Journal of Chemical Learning Innovation
Vol. 2, No. 2, December 2025, pp. 206~217
ISSN: 3063-0886, DOI:10.37251/jocli.v2i2.2845

Quantitative Evaluation of Oxidation Time, Dilute Acid Concentration and Acid Type on Indigo Yield from Indigofera Leaves: An Ethnochemical Approach to Sustainable Batik Dyeing

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Article Info

Article history:

Received Sep 5, 2025
Revised Oct 28, 2025
Accepted Nov 27, 2025
Online First Dec 30, 2025

Keywords:

Acid Concentration
Ethnochemistry
Indigofera Natural Dye
Mordant Effect
Oxidation Time

ABSTRACT

Purpose of the study: This study aims to analyze the effect of oxidation time, dilute acid concentration, and type of acid on the indigo content produced, as well as to determine the effect of the color-covering agent (mordant) on the color quality of batik cloth using natural dyes from the indigo plant (Indigofera).

Methodology: Analytical balance (Ohaus Pioneer), Pyrex beaker glass and volumetric flask, aeration system with Resun LP-40 air pump, pH indicator strips (Merck), and UV-Vis spectrophotometer (Shimadzu UV-1800) were used. Experimental laboratory method with oxidation-time and acid-variation design was applied. Data were processed using Microsoft Excel 2019 and SPSS 25. Literature review and observational approach supported analysis.

Main Findings: Indigo content increased with longer oxidation time, reaching 23.78 ppm at 12 hours. The optimal acid concentration was 0.01 M HCl (26.88 ppm), while 0.1 M significantly reduced yield (15.77 ppm). Sulfuric acid 0.01 M produced the highest indigo level (29.20 ppm). Mordant variation affected color quality: tunjung produced darker bluish-green tones, lime produced lighter blue, and alum maintained the original blue shade.

Novelty/Originality of this study: This study integrates ethnochemical perspectives with quantitative chemical analysis by systematically examining oxidation time, dilute acid concentration, acid type, and mordant effects within a traditional Indigofera-based batik framework. It advances knowledge by scientifically validating indigenous dyeing practices while providing measurable parameters to optimize natural indigo production and improve sustainable textile applications.

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1. INTRODUCTION

Indigo is one of the oldest and most historically significant natural dyes used in textile traditions worldwide [1], [2]. In Indonesia, indigo derived from Indigofera species plays a central role in batik production, representing not only a coloring material but also a manifestation of indigenous knowledge transmitted across generations [3], [4]. Traditional dyeing practices are deeply embedded in cultural identity, yet they simultaneously involve complex biochemical and physicochemical transformations that can be scientifically explained through

modern chemical principles [5], [6]. The preparation of indigo in traditional systems reflects empirical control over reaction conditions such as soaking duration, aeration, acidification, and fixation, even though these parameters are not formally described in chemical terminology.

Unlike many plant-based dyes that exist as ready-to-use pigments, indigo does not naturally occur in its final blue form within plant tissues [7], [8]. Instead, it is synthesized during the extraction process from precursor compounds, primarily indican (indoxyl-β-D-glucoside), a colorless secondary metabolite stored in plant vacuoles [9], [10]. The formation of indigo is a multistep biochemical and oxidative process involving precursor transformation, enzymatic hydrolysis, and molecular coupling reactions [11], [12]. Indican itself is not a dye; rather, it serves as a stable precursor that requires cleavage and oxidation to generate the characteristic blue pigment.

The first critical stage in this transformation is the hydrolysis of indican into indoxyl and glucose [13], [14]. This reaction may occur enzymatically through the action of β-glucosidase or be facilitated under acidic conditions through proton-mediated cleavage of the glycosidic bond. In traditional fermentation systems, β-glucosidase can originate from plant tissues or from microbial communities that develop during leaf steeping [15], [16]. The disruption of plant cell structures during soaking enables contact between indican and the enzyme, leading to the release of indoxyl into the solution [17], [18]. Under dilute acid conditions, hydrolysis may be accelerated by increased proton availability, which destabilizes the glycosidic linkage and enhances indoxyl formation. Thus, acid concentration and acid type may directly influence the rate and efficiency of precursor conversion.

