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Isolation and Identification of Lawsonia Content from the Leaves of Henna (*Lawsonia inermis*)

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ABSTRACT

Lawsone is an orange red dye mainly isolated from *Lawsonia inermis* plant leaves. *L. inermis* is commonly known as henna plant, Egyptian privet, or mignonette tree. In Pakistan, henna is known as *mehndi*. Paste from thick henna leaves has been used to dye hairs, fingernails, palms of hands, and eyebrows for more than 5000 years. The characteristic colour of henna is because of the natural dye lawsone present in it. In this study, lawsone was extracted from four samples of henna leaves collected from various parts of Pakistan. Moreover, the impact of the environment on the leaves was examined. Lawsone was extracted using diethyl ether, so that the maximum amount of lawsone could be isolated from the leaves. The maximum and minimum amount of lawsone isolated during this work was 1.01% and 0.745% of dry weight, respectively. Afterwards, IR and HPLC were used to identify the isolated reddish-brown substance known as lawsone which is useful in textile, cosmetics, and pharmaceutical sectors.

Keywords: dye, Henna, *Lawsonia inermis*, lawsone, plant

1. INTRODUCTION

Lawsonia inermis is commonly known as henna plant, Egyptian privet, or mignonette tree [1, 2]. In Pakistan, henna is known as mehndi. The principal colouring component of this plant is known as lawsone. This plant is grown on a large scale in Sindh and Baluchistan, two provinces of Pakistan [3]. However, due to its medicinal and cosmetic applications, it is

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cultivated throughout the world [4]. It is a significant medicinal plant that has drawn the attention of scientists from all over the world due to its amazing pharmacological properties [5]. It is a mid-sized, highly branched shrub. Several medicinal uses have been reported for its flower, seed, roots, and stem [6]. It is used to dye hair, skin, and nails [7]. Skin and hair colour are extracted from lawsone by using the technique based on Michael addition reaction theory. A non-permanent colouration that can last up to two to three weeks is produced when Lawsone makes a covalent link with the keratin protein present in skin and hair. The interaction between lawsone and keratin determines colour intensity [8]. Darker colouration results from a greater lawsone-keratin interaction. However, with the passage of time, lawsone is dissolved in water. As a result, lawsone concentration decreases and henna stain fades away [9].

Chemically, lawsone is a 2-hydroxy-1,4-naphthoquinone, also known as hennotannic acid [10]. Lawsone is an orange-red dye mainly found in *Lawsonia inermis* leaves. It is also present in other plant components, although the amount is quite small [11]. Lawsone gets its name from the plant it is derived from, that is, *Lawsonia inermis*. The genus *Lawsonia* includes only two species, that is, *L. inermis* and *L. odorata*. Lawsone is present in very small amounts in *Lawsonia odorata*. A minor quantity of lawsone was also observed in water hyacinth bloom [12].

Human beings have been using henna extracts containing lawsone for at least 5000 years to dye their hair and skin [13]. Lawsone shows an excellent antifungal, antibacterial, antitumor, antiviral, and pesticidal activity [14, 15]. The amount of lawsone in henna plant leaves varies by region. Geological conditions and a number of environmental factors affect its concentration [16]. Moreover, soil temperatures between 25°C and 30°C are ideal for the germination of this plant. Although, it may also survive in the temperature range of 35°C to 45°C [17]. In comparison to winter, a higher concentration of lawsone is observed in henna leaves during summer [18]. The current study highlights the effect of environmental conditions on lawsone content cultivated in different areas of Pakistan.

2. MATERIALS AND METHOD

Experimental work regarding the isolation and identification of lawsone content from the leaves of henna was carried out in the laboratory of Pakistan Council of Scientific and Industrial Research (PCSIR), Lahore.

HPLC instrument used in this experiment was designed by Shimadzu Corporation, Japan. While, the column used was LC-18 and SPD 6AV UV-detector, adjusted at 260nm. Henna leaves used in this work were collected from different areas of Sindh and Punjab. The identification of samples was confirmed by the Department of Botany, University of Education, Lahore. All the samples were shade dried.

Dried henna leaves sample (100g) was added in a 5L Erlenmeyer flask containing 5000ml distilled water and refluxed at 80^0C till the vapours stopped coming out of the flask. The suspension was stirred continuously with the help of magnetic stirrer to avoid any sort of coagulation. After approximately 3 hours, the colour of suspension turned brown and the reflux assembly was removed. Suspension was left overnight. Then, the suspension was made alkaline by adding sodium bicarbonate (20g). Alkaline suspension was then filtered and the filtrate was made acidic by adding 0.12M hydrochloric acid till the pH of the solution became 3. Lawsone was extracted by using diethyl ether. Excessive solvent was evaporated on a hot plate at 30^0C to avoid the burning of lawsone. Isolated reddish-brown material was then scratched and stored in a pre-weigh vial. The same procedure was reported for other samples and the isolated lawsone was stored in pre-weighed vials, separately (Figure 1 and 2) [16].

