Green Biotechnology for Aqueous Citric-Acid Extraction of Lawsone from Henna Leaves

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Abstract:
Henna leaves have been used as a natural hair dye due to the presence of lawsone, a red-orange naphthoquinone pigment. In this work, we investigate three extraction techniques: conventional solvent reflux extraction, Soxhlet extraction, and ultrasonic-assisted extraction (UAE) to isolate lawsone from henna leaf powder using water (with mild acid addition) as a green solvent. The effects of extraction temperature (25–70 °C), duration (2–9 h), and solution acidity (citric acid, up to 0.01 M) on lawsone yield were examined. UV-Vis spectroscopy was used to quantify lawsone content in the extracts. Optimal extraction conditions were identified at a moderate temperature of 50 °C, an extraction time of 5 h, and citric acid pH 3.2. Under these conditions, ultrasonic extraction achieved the highest lawsone yield, followed closely by heated solvent extraction, whereas Soxhlet extraction at 100 °C yielded comparatively less lawsone. The lawsone-rich extracts showed a characteristic absorbance peak around 350 confirming the target compound's presence. This study demonstrates that ultrasonic-assisted, mildly acidic aqueous extraction is an effective and eco-friendly approach to obtain lawsone from henna leaves, outperforming traditional methods in both yield and preservation of the active dye. These findings can facilitate the sustainable production of natural henna-based hair dyes and other cosmetic applications.

Keywords
Henna; Lawsone; natural dye; Reflux Solvent Extraction; Soxhlet; Ultrasonic-assisted extraction

1. Introduction

Since the early stages of human civilization, natural dyes for hair coloration have served not only for decoration, but also medicinal and religious purposes. As science advanced, synthetic dyes were invented using various chemicals that allowed color permanence, vibrance, and range. Nowadays, hair dyeing has gained worldwide popularity with demands from both genders. Common synthetic hair dyes available on the market nowadays can be categorized into three types: temporary, semipermanent, and permanent. Temporary and semipermanent hair dyes go under non-oxidative processes, but permanent ones require an oxidation step with an oxidizing agent, often being hydrogen peroxide (França A.S., 2015).

Temporary hair dyes do not penetrate the cortex but rather coat the cuticle with a layer of color molecule. Because of this characteristic, temporary hair dyes do not have a long-lasting period and are only capable of adding new nuances to the existing color as it does not interfere with the inner components of the hair. Semi-permanent hair dyes, on the other hand, somewhat interact with the cortical cells because the dye molecules have a strong affinity for hair keratin. Having a high molecular weight, these dyestuffs get absorbed into the hair through Van der Waals connections. Semi-permanent hair dyes typically have a longer fastness period, lasting for up to 4-6 shampoos. Permanent hair dyes allow the most long lasting and versatile color alteration, however, also most damaging to the hair. The procedure requires four critical components that make up the hair dye: coupling bases, reaction modifiers, alkalizing material, and an oxidizing agent. The process takes place in an alkaline environment, created by a substance with high pH (often ammonia) that opens the hair cuticle, allowing deep penetration into the cortical layer of the color molecules. The oxidizing agent (hydrogen peroxide) then starts reactions in the cortex. The process also often includes a bleaching step after the cuticle is opened where hydrogen peroxide oxidizes the melanin molecules that give the hair its pigment to remove the original color, leaving a yellow pale hue on the hair. Oxidation of couplers and reaction modifiers create intermediates (eg. PPD) that then react to form color molecules (França A.S., 2015).

Hair dyeing has been known to have many adverse effects, especially permanent dyeing products. A study was conducted by the Department of Pharmacology of the Sri Devaraj Urs Medical College in India to assess the knowledge, practice, and reactions among hair dye users. The study reveals that, of the participants, 76% believes that hair dyeing is unsafe, and almost half reports having suffered from side effects such as headaches and itching. Despite this, hair dyeing is still becoming increasingly popular, reaching not only those who felt it was fashionable, but also a large percentage that uses dyes to conceal gray hair (Narayana et al., 2013).