Indoxyl, once released, is chemically unstable and highly reactive. In the presence of dissolved oxygen, it undergoes spontaneous oxidative dimerization, forming indigotin (indigo), an insoluble blue pigment. This process involves radical-mediated coupling between two indoxyl molecules and is strongly influenced by oxygen availability, pH conditions, and oxidation duration [19], [20]. Insufficient aeration may result in incomplete dimerization, whereas excessive oxidative exposure can promote side reactions and reduce pigment purity [21], [22]. Therefore, oxidation time functions as a kinetic parameter governing the efficiency of indigo formation [21], [23]. The overall transformation pathway can be summarized as the sequential conversion of indican to indoxyl via enzymatic or acid-assisted hydrolysis, followed by oxygen-dependent oxidative dimerization to produce indigotin.

From an ethnochemical perspective, these transformations demonstrate that traditional indigo dyeing inherently manages hydrolysis kinetics, enzyme activity, proton concentration, and redox dynamics through empirically developed techniques [24], [25]. Indigenous practices such as prolonged soaking, controlled aeration, and selective acidification correspond directly to chemical parameters that regulate reaction efficiency [26], [27]. Ethnochemistry provides a framework for interpreting these local practices through scientific principles without detaching them from their cultural context [28], [29]. In the case of Indigofera-based batik dyeing, traditional knowledge aligns closely with acid–base chemistry, oxidation–reduction reactions, and dye–fiber interaction mechanisms.

Despite increasing global interest in natural dyes driven by sustainability concerns [7], [30], many previous studies emphasize extraction efficiency or microbial fermentation strategies without systematically evaluating acid-assisted hydrolysis within culturally embedded production systems [31], [32]. Although the biochemical pathway of indigo formation has been broadly described, the quantitative relationship between dilute mineral acid concentration, acid type, and indoxyl release efficiency remains insufficiently characterized. Furthermore, while oxidation is recognized as essential for pigment formation, limited research quantitatively investigates oxidation time as a kinetic variable influencing dimerization efficiency under controlled acid conditions [33], [34].

Another limitation in existing literature is the separation between chemical optimization and socio-cultural production frameworks. Few studies integrate mechanistic chemical analysis with ethnochemical interpretation, particularly within Indonesian batik processing systems [6], [35]. As a result, traditional acidification and mordanting practices often lack quantitative validation linking indigenous knowledge with measurable chemical parameters such as indigo concentration [36], [37]. Moreover, the combined effects of acid type (e.g., monoprotic versus diprotic strong acids), dilute concentration range, and controlled oxidation duration on indigo yield have not been comprehensively examined within a unified experimental design [24], [38].

In addition to extraction parameters, mordant application significantly influences final color expression and durability on textile fibers [39], [40]. Mordants alter dye–fiber interactions through coordination bonding, pH modification, and surface chemistry changes [41], [42]. Traditional batik artisans commonly employ lime, alum, and iron-containing compounds to modify shade intensity and fixation properties. However, systematic chemical evaluation of how these mordants affect the chromatic characteristics of Indigofera-derived indigo remains underexplored. Understanding these interactions is essential for improving reproducibility and enhancing the competitiveness of natural dyes in contemporary textile markets [43], [44].

The urgency of this research is reinforced by environmental considerations. Synthetic dye production contributes substantially to wastewater pollution and ecological degradation, whereas plant-based dyes offer

biodegradable and renewable alternatives [45], [46]. Nevertheless, for natural dyes to compete with synthetic counterparts, their extraction processes must be scientifically optimized and reproducible [47], [48]. Bridging indigenous knowledge systems with quantitative chemical validation can strengthen sustainable textile innovation while preserving cultural heritage.

Based on these considerations, this study aims to quantitatively evaluate the effects of oxidation time, dilute acid concentration, and acid type on indigo yield derived from Indigofera leaves, as well as to examine the influence of mordant agents on batik fabric color quality. By integrating reaction mechanism analysis with experimental validation within an ethnochemical framework, this research seeks to provide a scientifically grounded basis for optimizing traditional indigo dyeing practices while advancing sustainable and culturally informed textile science.

