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Original Article

Semi-Industrial Microwave-Assisted Extraction of an Alizarin-Rich Extract from Rubia tinctorum Roots: Process Development and UV–Vis/HPLC Characterization

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Abstract

Introduction: This study evaluates a semi-industrial microwave-assisted extraction (MAE) system for recovering an alizarin-rich extract from Rubia tinctorum roots.

Methods: A semi-industrial microwave extractor (IRAD-MEEO-140109) was used with ethanol/water (3:7 v/v). To minimize thermal degradation risk, controlled irradiation cycles and temperature (up to 60 °C) were applied. Optical properties were evaluated by UV–Vis, and relative purity was assessed by HPLC-UV against a Merck standard.

Results: The extraction showed good reproducibility (n=3, RSD < 10% for yield and peak height). From 400 g of plant material, 76 g of dried extract was obtained (yield 19% w/w). Alizarin content and absolute recovery were not determined. UV–Vis measurements were performed once per concentration (n=1), while extraction and HPLC were triplicate (n=3). UV–Vis showed peaks at ~330 and 420 nm. HPLC showed a major peak assigned to alizarin (t_R=2.9 min) with ~75% relative chromatographic area purity. Total energy consumption was estimated at 0.48 kWh (1.2 kWh/kg). A theoretical comparison with reflux extraction suggests lower energy demand; however, this is approximate and not experimentally validated.

Conclusion: This study provides a process development framework for semi-industrial MAE of alizarin, serving as a basis for future studies rather than a demonstration of therapeutic or industrial applicability.

Keywords: Alizarin; Rubia tinctorum; Microwave-assisted extraction; Semi-industrial extraction; HPLC characterization; Anthraquinones.

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Introduction

Efficient extraction of bioactive compounds from plant matrices is fundamental to developing modern pharmaceuticals and isolating potent therapeutic agents (1, 2). Plants remain indispensable biological reservoirs for discovering biomolecules and formulating therapeutic drugs (3). Among them, Rubia tinctorum (Indian Madder), a member of the Rubiaceae family, is recognized as a cornerstone of traditional pharmacopeias. Known in Iran as Majeshta and Manjeeth Iraani, it is highly regarded for its extensive medicinal properties (4, 5). Over 60 species of this genus have been identified globally, with R. tinctorum in Western Asia and the Mediterranean being particularly prominent due to its high concentration of therapeutic metabolites (6).

The roots of Rubia tinctorum contain a rich array of biologically active constituents, including iridoid glycosides, naphthoic acid regulators, and triterpenes. Most significantly, it contains anthraquinones, specifically alizarin and purpurin, which are the primary drivers of the plant's pharmacological efficacy (3, 6-10). These anthraquinone derivatives have been reported to exhibit a wide spectrum of pharmacological activities, including antibacterial, anticancer, antioxidant, and wound-healing effects (11, 12). Beyond their role as natural pigments, these compounds have attracted increasing attention in pharmaceutical research (13, 14). However, it should be emphasized that none of these biological activities were experimentally evaluated in the present study. These reports are cited only to highlight the potential pharmacological relevance of alizarin and to justify interest in its efficient extraction.

Recent pharmacological investigations have reported the potential of alizarin and purpurin in managing complex metabolic disorders. Purpurin has been suggested to inhibit adipocyte differentiation, reduce fat accumulation, and enhance AMPK activation in experimental models (15). Alizarin has also been reported to improve insulin resistance and lower blood glucose levels by upregulating GLUT4 and IRS-1 expression (16). Other studies have indicated that alizarin and purpurin may inhibit insulin fibril formation (17). Purpurin has been studied for its protective effects on liver damage in diabetic and non-diabetic rodents (18). Rubia tinctorum root extracts have been reported to be effective in managing type II diabetes mellitus (19). It should be noted that reference (20) concerns compounds obtained from pomelo peel rather than Rubia tinctorum, and is cited here only as a general example of anthraquinone-related antidiabetic activity. Purpurin has also been studied for its effects on diabetic nephropathy (21). It must be stressed that these findings are from previously published studies and were not evaluated in the present work; they are mentioned solely to provide context for the pharmacological importance of alizarin.

Despite their reported pharmacological potential,

the clinical utility of these compounds depends heavily on the efficiency and purity of the extraction method. Conventional techniques often require long extraction times and use excessive solvent, which can degrade sensitive bioactive molecules (6). To achieve a high alizarin-rich yield suitable for further pharmaceutical evaluation, researchers have explored various advanced methods, including solid-liquid extraction with organic solvents such as methanol, chloroform, or tetrahydrofuran (22-24) and microwave-assisted extraction (25, 26). It should be noted that reference 23 concerns alizarin extraction from *Morinda citrifolia* rather than Rubia tinctorum, and is cited here only as a general example of alizarin extraction from plant matrices.

Microwave-Assisted Extraction (MAE) is particularly advantageous for pharmaceutical applications because it uses dielectric heating to rapidly rupture plant cell walls, enabling faster diffusion and higher recovery of bioactive compounds (25, 26).

While laboratory-scale MAE is well documented, transitioning to semi-industrial scales is essential to provide the quantities required for drug formulation and clinical trials. However, it presents challenges in power distribution and thermal management.

Among the reported methods, the validated HPLC method of Derksen et al. for quantifying alizarin and other anthraquinones in Rubia tinctorum (24) and the microwave-assisted extraction study of Dabiri et al. for alizarin and purpurin (26) are particularly relevant to the present work.