Figure 1. Powdered Henna Leaves Sample Collected from Different Areas

3. RESULTS AND DISCUSSION

The amount of lawsone isolated from four different henna leaves samples was not more than 1% of the dry weight of samples (Table 1). The percentage of the isolated reddish-brown material depicted the effect of environmental conditions on the lawsone content. The material was identified by using IR and HPLC and the results were compared with the standard chemical lawsone.

Table 1. Percentage of Lawsone Isolated from Different Henna Samples

Henna Leaves Samples	Percentage of Lawsone Extracted
Sample leaves 1 (S1)	1.010
Sample leaves 2 (S2)	0.845
Sample leaves 3 (S3)	0.785
Sample leaves 4 (S4)	0.744

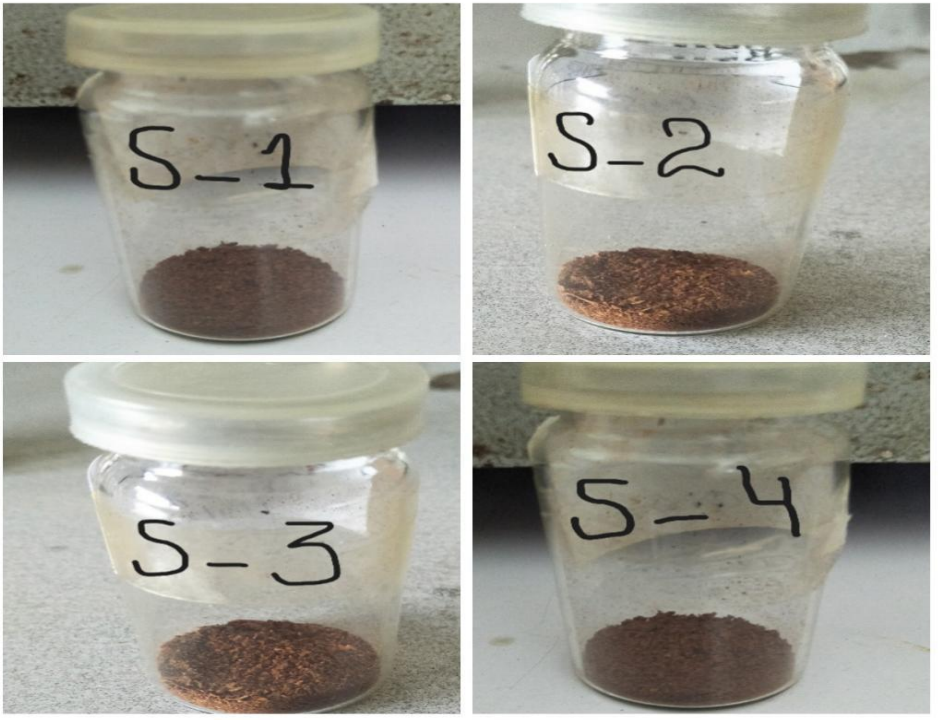

Figure 2. Reddish Brown Material Isolated from Different Henna Leaves Samples

3.1. IR Spectra

The IR spectra of the isolated material shows different peaks. Each peak corresponds to a specific functional group. The stretching of the O-H functional group, overlaid by the naphthalene ring's C-H vibrations, reached its maximum at 3159 cm^{-1}. Carbonyl stretching caused peak formation at 1675 cm-1 and 1636 cm^{-1}. These peaks were expected to divide due to internal hydrogen bonds. Peaks at 1216 cm^{-1} were related to the stretching C-O, while peaks at 1584 and 1510 cm^{-1} were related to the naphthalene ring's C=C vibrational bands. The IR spectra peaks confirmed the chemical nature of the reddish-brown substance, namely lawsone (Figure 3).