2. RESEARCH METHOD

2.1. Tools

This research utilized various laboratory equipment and supporting tools for the extraction and dyeing processes. The tools used included an analytical balance for precise weighing of materials, a beaker glass as a container for mixing and reaction processes, volumetric flasks and measuring cylinders for accurate volume measurements, and a watch glass as a weighing container or cover. A glass stirrer and spatula were used to assist the mixing and extraction process [49], [50]. A dropper was used for adding small amounts of solution. A knife and scissors were used for cutting the Indigofera plant material. A filter and filtration setup were used to separate the filtrate from the pulp. An air pump connected with a hose was used to support the aeration (oxidation) process of the dye solution. A pH indicator was used to monitor the acidity level of the solution during hydrolysis and alkalization stages [51], [52]. For quantitative analysis, a UV-Visible spectrophotometer was used to determine indigo content at its maximum absorption wavelength. A laboratory oven was used to dry the indigo precipitate until constant weight was obtained.

2.2. Materials

The materials used in this study consisted of *Indigofera* leaves as the main source of natural indigo dye [53], [54]. The chemicals used included hydrochloric acid (HCl) and sulfuric acid (H_2SO_4) as acidifying agents, lime and sodium hydroxide as alkalizing agents [55], [56], distilled water as solvent, brown sugar as a reducing agent, and alum and tunjung as mordants to improve color binding and fastness on fabric. All chemicals were of analytical grade and used without further purification.

2.3. Experimental Design

The experiment was arranged in a completely randomized factorial design consisting of Acid type: hydrochloric acid (HCl) and sulfuric acid (H_2SO_4), Acid concentration: 0.001 M, 0.01 M, and 0.1 M, Oxidation time: 4, 8, and 12 hours. Each treatment was conducted in triplicate to ensure reproducibility and statistical reliability [57], [58]. All experiments were performed under controlled laboratory conditions at ambient temperature (27–30°C). The volume of solution, mass of leaves, and aeration rate were kept constant across all treatments to minimize variability.

2.4. Tool Set for Oxidation Process

Prior to the oxidation process, a series of tools were designed and assembled to ensure optimal and controlled aeration. The oxidation system consisted of a glass beaker as a reaction vessel, a hose, and an air pump to supply continuous airflow into the solution. This setup allowed uniform oxygen distribution during oxidation, which is essential for the conversion of indoxyl into insoluble indigo (indigotin). The arrangement of the oxidation tool circuit is shown in Figure 1.

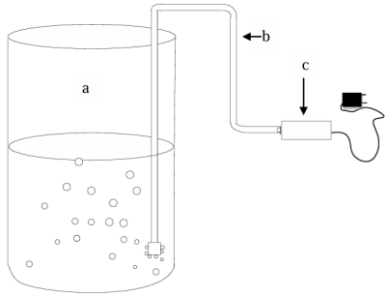

Figure 1. Oxidation Process Tool Circuit

Description:a = glass beaker b = hose c = air pump

During oxidation, airflow was maintained continuously and all treatments were carried out under the same ambient laboratory temperature (27–30°C) to ensure consistency.

2.5. Procedure

2.5.1. Variation of Hydrochloric Acid Concentration and Acid Type in Immersion Solution

Indigofera leaves were separated from the stems and washed thoroughly to remove dirt. A total of 400 grams of leaves were soaked in 1.2 L of acid solution for 24 hours at room temperature. The acid solutions used were hydrochloric acid with concentrations of 0.001 M, 0.01 M, and 0.1 M. The same procedure was also carried out using sulfuric acid at the same concentration levels as a comparison of acid type [59], [60]. After the soaking process was completed, the soaking liquid was separated from the leaf pulp through filtration. The filtrate then underwent an oxidation process by continuous aeration using an air pump for 12 hours.

Samples of 15 mL were taken at the 4th, 8th, and 12th hours during oxidation to observe the progression of indigo formation. For comparison between acid types and concentrations, final sampling was standardized at the 12th hour. After oxidation was complete, the sediment (blue precipitate) was separated from the filtrate, washed with distilled water to remove residual acid, and air-dried followed by oven drying at 60°C until constant weight was obtained. The dried precipitate was ground to obtain indigo powder. To ensure reliability of the results, each treatment was conducted in triplicate under identical experimental conditions.