It should be noted that the present study does not evaluate any pharmacological or therapeutic activity; it is limited to extraction process development and chemical characterization of the recovered alizarin. Although microwave-assisted extraction (MAE) of alizarin has been reported previously, the novelty of the present study lies in the following specific features: (i) the use of a semi-industrial microwave system with a 30 L reactor, (ii) the processing of a relatively large amount of plant material (400 g), which is considerably higher than typical laboratory-scale studies, (iii) the implementation of a pulsed irradiation strategy to control temperature and minimize the risk of thermal degradation, (iv) the use of a PT100 sensor for precise temperature control (maximum 60 °C), and (v) the evaluation of process reproducibility through three independent replicates (n=3). This study does not claim industrial production or absolute purity; rather, it provides a process development and characterization framework that may serve as a basis for future studies. In this study, a semi-industrial microwave-assisted system was employed for the extraction of an alizarin-rich extract. The total dried-extract yield, the relative chromatographic area purity of alizarin, and the reproducibility of the process were evaluated. The findings should be considered as a basis for future studies rather

than as a demonstration of industrial applicability.

Materials and Methods

Materials

Ground Rubia tinctorum powder (400 g, particle size 0.3–0.5 mm) was used as the raw material. The plant material was taxonomically identified and authenticated by a botanist, and a voucher specimen was deposited in the Herbarium of Shahid Sadoughi University of Medical Sciences under the accession number Rubia tinctorum SSU0145. The extraction was performed with a total solvent volume of 4 L of ethanol/water (3:7, v/v), corresponding to a solid-to-liquid ratio of 1:10 (g/mL). For validation, a high-purity alizarin standard (Merck, Cat. No. 106278, stated purity ≥98%) was used as the reference for both UV–Vis and HPLC analyses. The standard was stored according to the manufacturer's instructions and used without further purification.

Methods

Microwave extractor

The extraction was performed using the IRAD-MEEO-140109 semi-industrial microwave unit. The system's key technical specifications include:

- Power: Maximum output of 4800 W.

- Reactor: A 30 L borosilicate glass slurry tank, designed for high thermal resistance and transparency to microwave radiation.

Agitation: An acid-resistant stainless-steel agitator (blade diameter 0.25 m) operating at 30 RPM to ensure thermal homogeneity and minimize

the risk of localized overheating.

- Temperature Control: Monitoring via a PT100 sensor to maintain a maximum threshold of 60 °C.

- Extraction time: Total irradiation time of 6 min, consisting of 3 irradiation cycles (2 min each) with 30 s pauses between cycles.

- Actual operating power: 4800 W (maximum output).

- Temperature profile: The temperature was increased to a maximum of 60 °C and maintained at this level throughout the irradiation cycles.

- pH: The ethanol/water (3:7, v/v) solvent mixture had a pH of 6.8 (without adjustment).

- Number of independent experiments: The extraction was performed in three independent replicates (n=3) under identical conditions. Results are presented as mean ± standard deviation (SD), and the relative standard deviation (RSD%) was calculated for the evaluated parameters.

The system operates in a multimode configuration, allowing for a more uniform distribution of the electromagnetic field within the slurry tank. The process involved a two-step solvent loading to ensure complete wetting of

the powder and remove residual particles from the reactor walls.

As illustrated in Figure 1, the designed system operates in a multimode microwave configuration. The contents are fed into the glass slurry tank through the inlet pipe.

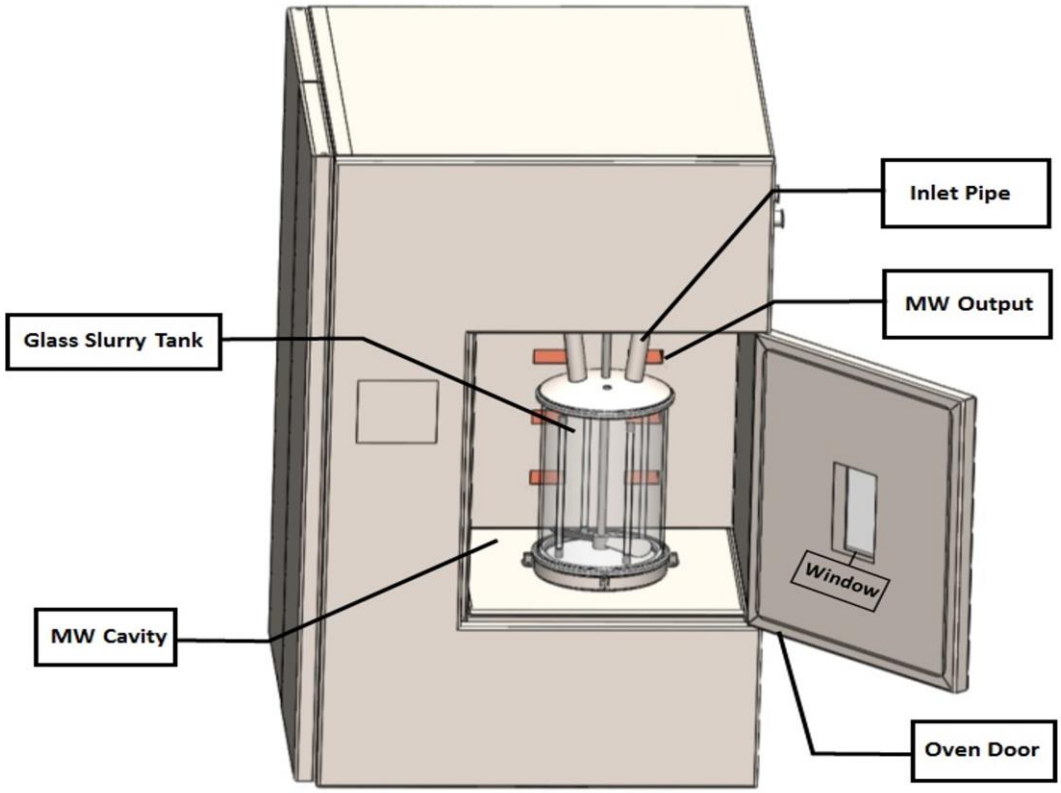


Figure 1. Schematic representation of the semi-industrial microwave extractor (IRAD-MEEO-140109).

