	Abu Arish	3,27
(VI)	Al Madaya	2.59
(VII)	Jazan	2.36
(VIII)	Natural henna	4.00

Detection of active constituents

Tests for alkaloids, flavonoids, resis, tanins, and saponine in Henna samples have been done according to the methods described in the experimental section.

The data in Table (2) indicate that Henna contain both flavonoids, alkaloids, resins, tannins. It does not contain saponines

Thin Layer Chromatography (TLC) test

Table (3) show the TLC data for Henna samples. the TLC test ,which contained the largest number of compounds appearing in TLC, it contained a number of compounds, with total of 3 pigments.

Determination of p-Phenylenediamine (PPD) content

Sodium 1,2-naphthoquinone-4-sulfonate (Folin's reagent) is a chemical reagent used to determine the amines and amino acids . The reagent produces a bright red color in alkaline solutions and is fluorescent. The main advantage of this procedure of Folin's was its simplicity and a color was developed at room temperature in slightly alkaline solution. Folin's reagent have been used for the determination of many amino compounds and a large number of substances of pharmaceutical interest. The mechanism of reaction between PPD and Folin's reagent are shown in the Figure. (1).

From Figure 1, we get an equation, $Y = mx + c$.

Y = Absorbance of the sample at 453 nm, m = Mass, x = concentration of PPD in solution g/L, c = Velocity constant

y = 1.4857x + 0.0487

Absorbance of market sample solution = 0.285, this gives x= 0.159 g PPD in the o.833g market sample. This gives PPD concentration in sample= 19%

This explain the harmful effect of this sample.

Table (2): Active constituents in Henna samples*

Test for constituent	Result obtained for sample :							
	(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)	(VIII)
Test for Alkaloids	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Test for Resins	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Test for Flavonoids	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Test for Tanins	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Test for Saponine	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)

Note: (+) is positive test result, (-) is negative test result

* Henna samples numbers are the same as Table (1)

Table(3): Results of TLC test

Sample name	Number of pigments	Dye color	Account
Abu Hajar	3	Yellow Brown Orange	0.3 0.8 0.7
Ahd almsarh	2	Yellow Orange	0.3 0.8
Abu Arish	2	Yellow Orange	0.3 0.8
Al Madaya	2	Yellow Orange	0.3 0.8
Al Ardih	3	Yellow Brown Orange	0.3 0.8 0.7
Al Tiwal	2	Yellow Orange	0.3 0.8
Jizan	3	Orange Green Yellow	0.8 0.5 0.73

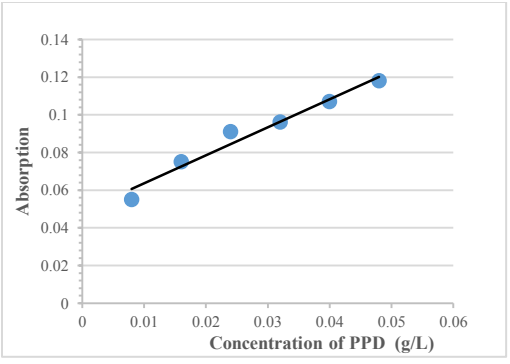
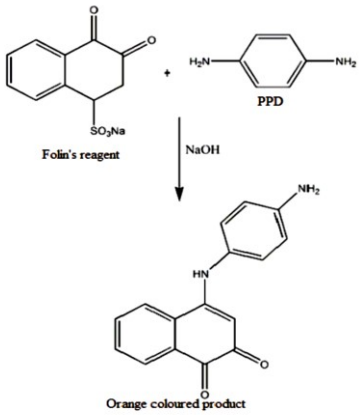


Figure (1): Standard absorption curve for PPD

Infrared analysis

The outcome of the FTIR spectra of all Henna Samples (Figure 2 are comparable . The I. R. spectra demonstrated characteristic of OH, C = O, C-H, and C = C, while the Jizan sample (Sample VII) showed the presence of NH group. This confirms the presence of PPD in the sample.



Scheme 2

In our work, we tried to find a natural substituent for PPD. We used Tea, Lemon , and Tamarined mixture. The pure Henna sample mixed sample with the natural substituent show the same I.R data as the untreated sample. The substituent mixed Henna sample show dark colour as black Henna.