2.5.2. Quantitative Determination of Indigo Content

The indigo powder obtained from each treatment was analyzed quantitatively. A known mass of indigo powder was dissolved in concentrated sulfuric acid to prepare a stock solution. The solution was then diluted appropriately for spectrophotometric analysis [61], [62]. Indigo concentration was determined using a UV-Visible spectrophotometer at a maximum wavelength of 611 nm. A calibration curve was prepared using standard indigo solutions within a defined concentration range. The indigo content of each sample was calculated based on the regression equation of the calibration curve. All measurements were performed in triplicate and expressed as mean ± standard deviation.

2.5.3. Application on Fabric

A total of 100 mL of sodium hydroxide solution was prepared and adjusted to reach pH 11. One gram of brown sugar and one gram of indigo powder were added to the solution while stirring until completely dissolved. White cotton fabric was dipped into the indigo dye solution and then air-dried to allow oxidation and color development. The cloth was then dipped into a lime solution consisting of 14 grams of lime in 200 mL of water and air-dried again.

The same procedure was carried out using alum and tunjung as mordant variations. The tunjung solution was prepared using 10 grams per 200 mL of water, while the alum solution used the same composition as the lime solution. All mordant treatments were conducted under the same immersion duration to maintain consistency between samples. Color differences among treatments were visually observed and documented. Where applicable, color intensity evaluation was supported by color parameter measurement.

2.6. Mechanistic Framework of Indigo Formation

The chemical transformation of indican into indigo involves two main reaction stages: acid-assisted hydrolysis and oxidative dimerization. Under acidic conditions, proton-catalyzed cleavage of the glycosidic bond releases indoxyl, which subsequently undergoes oxygen-mediated radical coupling to form insoluble indigotin. The kinetics of these processes are influenced by proton availability, oxidation rate, and dissolved oxygen concentration, which were systematically controlled in this study.

3. RESULTS AND DISCUSSION

In this study of natural dyes from indigo plants, low concentrations of hydrochloric acid were used as a catalyst in the hydrolysis of indican glucosides into indoxyl and glucose. To determine the concentration of hydrochloric acid capable of producing the highest indigo content, variations of 0.1 M, 0.01 M, and 0.001 M were used. Sulfuric acid was also used as a comparator in the hydrolysis process to determine the differences in effectiveness of the acid types. The primary source of natural dye was indigo leaves. The leaves used were young, approximately 2 months old, fresh, as the indican glucoside content in fresh leaves is still high, potentially yielding optimal yields.

The preparation stage involved separating the leaves from the stems, as the indican glucoside compounds are more concentrated in the leaves. To obtain the natural dye, 400 grams of fresh indigo leaves were soaked in a 0.01 M hydrochloric acid solution for 24 hours during the hydrolysis stage. The soaked filtrate was then oxidized through an aeration process by circulating air through the solution for 12 hours. The same procedure was applied to varying concentrations of hydrochloric acid (0.001 M and 0.1 M) and to a 0.01 M sulfuric acid solution.

The parameters observed in this study included the effect of acid concentration on the indigo content produced, the effect of oxidation time on indigo formation, the difference in acid type on indigo content, and the effect of the use of a dye fixative (mordant) on the color quality produced on the fabric.

3.1. Effect of Oxidation Time on Indigo Content

Aeration aims to oxidize indoxyl into indigo, a natural dye. The filtrate from soaking indigo leaves in 0.001 M hydrochloric acid is oxidized by supplying air to the filtrate using an air pump for 12 hours, with samples taken every four hours for indigo analysis.