The tank has a maximum capacity of 30 L. After loading the materials into the glass slurry tank, the microwave oven door is closed, and the irradiation process begins inside the microwave cavity to extract the compounds.

The temperature of the tank contents is monitored using a PT100 sensor. During loading, the microwave radiation is absorbed through the outer walls and across the mixture surface, initiating heating and surface evaporation. Once the agitator is activated, the slurry within the glass slurry tank is circulated continuously, ensuring a uniform temperature distribution throughout the system.

HPLC analysis

The chemical identity and relative purity of the extracted alizarin were evaluated using a reversed-phase HPLC system equipped with a

UV detector. Separation was achieved on a C18 column (25 cm × 4.6 mm, 5 µm particle size) under isocratic conditions using a mobile phase consisting of methanol/phosphate buffer (pH 3, 50 mM KH2PO4) at a ratio of 70:30 (v/v). The flow rate was 1 mL/min, the injection volume was 20 µL, and the column temperature was maintained at 25 °C. Detection was performed at 254 nm, and the total run time was 6 min. The detection wavelength of 254 nm was selected because alizarin exhibits strong UV absorption in the 250–280 nm range due to π→π* transitions of the aromatic ring system, in addition to its visible absorption bands at approximately 330 and 420 nm. This wavelength provides higher sensitivity and a lower detection limit for alizarin and related anthraquinones, and is consistent with previously validated HPLC-UV methods for Rubia tinctorum (24). The Merck alizarin standard (Cat. No. 106278) was prepared in methanol at 100 µg/mL, and the alizarin-rich extract was dissolved in the same solvent. Both samples were filtered through a 0.45 µm filter before injection.

Results

Physical Observations and Product Recovery

The extraction process began by loading half of the hydroalcoholic solvent into the borosilicate glass slurry tank, followed by adding Rubia tinctorum powder. The agitator was activated to ensure the initial dispersion of the powder. Subsequently, the remaining solvent was added; the mechanical action of the rotating blades effectively cleaned any residual powder from the tank walls, ensuring a homogeneous mixture and maximizing the contact area between the solvent and the plant matrix.

To mitigate the risk of thermal degradation and avoid "hot spots" caused by microwave-induced thermal shock, a pulsed irradiation strategy was implemented. The microwave extractor was operated at maximum power (4800 W) for 3 irradiation cycles of 2 min each, followed by a 30 s pause between cycles (total irradiation time: 6 min). This intermittent cycle allowed for the dissipation of excess heat and enabled visual monitoring of the slurry's consistency. The temperature was strictly maintained at a maximum of 60 °C to minimize the risk of thermal degradation. However, it should be noted that no stability testing or investigation of degradation products was performed in the present study. Therefore, the absence of thermal degradation cannot be definitively confirmed. The extraction was performed in three independent replicates (n=3) under identical conditions. Results are presented as mean ± standard deviation (SD), and the relative standard deviation (RSD%) was calculated for the evaluated parameters.

After irradiation, the crude extract was discharged from the microwave cavity (Figure 2a). The crude extract was purified in three sequential steps. First, the crude extract was gravity-filtered at room temperature

(approximately 27 °C) using a Whatman 0.45 µm filter to remove botanical debris and insoluble particles. Second, the filter cake was washed with distilled water to recover residual product. Third, the filtered extract was dried in a dark room at 27 °C for 24 hours to minimize photodegradation. The sample thickness during drying was approximately 3 mm. The drying endpoint was determined after 24 hours by visual observation of complete dryness and constant weight.

The final recovered product appeared as distinct, dark red particles (Figure 2b), as further evidenced in the collected sample shown in Figure 3 the physical appearance of these particles suggests a successful transition from a liquid extract to a stable, dried solid form, suitable for storage and further processing.



Figure 2a. Irradiated Rubia tinctorum product in ethanol/water solvent (3:7 v/v).



Figure 2b. Residual alizarin particles after filtration and drying in the hood for 24 hours.

As shown in Figure 2b, the alizarin particles are clearly visible. The remaining product was easily detached from the container and collected.



Figure 3. Extracted alizarin sample from Rubia tinctorum after drying.

Figure 3 shows the product collected from the container illustrated in Figure 2b. A portion of this sample was subsequently used for UV-Vis spectroscopic analysis

Product Recovery

The quantitative performance of the semi-industrial MAE process was evaluated. From 400 g of dried Rubia tinctorum powder and 4 L of ethanol/water (3:7, v/v), 76 g of dried extract was obtained, corresponding to a total dried-extract yield of 19% (w/w). It should be emphasized that this value represents the total dried-extract yield and not the alizarin recovery. The alizarin content in the dried extract and the absolute alizarin recovery from the plant material were not determined in the present study. The alizarin content in the dried extract could not be quantified because a calibrated HPLC method with an authentic standard was not available at the time of analysis. However, HPLC analysis of the alizarin-rich extract showed a single major peak assigned to alizarin, with a relative chromatographic area purity of approximately 75%. Absolute quantification of alizarin recovery (mg/g dry plant) therefore requires a preliminary linearity assessment curve, which is acknowledged as a limitation of the present study and is recommended for future work.

Table 1. Total dried-extract yield from Rubia tinctorum roots using the semi-industrial MAE system.

Parameter	Value
Mass of plant material (g)	400
Total solvent volume (L)	4
Solid-to-liquid ratio (g/mL)	01:10
Mass of dried extract (g)	76
Total dried-extract yield (%)	19
Alizarin content in dried extract	Not quantified (see text)
Alizarin recovery	Not determined (see text)

The reproducibility of the semi-industrial MAE process was evaluated by performing three independent extractions under identical conditions (n=3). The total dried-extract yields were 19.0%, 18.0%, and 20.25% (w/w), with a mean value of 19.1±1.1% (RSD=5.9%). The alizarin peak heights, determined by HPLC, were 0.74, 0.66, and 0.71 AU, with a mean of 0.70±0.04 AU (RSD=5.7%). The retention time of alizarin was consistent across all three replicates (2.90, 2.95, and 2.95 min; mean=2.93 min; RSD=0%). These results demonstrate good reproducibility of the extraction process and the HPLC procedure, with RSD values below 10% for all evaluated parameters. However, it should be emphasized that these reproducibility data refer to the extraction and HPLC procedures only, and not to the UV–Vis measurements, which were performed once per concentration (n=1).