In recent years, there is an ongoing demand for natural, safe, and environmentally friendly beauty products with growing global consumers more aware of the negative effects of synthetic dyes. While synthetic dyes can be harmful to many users, its alternative, natural dyes can provide semi-permanent coloring without unwanted effects. Certain types of phytochemicals, compounds found in plants produced for protection, are natural colorants that could be extracted to be used as dyes. For example, compounds such as quinones are found in Henna leaves (Lawsonia inermis L.) and walnut husks (Juglans regia L.) produce reddish brown color when extracted and used as a colorant. Other common phytochemicals are Indigo, Carotenoids, Curcuminoids, Tannins, and Flavonoids. Natural hair dyes not only reduce the risk of allergens and environmental biohazards, but some also provide health benefits as antioxidants and are capable of repairing damaged hair cuticles (Cui et al., 2022).

The growing environmental and health concerns associated with synthetic dyes have spurred interest in natural alternatives for cosmetics. The plant-based dyes are biodegradable and generally safer, making them attractive for sustainable use (Syafaatullah, 2023). Henna, a shrub native to tropical and subtropical regions, is a well-known source of natural dye. The dyeing property of henna is primarily due to lawsone (2-hydroxy-1,4-naphthoquinone), which is a red-orange pigment present in the leaves (Ashnagar, 2011). Lawsone readily binds to proteins, which enables it to impart a long-lasting stain on keratinous materials like hair and skin. Typically, dried henna leaf powder contains about 1–2% lawsone by weight (Sankar, 2023), although the exact content can vary with plant variety and growing conditions. In the henna plant, lawsone mostly exists in a precursor form (such as glycosides called "hennosides") rather than as free molecules. When the leaves are crushed and mixed with water, endogenous enzymes and mild acidity help convert these hennosides into the active aglycone (lawsone) that can bind to substrates (Sankar, 2023). This acidification facilitates the hydrolysis of the glycosides and stabilizes the reactive form of lawsone, thereby enhancing its availability.

A variety of extraction techniques can be employed to isolate lawsone and other bioactive compounds from plant materials. For henna, maceration in water or alcohol can extract lawsone, but prolonged extraction is needed to achieve high yield (Bennaceur, 2021). Soxhlet extraction is a classical method commonly used for plant metabolites, which repeatedly percolates hot solvent through the solid material. Soxhlet typically operates at the boiling point of the solvent, and when using water as the solvent, the extraction occurs around ~100 °C. In recent years, ultrasonic-assisted extraction (UAE) has gained attention as a technique to intensify the extraction of natural products. The UAE often achieves higher yields in shorter times and at lower temperatures compared to conventional methods (Altemimi, 2016).

The compound lawsone serves as a natural dye, and its extraction process is influenced by various factors. This study focuses on the extraction of lawsone from henna leaf powder using a solvent. Distilled water, with a small amount of citric acid as an acidifying agent, was chosen as the primary solvent to ensure the process is environmentally friendly and suitable for cosmetic applications. Extraction conditions, including temperature, time, and pH, were systematically varied to assess the impact of these factors on the efficiency of lawsone extraction. The aim is to develop an optimized extraction procedure and promote the use of green extraction methods in natural product biotechnology. In addition, the study aims to propose a hair dyeing process that is environmentally friendly, ensures natural color retention, and provides safety in the surrounding environment.

2. Experimental

Materials: Pure henna leaf powder was obtained from Prem Green Export Corporation (Rajasthan, India). Distilled water was used as the extraction solvent in all experiments. Citric acid monohydrate 99,8% was obtained from Xilong company.

Extraction Procedures: Three different extraction techniques were evaluated: (1) solvent extraction by reflux extraction, (2) Soxhlet extraction, and (3) ultrasonic-assisted extraction. In all cases, the solvent was water with a controlled amount of citric acid. A solid-to-liquid ratio of 1:20 (w/v) was employed for the extractions unless otherwise specified.

Reflux Extraction: For each experiment, 5g of henna powder was placed in a 250 mL Erlenmeyer flask, 100 mL of distilled water was added and refrigeration system was set up, The extraction was carried out at a set temperature (ambient ~30 °C up to 70 °C) for a specified duration (ranging from 2 to 8 hours). At the end of the extraction period, the mixture was allowed to cool and then filtered. The filtrate (henna extract) was collected for analysis.