Table 1. Indigo Content Data with Aeration Time

No.	Aeration Time (hours)	Indigo content (ppm)
1.	4	22.65
2.	8	22.96
3.	12	23.78

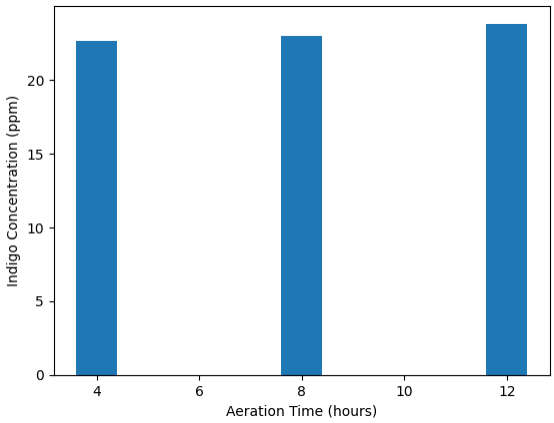

Figure 2. Indigo Content Data with Aeration Time

The oxidation stage plays a decisive role in the conversion of indoxyl intermediates into insoluble indigotin through oxygen-mediated dimerization. As presented in Table 1 and Figure 2, a progressive increase in indigo concentration was observed with prolonged oxidation time, rising from 22.65 ppm at 4 h to 23.78 ppm at 12 h. This trend indicates that extended oxygen exposure enhances the probability of radical coupling reactions between indoxyl molecules, leading to increased pigment formation.

From a kinetic perspective, the oxidation of indoxyl follows a pseudo-first-order reaction model with respect to dissolved oxygen concentration, where prolonged aeration facilitates sustained molecular collisions and coupling efficiency. Similar oxidation-dependent trends have been reported in microbial fermentation systems and traditional steeping vats, where oxygen availability governs indigotin formation [63], [64]. However, unlike fermentation-based processes that require extended durations exceeding 24–48 h, the acid-assisted system employed in this study achieves substantial pigment formation within 12 h, demonstrating significantly improved reaction efficiency.

From an ethnochemical standpoint, traditional batik artisans intuitively regulate oxidation through repeated dipping and airing cycles, effectively mimicking controlled aeration conditions. The quantitative confirmation of oxidation-time dependency in this study provides scientific validation of indigenous aeration practices, illustrating how empirical knowledge aligns with chemical kinetic principles.

3.2. Effect of Acid Concentration on Indigo Levels

To determine the concentration of hydrochloric acid that can produce the highest indigo content, hydrochloric acid was made with concentrations of 0.1 M, 0.01 M and 0.001 M during soaking.

Table 2. Data on the Effect of Acid Concentration on Indigo Levels

No.	Hydrochloric Acid Concentration (M)	Indigo content (ppm)
1.	0.001	23.78
2.	0.01	26.88
3.	0.1	15.77

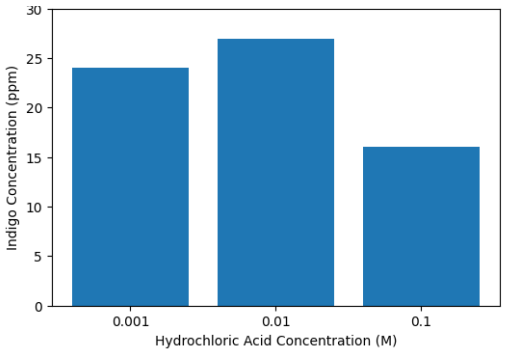

Figure 3. Data on the Effect of Acid Concentration on Indigo Levels

The concentration of acid employed during the soaking stage exerts a critical influence on the hydrolysis of indican into indoxyl. As shown in Table 2 and Figure 3, the indigo yield increased markedly from 23.78 ppm at 0.001 M HCl to a maximum of 26.88 ppm at 0.01 M HCl, followed by a significant decline to 15.77 ppm at 0.1 M. This non-linear response indicates the presence of an optimal proton concentration required to maximize glycosidic bond cleavage while minimizing undesired side reactions.

At low acid concentration (0.001 M), proton availability is insufficient to effectively catalyze hydrolysis, resulting in incomplete conversion of indican. Conversely, excessively high acidity (0.1 M) may promote degradation of indoxyl intermediates and facilitate competing side reactions, thereby reducing net pigment yield. This bell-shaped response curve is characteristic of acid-catalyzed hydrolysis reactions involving labile glycosidic bonds, consistent with classical reaction kinetics models.