Table 2. Reproducibility of the semi-industrial MAE process (n=3).

Parameter	n=1	n=2	n=3	Mean ± SD	RSD (%)
Mass of dried extract (g)	76	72	81	76.3 ± 4.5	5.9
Total dried-extract yield (%)	19.0	18.0	20.25	19.1 ± 1.1	5.9

Alizarin retention time (min)	2.90	2.95	2.95	2.93 ± 0.03	1.0
Alizarin peak height (AU)	0.74	0.66	0.71	0.70 ± 0.04	5.7

Spectroscopic Analysis and Optical Characterization

UV-Vis spectroscopic analysis

To evaluate the optical properties and spectral characteristics of the extracted alizarin, UV–Vis spectroscopic analysis was conducted. The relative purity of alizarin was assessed separately by HPLC-UV analysis (Section 3-2-3). A series of four successive dilutions were prepared in distilled water (final concentrations: 0.04, 0.08, 0.12, and 0.20 mg/mL). The final solutions had a pH of approximately 6.8–7.0 (without adjustment). All samples were visually inspected before measurement, and no precipitation, turbidity, or scattering was observed. The solutions remained clear and homogeneous throughout the analysis.

UV–Vis spectra were recorded using a double-beam spectrophotometer (wavelength range: 200–1100 nm) equipped with a 1 cm path length quartz cuvette. The scan speed was 600 nm/min, the spectral bandwidth was 2.0 nm, and the wavelength accuracy was ±0.5 nm. Distilled water was used as the blank, and baseline correction was performed before each measurement. The recorded spectra covered 300–700 nm.

It should be emphasized that each dilution was measured only once (n=1) for UV–Vis analysis; therefore, no statistical measures of variability are reported for the UV–Vis measurements, and the UV–Vis method should not be considered fully validated. In contrast, the extraction process and the HPLC analysis were performed in three independent replicates (n=3), and reproducibility data (RSD values below 10%) are reported in Section 3-1 and Table 2. These two aspects (process reproducibility and UV–Vis method validation) are distinct and should not be interpreted interchangeably.

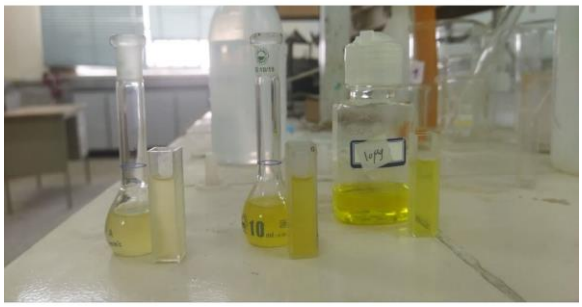


Figure 4. Alizarin extracted from Rubia tinctorum with four dilutions.

Figure 4 shows the extracted alizarin sample from *Rubia tinctorum* after four successive dilutions in distilled water. The spectroscopic data obtained from these samples are presented in Figure 5.

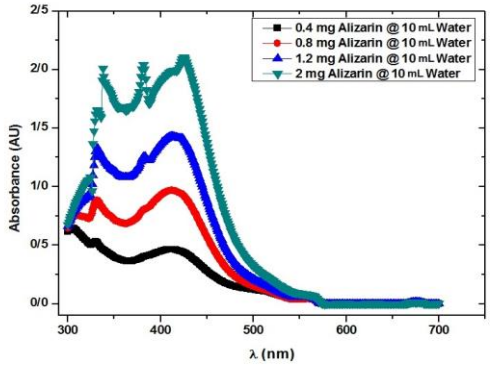


Figure 5. Absorption spectrum of alizarin extracted from Rubia tinctorum using the microwave-assisted extractor for various dilutions.

The absorption spectra in Figure 5 show a characteristic profile with two prominent absorption bands. The primary band, observed in the 330–340 nm range, is consistent with π→π* electronic transitions within the conjugated anthraquinone ring system, as reported for similar hydroxyanthraquinones (13, 27, 28). The secondary band, located between 420 and 440 nm, can be assigned to n→π* transitions and charge-transfer interactions involving the phenolic hydroxyl groups, which are sensitive to the degree of ionization and the polarity of the medium (8-10). It should be noted that the exact position and intensity of these bands are influenced by both structural electronic transitions and solvent/pH effects (22, 23, 26), as further discussed in Section 3-3. Therefore, the assignments presented here are tentative and based on literature comparison, and more advanced computational studies (e.g., TD-DFT) would be required for definitive assignment.

A critical observation in Figure 5is the stability of the peak positions across different concentrations. As the concentration increases from 0.4 to 2 mg/10 mL, the absorbance intensity increases linearly without any bathochromic or hypsochromic shifts. The linear relationship between absorbance and concentration demonstrates linearity within the tested concentration range (0.04–0.20 mg/mL). This linearity is consistent with the Beer–Lambert relationship under the tested conditions, but it does not, by itself, fully validate the Beer–Lambert law for this system. Since the concentration is expressed in mass units (mg/mL), the relationship can be expressed as $A=a\cdot b\cdot c$, where A is absorbance, a is the mass absorptivity coefficient ($mL\cdot mg^{-1}\cdot cm^{-1}$), b is the path length (cm). C is the concentration (mg/mL). The detailed calibration curve and its regression parameters are presented in Section 3-2-2 and Figure 6. This suggests that the solution remains homogeneous and that the alizarin molecules do not undergo significant aggregation or chemical transformation within this range. However, replicate measurements were not performed in the present study, and further validation is recommended.