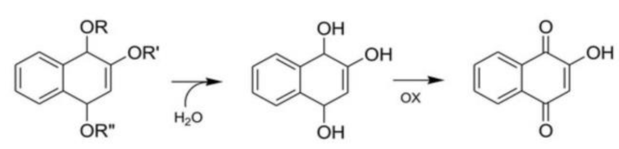
Soxhlet Extraction: About 5 g of henna powder was loaded into a porous cellulose thimble, which was then placed in the Soxhlet extractor chamber. Distilled water (approximately 350 mL) with the desired citric acid concentration was added to a 500 mL round-bottom flask attached below the Soxhlet chamber (Liew, 2022). The setup was heated on an electric heating mantle to bring the water to gentle boiling. The cycle was allowed to repeat for 5 hours to ensure extensive contact. The aqueous extract in the round-bottom flask was carefully decanted.

Ultrasonic-Assisted Extraction: Ultrasonic extractions were conducted using an ultrasonic cleaning bath (40 kHz frequency, 300 W power) large enough to accommodate the sample flask. In each ultrasonic trial, 5.0 g of henna powder was mixed with 100 mL of distilled water (acidified to the desired concentration) in a 150 mL beaker. The beaker was placed in the ultrasonic bath, which was filled with water and maintained at the target temperature. The sample was sonicated for a set time

Analysis of Extracts: The primary analytical technique for quantifying lawsone in the extracts was UV-Visible spectroscopy. A UV-Vis spectrophotometer (scanning from 200 to 500 nm) was used to record the absorption spectrum of each extract.

3. Results and Discussion

Some recent studies indicate that the orange dye "lawsone" (2-hydroxy-1,4-naphthoquinone) in henna is largely generated during processing rather than stored as the free aglycone in intact leaves. In dried leaf tissue, the dominant constituents are glycosylated precursors—hennosides, upon aqueous maceration, are hydrolyzed by endogenous β-glucosidase to the aglycone. Thus, experimental factors significantly influence the hydrolysis that produces lawsone.



3.1 Reflux Extraction

Effect of Temperature on Extraction

Temperature was found to be a critical factor affecting the efficiency of lawsone extraction. Figure 1 illustrates the effectof extraction temperature (investigated at 25 °C, 40 °C, 50 °C, 60 °C, and 70 °C for a 4 hour extraction in 0.07 M acid) on the yield of lawsone.

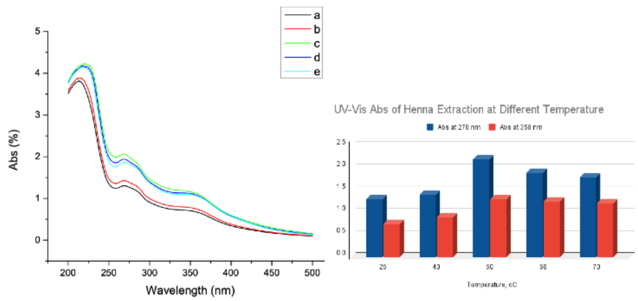


Figure 1. UV Vis spectra for the impact of temperature on the extraction process of Henna during reflux: a. 25 °C, b. 40 °C, c. 50 °C, d. 60 °C, and e. 70 °C.

As the temperature increased from ambient (~25 °C) to 50 °C, the intensity of the peaks at 270 nm and 380-420 nm, corresponding to polyphenol and lawsone, respectively, increased. Warmer temperatures generally enhance the diffusion of solvent into plant material and the solubility of many organic compounds, which can accelerate the release of polyphenol and lawsone from henna leaves. However, a further increase to 70 °C led to a decrease in polyphenol and lawsone yield. This decline at higher temperature is attributed to the thermal instability of lawsone and related compounds. Lawsone can undergo degradation or oxidative reactions when exposed to heat for prolonged periods. Bennaceur et al. observed an inverse relationship between drying temperature and lawsone content, indicating that excessive heat can destroy some of the pigment (Bennaceur, 2021). The optimal temperature of 50 °C strikes a balance: it is sufficiently high to improve mass transfer and solubilize the dye, yet mild enough to avoid significant thermal degradation of lawsone. This finding is in line with other reports that recommend moderate temperatures for extracting natural dyeing agents.