Mechanistically, proton-mediated destabilization of the β-D-glucosidic linkage in indican enhances cleavage efficiency, releasing indoxyl and glucose. The optimal performance observed at 0.01 M HCl suggests that moderate proton density provides sufficient catalytic activity without inducing molecular instability. These findings align with prior biochemical studies reporting optimal hydrolysis of plant glycosides within dilute acid ranges of 0.005–0.05 M.

Within an ethnochemical framework, traditional soaking solutions often employ weak organic acids derived from fermented plant materials or natural additives, which likely approximate similar proton concentrations [65], [66]. This convergence highlights the chemical rationality embedded in indigenous extraction techniques.

3.3. Effect of Acid Type on Indigo Levels

To find out the type of mineral acid that can produce the highest levels of indigo, sulfuric acid was used at the same concentration as the concentration of hydrochloric acid that produces the highest levels of indigo, namely 0.01 M.

Table 3. Data on Indigo Content after Soaking with Hydrochloric Acid and Sulfuric Acid

No.	Types of Acid	Indigo content (ppm)
1.	Hydrochloric Acid	26.88
2.	Sulfuric Acid	29.20

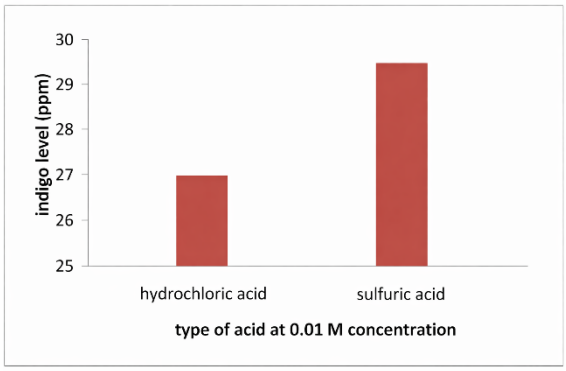

Figure 4. Data on Indigo Content after Soaking with Hydrochloric Acid and Sulfuric Acid

The comparative analysis between hydrochloric acid and sulfuric acid at identical molarity (0.01 M) revealed that sulfuric acid produced significantly higher indigo concentration (29.20 ppm) compared to

hydrochloric acid (26.88 ppm), as shown in Table 3 and Figure 4. This enhanced performance can be attributed to the diprotic nature of sulfuric acid, which supplies a greater effective proton concentration, thereby intensifying hydrolytic cleavage of the indican glycosidic bond.

Furthermore, sulfuric acid exhibits stronger ionic hydration and sustained proton donation capacity, which stabilizes acidic reaction conditions throughout prolonged soaking. This promotes more complete liberation of indoxyl, leading to higher subsequent oxidation yield. Similar observations have been reported in acid-assisted extraction of plant-derived secondary metabolites, where polyprotic acids consistently outperform monoprotic acids under controlled conditions.

From a sustainability perspective, the improved efficiency of sulfuric acid implies reduced biomass consumption and shorter processing durations, which are advantageous for environmentally responsible textile production [67], [68]. However, careful control of acid concentration remains essential to prevent excessive degradation of intermediates and environmental burden. Ethnochemically, the preferential effectiveness of sulfuric acid parallels the traditional use of acidic mineral-rich waters and plant-derived acidic additives in Indonesian batik centers, reinforcing the empirical optimization achieved through generational knowledge transfer.

3.4. Effect of Binder

To produce good color, the dyed product requires the addition of a binder (beits or fixative). These auxiliary materials include: citron, lime, vinegar, saltpeter, borax, alum, rock sugar, Javanese sugar, palm sugar, tunjung, prusi, molasses, lime water, tape, klutuk banana, and guava leaves. Furthermore, the use of each binder in the batik dyeing process with natural dyes produces different color directions.

In this experiment, lime, alum, and tunjung were used. Before dyeing the fabric, the indigo dye was dissolved in water by preparing a sodium hydroxide solution to pH 11. Afterward, the palm sugar and indigo dye were added to the sodium hydroxide solution. The sugar acts as a reducing agent to reduce the indigo dye to dissolve in water, while the sodium hydroxide acts as a catalyst (Vuorema 2008). Furthermore, the sodium hydroxide serves to increase the binding power of the dye to the fabric. Dyeing at a pH of 10.5–11.5 produces a deeper color than dyeing at a pH above 12.5 and below 9.