Furthermore, the increased intensity of the 420 nm peak at higher concentrations may suggest the onset of weak intermolecular interactions, such as π–π stacking or hydrogen-bonded dimer formation. However, it should be emphasized that no controlled experiment was performed using a pure alizarin standard at the same

concentration in different solvents. Therefore, the observed spectral changes cannot be definitively attributed to solvent effects alone, as differences in concentration, pH, and extract-matrix composition may also influence the absorption spectrum. These observations are presented as a tentative interpretation only.

Calibration curve and preliminary linearity assessment

To evaluate the linearity of the UV–Vis response, a calibration curve was constructed using four concentrations of the alizarin-rich extract (0.04, 0.08, 0.12, and 0.20 mg/mL) in distilled water. The absorbance was measured at 420 nm, which corresponds to the main absorption band of alizarin. The calibration curve is presented in Figure 6. The linear regression equation was y=9.66x + 0.14, with an R^2 value of 0.983. The linear range was 0.04–0.20 mg/mL. The regression line showed a linear relationship between absorbance and concentration over the tested range.

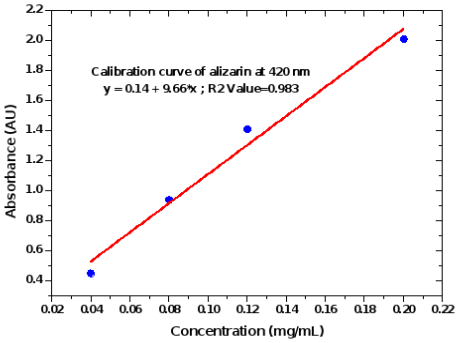


Figure 6. Calibration curve of the alizarin-rich extract at 420 nm.

It should be noted that each concentration was measured once (n=1); therefore, this should be considered a preliminary linearity assessment rather than a fully validated analytical calibration. This calibration curve was used only for the preliminary linearity assessment and was not used to determine the actual alizarin content or its absolute purity. The Beer-Lambert relationship for this system can be expressed as A=a·b·c, where A is absorbance, a is the mass absorptivity coefficient ($mL \cdot mg^{-1} \cdot cm^{-1}$), b is the path length (cm), and c is the concentration (mg/mL). Since the concentration is expressed in mass units (mg/mL), the mass absorptivity coefficient (a) is used instead of the molar absorptivity (ε). A fully validated analytical calibration with replicate measurements (n≥3), recovery, accuracy, precision, and LOD/LOQ data is recommended for future studies.

Table 3. Preliminary concentration–absorbance data for the alizarin-rich extract at 420 nm.

Concentration (mg/mL)	Absorbance (AU)
0.04	0.45
0.08	0.94
0.12	1.41
0.20	2.01

HPLC analysis

Following the purification step, HPLC analysis of the extracted sample showed a single major peak at t_R=2.9 min, which is close to the retention time of the Merck alizarin standard (t_R=2.6 min). The difference of 0.3 min is larger

than typical run-to-run variation and is attributed to matrix effects from the plant extract. To further support the peak assignment, the UV-Vis absorption maxima of the extracted compound (approximately 330 and 420 nm, Figure 5) were compared with the reported spectral characteristics of the Merck alizarin standard, and a high degree of consistency was observed. The peak was therefore assigned to alizarin based on (i) proximity of retention time to the Merck standard, (ii) UV–Vis spectral correlation, and (iii) comparison with literature data. It should be emphasized that definitive confirmation of the peak assigned to alizarin would require co-injection/spiking experiments, diode-array detection (DAD), or LC-MS analysis, which were not available in the present study. Minor peaks were observed at t_R=1.2, 1.4, 2.4, and 4.4 min, indicating trace amounts of co-extracted compounds. The peak areas were calculated from the raw chromatographic data using the trapezoidal rule. The relative chromatographic area purity of alizarin was determined by dividing the alizarin peak area by the total integrated peak area of the major detected compounds. The purity was calculated as approximately 75% (mean of three independent replicates, n=3). All major peaks detected in the chromatogram were integrated and included in the calculation. It should be emphasized that this value represents relative chromatographic area purity and not absolute chemical purity or assay value. Absolute purity determination would require a fully validated HPLC method with calibration, recovery, accuracy, precision, and LOD/LOQ data, which is recommended for future studies.

Discussion

Comparative Analysis

The spectral characteristics of the extracted compound (Figure 5) were compared with the reported absorption bands of the Merck alizarin standard. The observed maxima and spectral profile were consistent, supporting the assignment of the extracted pigment as an alizarin-rich extract that retains alizarin's fundamental electronic structure and optical properties. However, it should be noted that UV–Vis spectroscopy alone cannot definitively confirm the absence of other anthraquinones, since compounds may be present at concentrations below the UV–Vis detection limit or may exhibit overlapping absorption bands. Typical natural extracts often show additional absorption peaks in the 500–550 nm region due to other anthraquinones, such as purpurin and munjistin. In the present study, no major additional absorption bands were observed in the UV–Vis spectrum; however, UV–Vis spectroscopy alone cannot rule out the presence of minor co-extracted compounds, and chromatographic analysis was therefore required. It should be noted that the relative purity of alizarin was determined by HPLC-UV analysis (Section 3-2-3) and not by UV–Vis spectroscopy. It should also be noted that a controlled solvatochromic study using standard

alizarin in different solvents was not performed in the present study, and this is acknowledged as a limitation.