GC-MS analysis

Gas Chromatogarphy – Mass Spectrometry (GC – MS) is a method that combines the features of gas liquid chromatography and mass spectrometry to identify different substances with in a test sample. Applications of GC/MS include drug detection, fire investigation, environmental analysis, explosives investigation and identification of unknown samples. [20]

The GC-MS analysis have been performed for pure Henna sample, for Jizan sample (sample VII), and for Henna mixed with natural substituent.

The data shown in Figure (3), show that both pure Henna and natural substituent mixed Henna show nearly the same compounds with the same RT

and Area value. However the ample proven to contain PPD (sample VII) show different GC-MS curve due to different constitution.

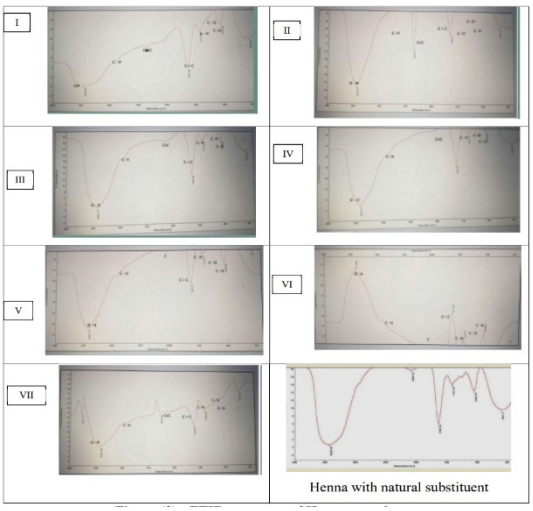


Figure (2) : FTIR spectrum of Henna samples

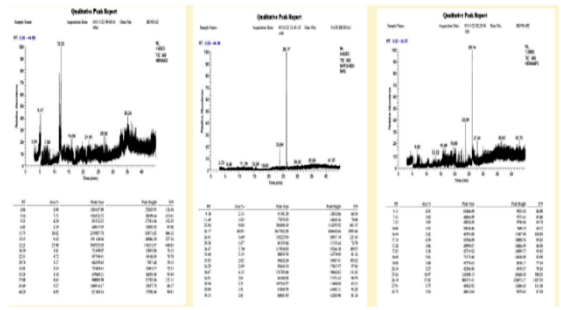


Figure (2) : GC-MS analysis data of Henna samples

Conclusions

- Henna contain both flavonoids, alkaloids, resins, tannins. It does not contain saponines.

- Some of the local market Henna pastes used for Tatooes have low pH value and contain PPD that is very harmful for skin use, and may cause skin burn.

- A natural substituent is suggested that give henna pastes dark colour, and safe for use.

Funds

No fund

Conflict of Interest

The authors declared no competing interests in the publication of this article

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دراسة المواد المضافة للحناء في سوق جازان المحلي

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المستخلص .

هدفت هذه الدراسة إلى التعرف على المواد المضافة الموجودة في معاجين الحناء التجارية في سوق جازان، والتحقق مما إذا كانت تحتوي على مواد ضارة مثل البارافينيلين ديامين .*(PPD)* وجد أن قيمة الأس الهيدروجيني لعينات الحناء قابلة للمقارنة باستثناء بعض المعاجين التي قد تثبت تأثيرها الضارعند الاستخدام .وجد أن عينات الحناء تحتوي على كل من القلويدات والراتنجات والفلافونويدات والتانينات و لا تحتوي على صابونين .أظهر التحليل الطيفي *FTIR* لعينات الحناء امتصاصًا مميزًا لـ *OH* و *C = O* و *C-H* و *C = C* ، بينما تم الكشف عن ذروة *NH*فقط لعينة جازان .وجد أن هذه العينة تحتوي على محتوى 19٪ من*PPD* كما كشف تحليل *GC-MS* أيضًا عن اختلاف هذه العينة عند مقارنتها بمسحوق الحناء النقي.كمايقترح البحث بديل طبيعي يعطي معاجين الحناء لونًا داكنًا وآمنًا للاستخدام و تمت مقارنته مع العينات الموجوده في السوق المحلي بجازان .

الكلمات المفتاحية: الحناء ، البارافينيلين ديامين ، بديل طبيعي.