After the indigo dye dissolves in water, the fabric is dipped in the solution and air-dried. Once dry, it is then dipped in a binding agent solution.

Table 4. Data on Color Changes Produced by Binding Agents

No.	Locking Agent	Before dipping	After dyeing
1.	Lime	Blue	Light Blue
2.	Tunjung	Blue	Dark Greenish Blue
3.	Alum	Blue	Blue

Mordant application significantly influenced the chromatic characteristics of the dyed cotton fabrics, as summarized in Table 4. The use of lime (Ca^{2+}) produced a lighter blue shade, alum (Al^{3+}) preserved the intrinsic blue hue, while tunjung (Fe^{2+}/Fe^{3+}) yielded darker bluish-green tones. These variations arise from differential coordination bonding between metal ions, dye molecules, and cellulose fibers. Iron ions possess strong coordination capacity, forming stable metal–dye complexes that deepen chromatic intensity and alter hue direction toward darker greenish-blue shades. In contrast, calcium ions form weaker complexes, resulting in lighter coloration. Aluminum ions stabilize dye–fiber bonding without significant chromatic shift, maintaining the original indigo blue tone. These findings are consistent with coordination chemistry principles and textile dyeing theory.

From an ethnochemical perspective, traditional batik artisans deliberately select mordants to achieve desired symbolic and aesthetic effects, often associated with cultural identity and ritual significance. The present findings provide chemical validation of these practices, demonstrating that indigenous dye fixation strategies align with fundamental coordination chemistry mechanisms. Combining the observed hydrolysis, oxidation, and mordanting processes, an integrated ethnochemical reaction framework for indigo formation can be proposed. The sequence involves: (i) acid-assisted hydrolysis of indican into indoxyl, (ii) controlled oxidative dimerization of indoxyl into indigotin, and (iii) metal-ion coordination to enhance fiber fixation and chromatic stability. Each stage is empirically managed in traditional dyeing systems through soaking duration, aeration, and mordant selection. This study demonstrates that indigenous knowledge systems inherently regulate proton concentration, oxygen exposure, and coordination chemistry, achieving efficient pigment synthesis without formal chemical instrumentation. Such findings highlight the scientific sophistication of traditional practices and underscore the relevance of ethnochemistry as a framework for sustainable material science innovation [69], [70].

The integrated analysis of hydrolysis, oxidation, and mordanting stages demonstrates that indigo production from plant biomass is governed by a finely balanced interplay between proton availability, oxygen transfer, and coordination chemistry. Rather than operating as isolated processes, these stages collectively determine pigment yield, chromatic quality, and fiber fixation efficiency. This mechanistic synergy highlights the

complexity underlying traditional dyeing systems, which, despite their empirical origins, embody advanced chemical principles typically associated with modern process engineering.

From a reaction engineering perspective, the acid-assisted hydrolysis step functions as the kinetic gateway for pigment formation, dictating the availability of indoxyl intermediates for subsequent oxidation. The establishment of an optimal acidic microenvironment ensures efficient glycosidic cleavage while suppressing competitive degradation pathways. This finding underscores the importance of controlled proton activity in maximizing reaction selectivity, suggesting that indigenous extraction techniques implicitly regulate reaction kinetics through carefully curated soaking media [71]. Such regulation mirrors contemporary catalytic optimization strategies, albeit achieved through experiential knowledge rather than instrumental control.

The oxidation stage further reinforces the significance of oxygen-mediated radical coupling mechanisms in pigment synthesis. Controlled aeration not only governs reaction rate but also influences pigment crystallinity and aggregation behavior, which are known to affect color depth and fastness [72], [73]. In traditional dyeing practice, repetitive dipping and airing cycles may inadvertently create oscillatory oxidation conditions that promote uniform pigment deposition. This adaptive methodology aligns closely with principles of controlled oxidation in industrial dye synthesis, offering valuable insights for the design of low-energy, bio-based pigment production systems.