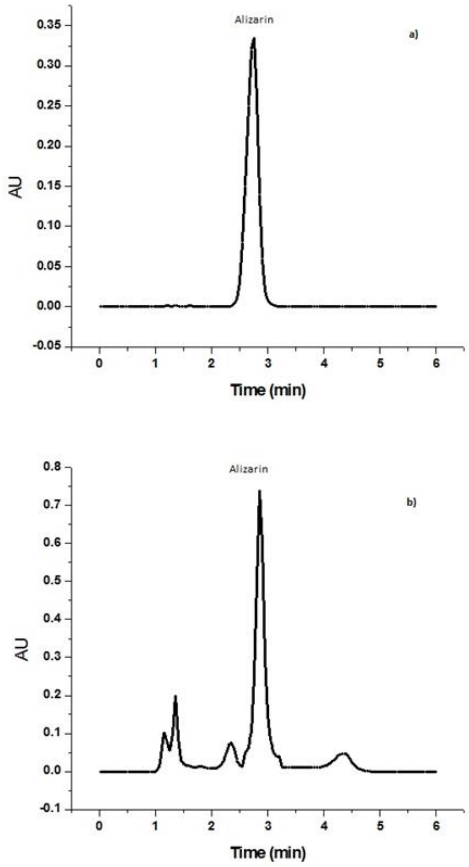


Figure 7. HPLC-UV chromatograms of (a) the Merck alizarin standard (t_R=2.6 min) and (b) the alizarin-rich extract obtained from Rubia tinctorum using the semi-industrial MAE system (peak assigned to alizarin at t_R=2.9 min). Detection was performed at 254 nm. The x-axis represents retention time (min), and the y-axis represents absorbance (AU). Panel (b) shows minor peaks corresponding to trace co-extracted compounds.

As shown in Figure 7 (HPLC analysis), the extract exhibited a single major peak assigned to alizarin (t_R=2.9 min), with only trace amounts of other compounds detected. These chromatographic results support the semi-industrial MAE process and support the assignment of the major peak as alizarin, along with its relative purity.

Overall, the combination of UV-Vis and HPLC analyses indicates that semi-industrial microwave-assisted extraction accelerates recovery and yields an alizarin-rich extract with a relative chromatographic area purity of approximately 75%. However, absolute purity and selectivity should be further confirmed by more advanced chromatographic methods (e.g., LC-MS) in future studies. HPLC analysis was performed in three independent replicates (n=3), and reproducibility data (RSD < 10%) are reported in Section 3-1 and Table 2.

Although HPLC-UV analysis showed a single major peak corresponding to alizarin with only trace amounts of other compounds, the complete absence of minor co-eluting compounds below the detection limit or unresolved under the current chromatographic conditions cannot be excluded without a fully validated method (e.g., LC-MS), which is recommended for future studies.

Energy consumption analysis

The energy consumption of the semi-industrial MAE process was calculated based on the actual operating power and total irradiation time. The microwave extractor was operated at a nominal maximum output power of 4800 W (4.8 kW) for a total irradiation time of 6 min (0.1 h). Based

on this nominal output power and irradiation time, energy consumption was estimated at 0.48 kWh. It should be noted that this value is an estimate based on the stated power and time, as the electrical input power was not directly measured. Normalized to the amount of plant material processed, this corresponds to 1.2 kWh/kg of dry plant material, or 6.3 kWh/kg of dried extract.

For comparison, the energy consumption of conventional reflux extraction was estimated based on a typical laboratory-scale setup (1 kW heating mantle, 6 h), which would consume approximately 6 kWh. This comparison is presented as an approximate theoretical estimate based on typical laboratory equipment and is not intended as an experimentally measured energy advantage. It should be emphasized that the comparison between the present MAE process and conventional reflux extraction is limited to energy consumption only. Other parameters such as total dried-extract yield, alizarin recovery, solvent consumption, and extraction time are not directly comparable across these methods, since they depend on different experimental conditions and were not measured under identical conditions in the present study. Similarly, the estimated energy consumption of Soxhlet extraction (1 kW, 16 h) would be approximately 16 kWh. These estimates suggest that the semi-industrial MAE system may substantially reduce energy consumption compared with conventional extraction methods. However, these values are approximate, depend on the specific equipment and operating conditions, and were not experimentally measured in the present study. Future studies should experimentally measure energy consumption for both methods under comparable conditions to validate this estimate.

It should be emphasized that total dried-extract yield, alizarin recovery, solvent consumption, extraction time, and energy consumption are distinct parameters that should not be treated as interchangeable measures of extraction performance. In the present study:

- The total dried-extract yield (19% w/w) represents the total mass of dried extract, not the alizarin recovery.

- The alizarin recovery from the plant material was not determined.

- The relative chromatographic area purity (approximately 75%) represents a relative measure and not an absolute purity value.

- The energy comparison with reflux extraction is a theoretical estimate and not an experimentally measured advantage.

These distinctions are essential for correctly interpreting process performance and should be considered in future comparative studies.

Safety and Toxicity Considerations

It should be emphasized that although alizarin and related anthraquinones have been reported to possess various pharmacological activities, their safety profile is not uniformly favorable.

Published studies have reported potential genotoxic and mutagenic effects for some Rubia-derived anthraquinones (29), and have also raised concerns about hepatic and renal effects at high doses (19). Therefore, these compounds require careful evaluation for biocompatibility and long-term safety. In the present study, no toxicity or biocompatibility assessment was performed, and the pharmacological relevance of alizarin is discussed solely based on previously published literature.

Conclusion

This study demonstrates the semi-industrial microwave-assisted extraction of an alizarin-rich extract from the medicinal plant Rubia tinctorum. From 400 g of dried plant material and 4 L of ethanol/water (3:7, v/v), 76 g of dried extract was obtained, corresponding to a total dried-extract yield of 19% (w/w). This value represents the total dried-extract yield and not the alizarin recovery, which was not determined in the present study. Using a multimode microwave reactor with pulsed irradiation enabled recovery of an alizarin-rich extract while minimizing thermal degradation.