Effect of Extraction Time: The extraction time influences the approach to equilibrium in the process. Figure 2 shows the effect of time on lawsone yield using the solvent extraction method at 50 °C and pH 3.2. During the initial stages (up to 4 hours), polyphenol and lawsone are rapidly released from easily accessible sites (e.g., surface pigments and those in broken cells). As extraction time increases, the solvent penetrates deeper into the plant matrix, extracting additional lawsone. By 5 hours, no significant improvement in yield was observed, suggesting that equilibrium or the complete extraction of readily available lawsone was reached by this point. Extending the extraction time beyond 5 hours did not result in a higher lawsone content. While a longer extraction time may extract more polyphenols, some of the lawsone undergoes degradation. Further extension of the extraction time could potentially lead to a slight decrease in yield if lawsone decomposes slowly or if prolonged heating induces side reactions. No significant decrease in yield was observed, even at 9 hours, aside from the lack of additional gains.

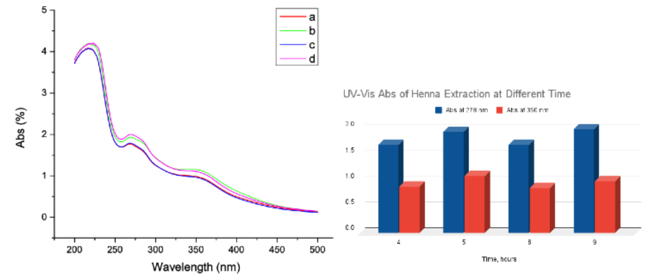


Figure 2. UV Vis spectra for the impact of time on the extraction process of Henna during reflux with a. 4h, b. 5h, c. 6h, d. 9h

Effect of Acidity (Citric Acid Concentration): The addition of a mild acid had a pronounced effect on lawsone extraction. Figure 3 summarizes the yields obtained at varying citric acid concentrations (tested in the range of pH 3-4) for a 5 h extraction at 50 °C.

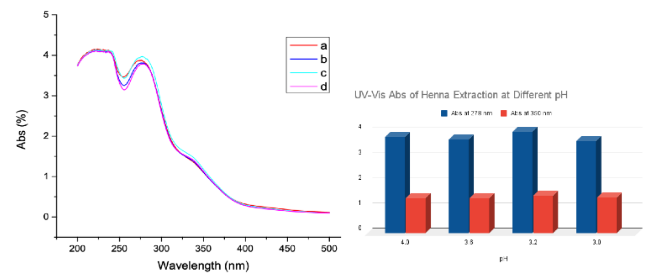


Figure 3. UV Vis spectra for the impact of acidity on the extraction process of Henna during reflux with pH 4, pH 3.6, pH 3.2, pH 3.0

Introducing a small amount of citric acid to adjust the pH into the mildly acidic range significantly enhanced the extraction yield. The optimal pH was found to be around 3.2, where the absorbance at both 278 nm and 350 nm reached their highest values. This corresponds to the maximum release of polyphenolic compounds and lawsone, as indicated by the increased absorbance at these diagnostic wavelengths. Further acidification beyond pH 3.2 did not yield further improvement. There are several reasons why a mild acid environment boosts the extraction of lawsone: (i) as mentioned earlier, henna leaves contain lawsone precursors (hennosides) that require acidic hydrolysis to break down into free lawsone. A low pH helps cleave the glycosidic bonds, releasing the aglycone form of lawsone which can then diffuse out of the plant cells. (ii) Acid may help to break down or soften the plant cell walls and associated pectins/cellulose, making it easier for the solvent to penetrate and for compounds to leach out. (iii) Lawsone itself is a weak acid (with a phenolic –OH); in neutral or basic conditions it might form salts or bind to other components, whereas in acidic conditions it remains in a neutral form that may partition more readily into an organic phase, which could favor its interaction with the relatively non-polar interior of cell membranes, aiding its release. Thus, controlling the pH is an effective strategy to optimize extraction yield.