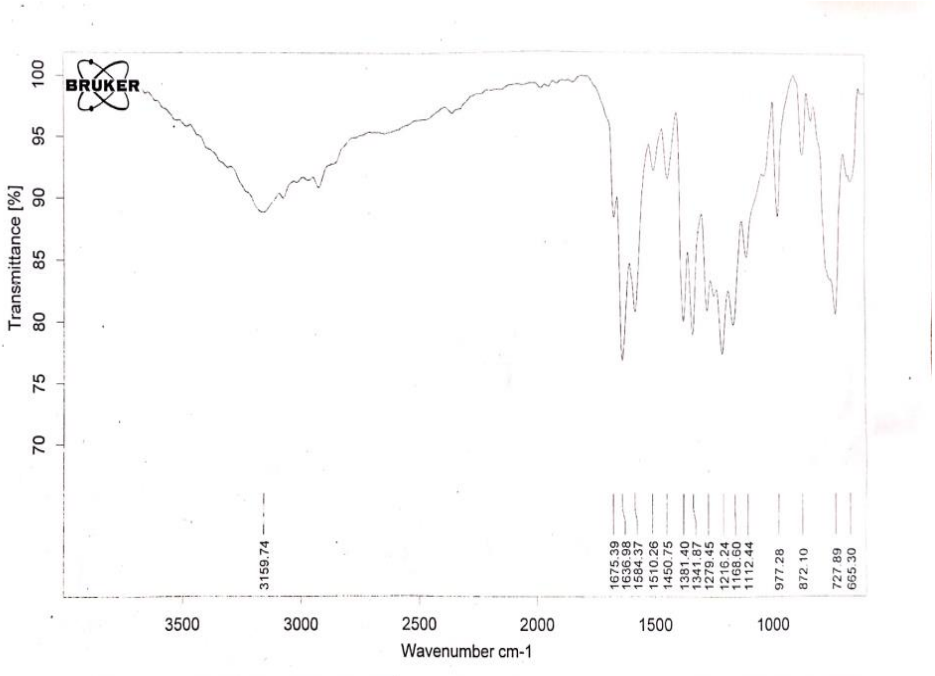

Figure 3. IR Spectra of Isolated Reddish-Brown Material

3.2. HPLC Analysis

The nature of the isolated material was further confirmed by HPLC. For this purpose, standard lawsone was purchased from Merck. A total of 50mg of standard and isolated material was used to prepare standard and sample solutions, respectively. Mobile phase used during the experiment was 70% methanol and 30% water. A significant resemblance between the separated

material's HPLC spectra and the standard lawsone HPLC spectra was observed. This resemblance proved that the isolated chemical was lawsone (Figure 4).

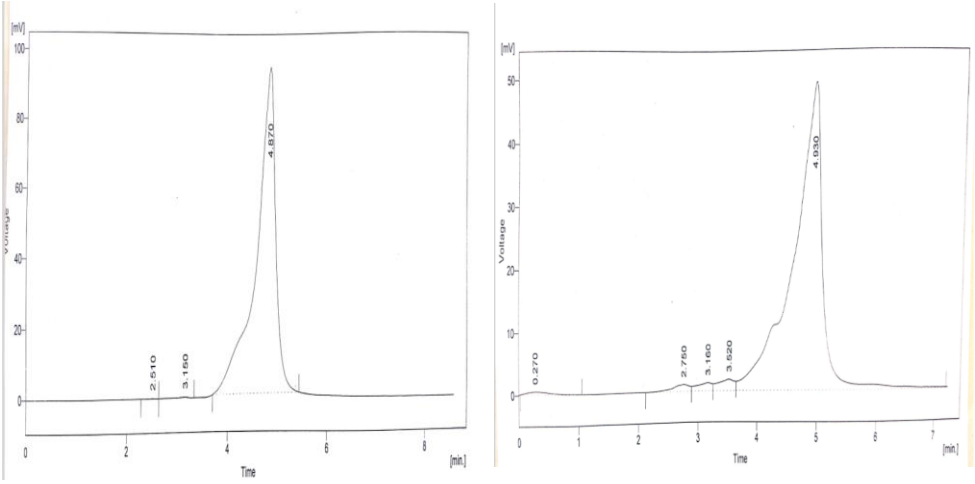

Figure 4. HPLC Spectra of Standard and Isolated Reddish-Brown Material

3.3. Effect of Geological Conditions on Lawsone Content

Henna leaves collected from areas with a high temperature and dry air have more lawsone. High temperature is favorable for lawsone content in henna leaves. Moreover, henna plants cultivated during summer have more lawsone content in their leaves than those cultivated in winter.

3.4. Factors Effecting Lawsone Content

3.4.1. Effect of Temperature. Temperature has a strong influence on the amount of lawsone extracted from *Lawsonia inermis* leaves [19]. This is because if the extraction temperature exceeds 80^0C, then most of lawsone is either dissolved in water or burned out. Similarly, if the temperature exceeds the boiling temperature of the solvent during the isolation of lawsone from diethyl ether, then all the lawsone is burnt.

3.4.2. Effect of pH. Change in pH does affect lawsone concentration but its effects are less harsh as compared to temperature. A slight change in pH can be neglected but a major change does affect the lawsone yield. The greater the pH of solution the lesser is the amount of the isolated lawsone. pH also affects the colour of the extract [20].

4. CONCLUSION

The primary colouring component of the *Lawsonia inermis* plant is lawsone. The identification of environmental conditions and their impact on lawsone content were studied in this research. Lawsone was extracted from four different kinds of henna leaves samples. The maximum and minimum amount of lawsone isolated during this work was 1.01% and 0.745% of dry weight, respectively. The percentage yield made it evident that sample leaves from Sindh contained more lawsone than sample leaves from Punjab. Thus, it was concluded that the weather conditions of Sindh are in favor of lawsone content in *Lawsonia inermis* leaves. Moreover, it was also determined that henna plants harvested in the summer season have more lawsone than those harvested in winters. Thus, isolated lawsone can be used in the pharmaceutical industry and as a dying agent in cosmetic and textile industries.

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