This semi-industrial approach may offer advantages in processing time, solvent use, and energy consumption. However, it should be emphasized that no direct assessment of solvent consumption, waste generation, or environmental impact was performed in the present study. Therefore, these aspects should be considered as potential advantages rather than demonstrated outcomes. This extract may be considered as a potential precursor for future pharmaceutical applications; however, such applications would require additional purification, quantitative assay, safety testing, reproducibility studies, and pharmaceutical quality assessment, which were not performed in the present study. Further studies are recommended to evaluate the scalability, long-term stability, and absolute quantification of the final alizarin-rich extract.

Overall, this work provides a process development and characterization framework for semi-industrial MAE of alizarin. The findings should be considered as a basis for future studies rather than a demonstration of therapeutic or industrial applicability.

Limitations

The present study has several limitations that should be acknowledged.

First, absolute quantification of alizarin recovery was not possible because a validated HPLC calibration curve with an authentic standard was not available. Therefore, the alizarin content in the extract and the absolute alizarin recovery from the plant material were not determined. Future studies should include full quantitative validation.

Second, the total dried-extract yield (19% w/w) represents the total mass of dried extract and should not be interpreted as the alizarin

recovery. These two parameters are distinct and should not be treated as interchangeable measures of extraction performance.

Third, the relative chromatographic area purity of alizarin (approximately 75%) should be considered a preliminary estimate, not absolute chemical purity or a quantitatively established alizarin assay. Establishing absolute purity and the actual alizarin content would require a valid quantitative method, an appropriate reference standard, and analytical validation data, which were not available in the present study. A fully validated HPLC method with calibration, recovery, accuracy, precision, and LOD/LOQ data is recommended for future studies.

Fourth, the extraction process and the HPLC analysis were performed in three independent replicates (n=3), with RSD values below 10%. In contrast, the UV–Vis measurements were performed only once per concentration (n=1), and the calibration curve was constructed from single measurements. Therefore, the reproducibility data refer only to the extraction and HPLC procedures and should not be interpreted as a complete validation of the UV–Vis method or the overall analytical procedure. Future studies should include replicate UV–Vis measurements for full validation.

Fifth, the calibration curve was constructed from single measurements per concentration (n=1) and represents a preliminary linearity assessment rather than a fully validated analytical method. It was not used to determine the actual alizarin content or its absolute purity.

Sixth, UV–Vis spectroscopy was used in this study only for spectral characterization and investigation of the optical properties of the extracted alizarin. The relative purity of alizarin was assessed by HPLC-UV analysis (Section 3-2-3) and not by UV–Vis spectroscopy.

Seventh, the assignment of UV–Vis absorption bands were based on literature comparison and remains tentative. Definitive assignment would require TD-DFT calculations, which were not performed in the present study.

Eighth, a controlled solvatochromic study using standard alizarin at the same concentration in different solvents was not performed. Therefore, the observed spectral changes in the UV–Vis spectra are presented as a tentative interpretation and cannot be definitively attributed to solvent effects alone, as differences in concentration, pH, and extract-matrix composition may also influence the absorption spectrum.

Ninth, the difference in retention time between the Merck alizarin standard (2.6 min) and the extracted sample (2.9 min) exceeds typical run-to-run variation. Although the peak was assigned to alizarin based on (i) proximity of retention time, (ii) UV–Vis spectral correlation, and (iii) comparison with literature data, definitive confirmation of the peak assigned to alizarin would require co-injection/spiking experiments, diode-array detection (DAD), or LC-MS analysis, which were not available in the present study.

Tenth, no stability testing or investigation of degradation products was performed. Therefore, although the temperature was controlled at a maximum of 60 °C to minimize the risk of thermal degradation, the absence of thermal degradation cannot be definitively confirmed.

Eleventh, the comparison of energy consumption with conventional reflux extraction is based on theoretical estimates rather than experimental measurements. Future studies should experimentally measure energy consumption for both methods under comparable conditions to validate this estimate. Furthermore, no direct assessment of solvent consumption, waste generation, energy use, or environmental impact was performed. Therefore, the environmental advantages of the semi-industrial MAE process should be considered as potential advantages rather than demonstrated outcomes.

Finally, the long-term stability and pharmaceutical-grade quality of the product require further investigation. Further optimization of the filtration washing step is also recommended to ensure full reproducibility.

Declarations

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Author Contributions

Mohsen Askarbioki designed the study and interpreted the data. Seyed Mohammad Mosavi Hasanabad and Mahdi Eslami performed the extraction experiments. Abdolhamid Amooee supervised the work and reviewed the manuscript. Seyed Pezhman Shirmardi and Saeed Kargar conducted the spectral analysis and contributed to the writing process. All authors have read and approved the final version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest regarding the publication of this manuscript.

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Ethical Approval / Protection of Human and Animal Subjects

No human or animal subjects were involved in the present study; therefore, ethical approval was not required.

Data Availability

The data supporting the findings of this study are included within the article.

Use of Artificial Intelligence

AI language models were used solely for language editing and improving the clarity of the text. The authors take full responsibility for the content, data analysis, and conclusions of the manuscript.

References

1.Builders P. Herbal medicine: BoD–Books on Demand; 2019.

2.Bone K, Mills S. Principles and practice of phytotherapy: modern herbal medicine: Elsevier Health Sciences; 2013.

3.Deshkar N, Tilloo S, Pande V. A comprehensive review of Rubia cordifolia Linn. Pharmacognosy Reviews. 2008;2(3):124.

4.Kirtikar KR, Basu BD. Indian medicinal plants: publisher not identified Basu, Bhuwaneśwari Âśrama; 1918.

5.Khare C. Encyclopedia of Indian medicinal plants: rational western therapy, ayurvedic and other traditional usage, botany: Springer; 2004.

