

## Isolation, identification and characterization of lawsone from henna leaves powder with soxhlet technique

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### Abstract

Lawsone, a natural pigment present in the henna leaves, has been used as a skin and hair dye since 1400 BC. The concentration of this natural compound in leaves varies from place to place depending upon many of the environmental factors and the highest quantity reported so far is about 1% of the dry mass. Heretofore, it has been reported that natural colored extracts isolate from Henna leaves with many methods such as maceration, digestion, microwave and infusion. In this paper, regarding the therapeutic effects and traditional applications of henna, it was tried to isolate and characterize Lawsone from the henna leaves marketed in Tabriz city of Iran by soxhlet extraction technique in methanol solvent. The advantage of this technique is the isolation of large amounts of lawsone (720 mg from 40 g henna leaves powder) with smaller quantity of methanol.

**Keywords:** Henna; *Lawsonia inermis* L.; lawsone; isolation; soxhlet extractor.

### Introduction

The henna plant is a tall flowering tree standing about 5 m tall, native to tropical and subtropical regions of Africa, Asia and Northern

Australia [1]. The practical use of henna leaves powder is as a dye for colouring hair and nails and for decoration of parts of the body temporarily [2]. Naturally, henna colo-

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rations are considered as harmless. Some people in North Africa apply henna paste on the skin as a protection against the sun [3]. This is possible because coloured compound of henna plant strongly absorbs UV radiation and so do its covalent reaction products with the protein keratin in the skin. Henna pierces the dead cells of the horny outer layer of the skin [4]. The henna leaves have been used in medicine as an astringent, antiseptic and antipyretic [5, 6]. In ancient times, henna was used to treat serious diseases (leprosy, smallpox, chickenpox, tumours) by Arab doctors [7]. Also, henna's some physiological effects have been confirmed as bactericidal and fungicidal actions [8]. There are several natural compounds in the henna leaves. The well-known compound is lawsone occurred in the henna plant leaves. Phytochemists gave the compound the trivial name lawsone due to its origin, the henna plant *Lawsonia inermis* L.. Lawsone is an intact glycosidase, able to split the glycosidic bond, when brought into contact with hot water [9]. Heretofore, lawsone has been extracted by means of maceration, digestion and infusion [10]. This paper deals with an efficacious isolation and characterization of the lawsone. In this work, we first extracted lawsone by soxhlet extractor method [11]. This material can be further purified by recrystallization. Depending on the

shape and size of the crystals formed, the colour of lawsone differs from an intense yellow to dark red [12].

### **Experimental**

All solvents were purchased from Merck Company. They were distilled before use and stored over a drying agent. IR spectra were recorded with a Shimadzu FTIR-408 spectrophotometer as KBr pellets.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker 250 AC spectrometer in DMSO as a solvent at room temperature. The  $\lambda_{\text{max}}$  and colour intensity of lawsone were determined on a Philips PU 8620 UV spectrophotometer in DMSO solvent using a 1-cm quartz cell. Mass spectrum was attained by double-focusing mass spectrometer. TLC was performed by the use of Merck's silica gel.

### **Isolation and characterization of lawsone from henna plant powder**

40 g of dried henna leaves powder is placed in a large beaker and 1 L distilled n-hexane is added together with a magnetic stirring rod. The suspension is stirred on a magnetic stirrer for 1 week. Then, the suspension is filtered and placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber of the soxhlet apparatus. 320 mL methanol in flask (500 mL) is heated, and its vapors are condensed in condenser. The condensed extractor drips into the thimble con-

taining the henna powder, and extracts it by contact. When the level of liquid in chamber rises to the top of siphon tube, the liquid contents of chamber flood into flask. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. After 2 days, the solvent is evaporated by rotary apparatus. Then, it is dissolved in 100 mL toluene. The solution is poured into separatory funnel. Then, 100 mL NaOH 0.2 M is added to the solution and shaken for minutes. The aqueous phase is collected and acidified to pH 3 by HCl 0.2 M. The brown extract undergoes a clarification in this step and turns slightly cloudy. The filtrate is extracted with diethyl ether ( $3 \times 100$  mL). In the final extraction, the ether turns to a very pale yellow, indicating the end of the extraction. The combined ethereal phases are washed with 30 mL water and dried over  $\text{MgSO}_4$ . The ether is removed completely in vacuum to leave a reddish brown solid (720 mg) as crude product. The crude lawsone is purified by thin layer chromatography. The product (Figure 1) was chromatographed over silica gel by ethanol:ethyl acetate in a ratio of 1:2 v/v. The melting point of the isolated, pure lawsone was in the range of 192-195 °C which is same as the literature value, 195 °C [13].

UV-Vis spectrum of lawsone in DMSO solvent is recorded. The transitions are obtained in 296, 339, 416 and 448 wavelengths. The commonly observed transitions are  $n \rightarrow \pi^*$  or  $\pi \rightarrow \pi^*$ . We saw conjugation causes absorption signatures shift to longer wavelengths because the  $\pi \rightarrow \pi^*$  transitions are more intense than  $n \rightarrow \pi^*$  transitions.