Optimization and Synergy of Factors: Through the above investigations, the conditions identified as optimal for extracting lawsone in Reflux system were temperature ~50 °C, extraction time ~5 h, and citric acid with pH 3.2. These optimal conditions were then applied to each of the other extraction techniques (Soxhlet and Ultrasonic) in comparative experiments to evaluate their performance differences. It is worth mentioning that these factors can have interactive effects but for consistency in comparing methods, we used the same baseline conditions for all methods and leveraged the inherent differences of the methods themselves as the variable.

3.2. Ultrasonic-Assisted Extraction

Building on the reflux results, we evaluated ultrasound-assisted extraction (UAE) at 50 °C under acidic for 15, 30, 60, and 90 min (UV–Vis data in Fig. 4). UAE shortened the time to reach comparable dye release: spectra after 60 and 90 min were essentially superimposable at the diagnostic bands for polyphenols and lawsone, indicating near-plateau recovery. This time-efficiency is consistent with acoustic cavitation, which disrupts cell walls and accelerates mass transfer at mild temperatures, limiting thermal/oxidative loss relative to conventional heating (Hiranpradith, 2025). The absorbance peaks at 278 nm and 350 nm suggest the extraction of polyphenolic compounds and lawsone. The absorbance observed at 350 nm further supports the lawsone absorption band which is consistent with the deprotonation of lawsone and other related compounds (Monroy-Cárdenas, 2021). Overall, UAE at 50 °C provides an efficient route to henna extracts with high apparent lawsone signal while reducing process time versus reflux, in agreement with prior studies on henna and with general UAE advantages for botanicals (Bennaceur, 2021). Moreover, UAE is considered a non-thermal or low-thermal technique, so it avoids the prolonged high-temperature exposure that might degrade sensitive compounds (Abidin, 2020)

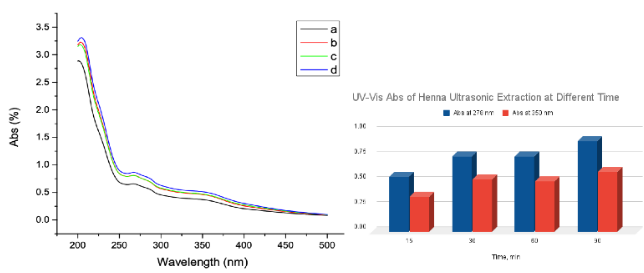


Figure 4. UV Vis spectra for the impact of time on the extraction process of Henna during Ultrasonic: a. 15 min, b. 30 min, c. 60 min, and d. 90 min

3.3. Soxhlet Extraction

The Soxhlet extraction results in Figure 5 correspond to extraction times of 1.5 h and 5 h. Extending the extraction time increased the extract yield. However, no UV absorption band at 350–420 nm—characteristic of lawsone—was detected, indicating that conversion to lawsone was not observed. This outcome may reflect: (i) operation at the boiling temperature of water, where lawsone can decompose at elevated temperatures (Bennaceur, 2021). In contrast, the reflux extraction at 50 °C and the ultrasonic extraction at 50 °C avoided that harsh condition. (ii) Limited Soxhlet efficiency because water evaporates and condenses more slowly than volatile organic solvents, thereby reducing the number of effective reflux cycles. This could lead to incomplete extraction. The combination of thermal and mass-transfer limitations likely explains the lower performance of Soxhlet here. We infer that while Soxhlet is a powerful method with organic solvents, its application with water at atmospheric boiling conditions is suboptimal for lawsone.