6.Murthy HN, Joseph KS, Paek KY, Park SY. Anthraquinone production from cell and organ cultures of Rubia species: An overview. Metabolites. 2022;13(1):39.

7.Abou Elmaaty T, Sayed-Ahmed K, Magdi M, Elsisi H. An eco-friendly method of extracting alizarin from Rubia tinctorum roots under supercritical carbon dioxide and its application to wool dyeing. Scientific reports. 2023;13(1):30.

8.Itokawa H, Mihara K, Takeya K. Studies on a novel anthraquinone and its glycosides isolated from Rubia cordifolia and R. akane. Chemical and pharmaceutical bulletin. 1983;31(7):2353-8.

9.Singh R. Isolation and synthesis of anthraquinones and related compounds of Rubia cordifolia. J Serb Chem Soc. 2005;70:937-42.

10.Gandhi M, Reegan A, Ganesan P, Sivasankaran K, Paulraj M, Balakrishna K, et al. Larvicidal and Pupicidal Activities of Alizarin Isolated from Roots of Rubia cordifolia Against Culex quinquefasciatus Say and Aedes aegypti (L.)(Diptera: Culicidae) Gandhi et al. Neotropical Entomology. 2016;45(4):441-8.

11.Xu K, Wang P, Wang L, Liu C, Xu S, Cheng Y, et al. Quinone derivatives from the genus Rubia and their bioactivities. Chemistry & biodiversity. 2014;11(3):341-63.

12.Patil R, Mohan M, Kasture V, Kasture S. Rubia cordifolia: a review. Orient Pharm Exp Med. 2009;9(1):1-13.

13.Caro Y, Anamale L, Fouillaud M, Laurent P, Petit T, Dufossé L. Natural hydroxyanthraquinoid pigments as potent food grade colorants: an overview. Natural products and bioprospecting. 2012;2(5):174-93.

14.Yusuf M, Shabbir M, Mohammad F. Natural colorants: Historical, processing and sustainable prospects. Natural products and bioprospecting. 2017;7(1):123-45.

15.Nam W, Nam SH, Kim SP, Levin C, Friedman M. Anti-adipogenic and anti-obesity activities of purpurin in 3T3-L1 preadipocyte cells and in mice fed a high-fat diet. BMC complementary and alternative medicine. 2019;19(1):364.

16.Xu L, Xing M, Xu X, Saadeldeen FS, Liu Z, Wei J, et al. Alizarin increase glucose uptake through PI3K/Akt signaling and improve alloxan-induced diabetic mice. Future Medicinal Chemistry. 2019;11(5):395-406.

17.Wang W, Zhang J, Qi W, Su R, He Z, Peng X. Alizarin and purpurin from Rubia tinctorum L. suppress insulin fibrillation and reduce the amyloid-induced cytotoxicity. ACS Chemical Neuroscience. 2021;12(12):2182-93.

18.Bedi O, Srivastava N, Parsad D, Hooda T, Lather A, Grewal AK, et al. Protective effects of pterostilbene, purpurin, and arbutin on liver damage in diabetic and non-diabetic rodents. Journal of Applied Pharmaceutical Science. 2025;15(6):295.

19.Eltamany EE, Nafie MS, Khodeer DM, El-Tanahy AH, Abdel-Kader MS, Badr JM, et al. Rubia tinctorum root extracts: chemical profile and management of type II diabetes mellitus. RSC advances. 2020;10(41):24159-68.

20.Zheng Z, Xu Y, Qu H, Zhou H, Yang H. Enhancement of anti-diabetic activity of pomelo peel by the fermentation of Aspergillus oryzae CGMCC23295: In vitro and in silico docking studies. Food Chemistry. 2024;432:137195.

21.Shang Y, Mohideen AP. Purpurin, a natural anthroquinone ameliorates streptozotocin-induced diabetic nephropathy via attenuating hyperglycemia, dyslipidemia, and inflammation in rats. Pharmacognosy Magazine. 2023;19(2):461-72.

22.Essaidi I, Snoussi A, Ben Haj Koubaier H, Casabianca H, Bouzouita N. Effect of acid hydrolysis on alizarin content, antioxidant and antimicrobial activities of Rubia tinctorum extracts. Pigment & Resin Technology. 2017;46(5):379-84.

23.Jamaludin R, Kim D-S, Salleh LM, Lim S-B. Kinetic Study of Subcritical Water Extraction of Scopoletin, Alizarin, and Rutin from *Morinda citrifolia*. Foods. 2021;10(10):2260.

24.Derksen GC, Lelyveld GP, van Beek TA, Capelle A, de Groot Æ. Two validated HPLC methods for the quantification of alizarin and other anthraquinones in Rubia tinctorum cultivars. Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques. 2004;15(6):397-406.

25.Chajii O, Chemchame Y, Zourif A, Azoubi Z, Achahboune R, Dalal M, et al. Microwave-assisted extraction of natural dye from Rubia tinctorum L. plant for dyeing wool fabrics with multiple effects. Fibers and Polymers. 2025;26(6):2427-48.

26.Dabiri M, Salimi S, Ghassempour A, Rassouli A, Talebi M. Optimization of microwave-assisted extraction for alizarin and purpurin in Rubiaceae plants and its comparison with conventional extraction methods. Journal of separation science. 2005;28(4):387-96.

27.Shan M, Yu S, Yan H, Chen P, Zhang L, Ding A. A review of the botany, phytochemistry, pharmacology and toxicology of rubiae radix et rhizoma. Molecules. 2016;21(12):1747.

28.Singh R, Geetanjali, Chauhan S. 9, 10-Anthraquinones and other biologically active compounds from the genus Rubia. Chemistry & biodiversity. 2004;1(9):1241-64.

29.Marec F, Kollárová I, Jegorov A. Mutagenicity of natural anthraquinones from Rubia tinctorum in the Drosophila wing spot test. Planta medica. 2001;67(02):127-31.