IR (neat,  $\text{cm}^{-1}$ ): 3170 (stretching O-H which overlays the C-H vibrations), 1680 and 1641 (stretching carbonyl, this splitting could be due to some contribution of an internal hydrogen bond), 1578 and 1592 (C=C vibrational bands of the naphthalene ring) and 1215 (stretching C-O).

$^1\text{H}$  NMR (FT-250 MHz,  $\text{DMSO-d}_6$ ):  $\delta$ ; 7.78-8.02 (m, 4H of benzene ring) and 6.17 (s, 1H<sub>3</sub>).

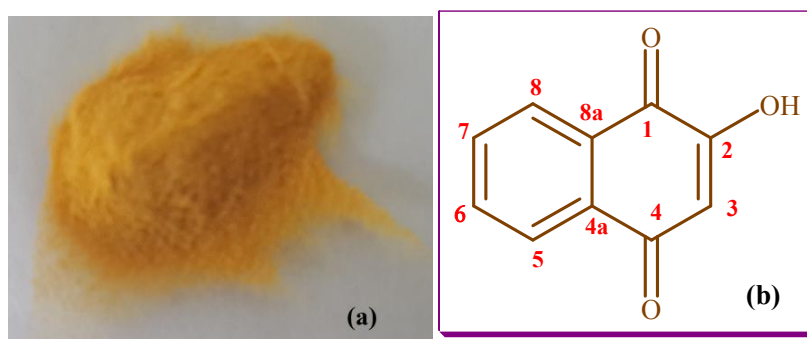
$^{13}\text{C}$  NMR (FT-250 MHz,  $\text{DMSO-d}_6$ ):  $\delta$ ; 111 (C<sub>3</sub>), 125 (C<sub>5</sub>), 126 (C<sub>8</sub>), 131 (C<sub>8a</sub>), 132 (C<sub>4a</sub>), 133 (C<sub>7</sub>), 135 (C<sub>6</sub>), 181 (C<sub>1</sub>), C-1 and C-4 chemical shifts don't appear in spectrum.

MS:  $m/z = 174$  [M]<sup>+</sup>, 146, 118, 105, 89 and 77. The mass spectrum gives the molecular ion as the base peak, pointing to the stability of this naphthoquinone. The loss of CO leads to the ion with  $m/z = 146$ . The ion with  $m/z = 146$  can form the benzoyl ion with  $m/z = 105$ , which finally loses CO to give the phenyl ion with  $m/z = 77$  (Scheme 1).

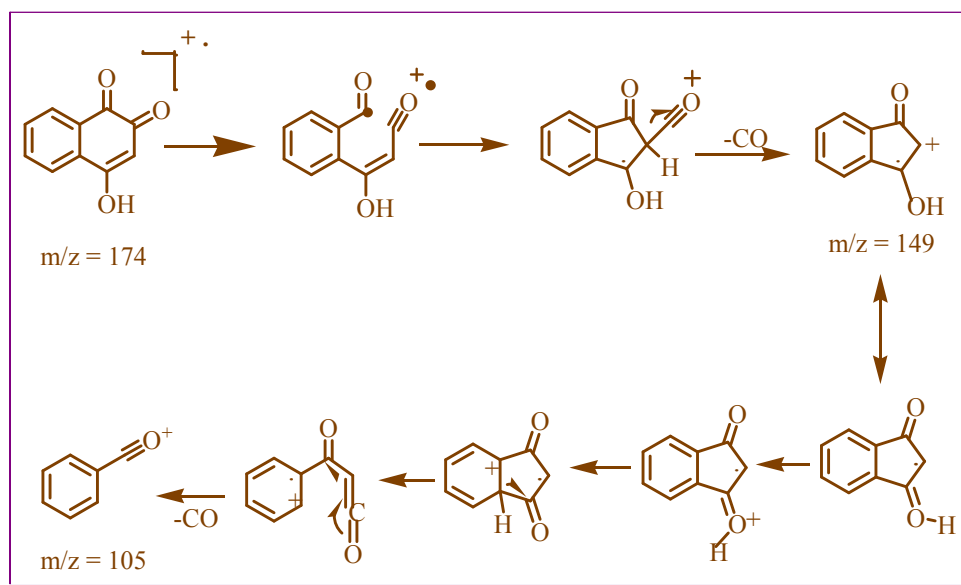
## Results and discussion

We have demonstrated that color of Henna is lawsone compound. Lawsone is main compound of this plant. This natural compound has many uses such as hair dye and medicine. Before this time, lawsone had been extracted by means of maceration, digestion, microwave and infusion. In this work, we extracted the lawsone by soxhlet extractor method. It is

characterized by spectroscopy methods such as UV-Vis, FTIR, Mass and NMR analysis. The advantage of this method, compared to previously described methods, is that large amounts of lawsone can be extracted with a much smaller quantity of solvent. This affects tremendous economy in terms of time, energy and consequently financial inputs.



**Figure 1.** (a) Lawsone compound powder, (b) Structure of lawsone



**Scheme 1.** Fragments of lawsone mass spectrum

## Conclusions

In this study, we extracted lawsone natural compound by Soxhlet extractor. Then, other many compounds in the extract were removed by solvent-solvent extraction. The lawsone was purified by TLC and characterized with spectroscopy methods. The advantage of our method is the extraction of large amounts of lawsone with smaller quantity of methanol.

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