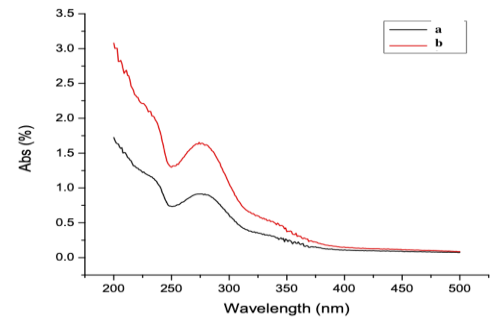


Figure 5. UV Vis spectra for the impact of time on the extraction process of Henna during Soxhlet: a. 1.5h, b. 5h

In summary, The UV–Vis spectra of the three henna extracts indicate that the extraction technique significantly influences both phenolic precursor release and lawsone formation (Figure 5). Among the methods, UAE exhibits the highest absorbance, followed by reflux, with Soxhlet showing a much lower signal. In the 250–300 nm region, associated with aromatic polyphenols and precursors (Syafaatullah, 2023), UAE shows the strongest peak, suggesting efficient mobilization of phenolic compounds. The reflux sample also shows a pronounced peak but with lower intensity, indicating that prolonged heating enhances diffusion while inducing side reactions. The Soxhlet sample, however, demonstrates a weak signal, implying limited phenolic extraction.

In the 380–420 nm region, characteristic of lawsone (Lew, 2022), the UAE sample shows a clear visible band, while reflux presents a weaker band, and Soxhlet approaches baseline, indicating that only UAE and reflux enable conversion of precursors to lawsone. UAE's effectiveness is attributed to acoustic cavitation, which disrupts cell walls and enhances mass transfer without excessive heat, preserving the lawsone band. In contrast, reflux facilitates precursor hydrolysis and oxidation, but prolonged heating promotes unwanted reactions, reducing lawsone absorbance. Aqueous Soxhlet, operating at ~100 °C with slow cycles, favors degradation and secondary reactions, leading to a weak lawsone signal.

The UAE and reflux samples exhibit similar spectral shapes, indicating a common mechanism of phenolic release followed by oxidation to lawsone, with UAE achieving higher efficiency in a shorter time. The weak Soxhlet signal suggests that lawsone is either degraded or its conversion is suppressed under these conditions. Overall, the effectiveness of extraction methods for recovering both precursors and lawsone follows the order: UAE ≥ reflux ≫ Soxhlet. UAE provides the best balance of mass-transfer enhancement and chromophore stability, while reflux can be effective if carefully timed or assisted by controlled acidity. Aqueous Soxhlet is less effective due to its thermal conditions, which hinder lawsone formation and stability. Thus, maximizing about 278nm band and protecting the 350 nm chromophore from thermal/oxidative loss are key to optimizing the extraction process, with UAE offering the superior outcome.

4. Conclusions

This study demonstrated an effective approach to extract the natural dye compound lawsone from Lawsonia inermis (henna) leaf powder using environmentally friendly methods. Among the parameters investigated, a moderate extraction temperature (around 50 °C), sufficient time (approximately 5 hours), and mild acidity (citric acid pH 3.2) were identified as optimal for maximizing lawsone yield in an aqueous extraction medium. Under these optimized conditions, a comparison of three extraction techniques yielded the following conclusions:

- Ultrasonic-Assisted Extraction is the most efficient method for isolating lawsone. The use of power ultrasound significantly enhanced the release of lawsone, achieving the highest extraction yield. Ultrasonication accelerated the extraction process and allowed for nearly complete recovery of lawsone from the henna powder while operating at a relatively low temperature.
- Conventional Solvent Reflux Extraction under optimized conditions also proved capable of extracting a large fraction of lawsone, albeit at a slower rate than UAE. By the 5-hour mark, the yield from the hot solvent extraction was close to that of the ultrasonic method, affirming that given enough time and proper conditions, a simple heated water extraction can be effective.
- Soxhlet Extraction with water, in its basic implementation, was the least effective of the methods tested. The prolonged high-temperature exposure (boiling water at ~100 °C for 5 h) likely led to partial degradation of lawsone and did not extract the dye as completely as the other methods, presumably due to a combination of thermal loss and less mechanical agitation.

In conclusion, the research findings support the use of ultrasonic-assisted extraction in a mildly acidic aqueous medium as an optimal strategy for obtaining lawsone from henna leaves. This method is not only effective in yield but also aligns with sustainable and safe processing (using only water and food-grade acid). The work contributes valuable insights for the production of natural hair dyes and other henna-based products, demonstrating that it is feasible to replace or augment traditional extraction methods with more efficient, green technologies

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