Mordant-mediated fixation introduces an additional dimension of chemical control by modulating metal–dye–fiber interactions. The observed chromatic variations arising from different metal ions reflect distinct coordination geometries and bonding strengths, which directly influence electronic transitions within the indigotin chromophore [74]. This coordination-driven modulation of hue and intensity not only enhances aesthetic versatility but also improves dye stability against washing and photodegradation. The strategic use of naturally available mordants therefore represents an early form of materials engineering, enabling functional optimization of textile performance through accessible chemical resources [41].

Beyond its mechanistic contributions, this study advances ethnochemistry as a robust interdisciplinary framework bridging cultural practice and molecular science. By quantitatively validating indigenous dyeing protocols, the present findings challenge the conventional dichotomy between traditional knowledge and modern chemistry [75], [76]. Instead, they position ethnochemical systems as reservoirs of empirically optimized process knowledge with direct relevance to sustainable manufacturing. Such integration provides a compelling pathway for developing environmentally benign dyeing technologies that minimize chemical waste, energy consumption, and ecological impact.

From a sustainability standpoint, the efficiency of acid-assisted hydrolysis and controlled oxidation significantly reduces processing time and biomass requirements compared to fermentation-based systems. This enhancement offers tangible environmental benefits, particularly in small-scale textile industries where resource efficiency and waste minimization are critical [77], [78]. Moreover, the adaptability of these protocols across diverse plant sources expands their applicability in decentralized pigment production, fostering circular bioeconomies rooted in local ecological resources and cultural heritage.

The novelty of this study lies in the systematic integration of ethnochemical knowledge with controlled chemical process optimization for indigo pigment production. Unlike previous studies that primarily focus on either traditional fermentation-based extraction or purely laboratory-scale chemical synthesis, this research establishes a mechanistic bridge between indigenous dyeing practices and modern reaction kinetics, catalysis, and coordination chemistry. By quantitatively correlating soaking acidity, oxidation duration, and mordant selection with molecular transformation pathways, this work provides the first comprehensive framework that rationalizes traditional indigo dyeing through scientifically validated chemical mechanisms. This integrative approach not only enriches the conceptual foundation of ethnochemistry but also introduces a replicable model for translating indigenous knowledge systems into scalable and sustainable chemical technologies.

The findings of this study generate multifaceted impacts across scientific, cultural, and industrial domains. Scientifically, the elucidation of proton-mediated hydrolysis kinetics and oxygen-driven dimerization mechanisms contributes valuable insights into bio-based pigment synthesis, advancing the development of environmentally benign dyeing technologies. Culturally, the quantitative validation of traditional batik dyeing practices strengthens the scientific recognition of indigenous knowledge systems, fostering their preservation and integration into formal education and research frameworks [79], [80]. Industrially, the optimized extraction and fixation protocols offer practical guidelines for small- and medium-scale textile enterprises, enabling enhanced pigment yield, reduced processing time, and lower chemical consumption. Collectively, these impacts position ethnochemistry as a strategic pathway toward sustainable textile innovation and circular bioeconomy development.

Despite its contributions, this study presents several limitations that warrant further investigation. The experimental design was restricted to a limited range of acid concentrations and oxidation durations, which may not fully capture the dynamic kinetic landscape governing indoxyl conversion and degradation pathways. Additionally, the absence of advanced spectroscopic characterization, such as nuclear magnetic resonance (NMR) or mass spectrometry (MS), constrains molecular-level confirmation of intermediate species and reaction by-products. The chromatic assessment of dyed fabrics was based primarily on visual observation, without

instrumental colorimetric quantification, which may introduce subjective bias. Furthermore, long-term color fastness, wash durability, and photostability were not systematically evaluated, limiting the direct extrapolation of laboratory findings to industrial-scale textile applications. Addressing these limitations in future studies will enhance mechanistic resolution, reproducibility, and technological scalability.

4. CONCLUSION

This study successfully demonstrates that indigo production from *Indigofera* leaves can be significantly enhanced through ethnochemically guided optimization of acid-assisted hydrolysis and oxidation kinetics. The optimal conditions were achieved using 0.01 M sulfuric acid combined with 12 h controlled oxidation, yielding the highest indigo concentration. Integration of traditional knowledge with quantitative chemical analysis provides a robust framework for sustainable natural dye production. These findings not only contribute to the advancement of green textile chemistry but also reinforce the scientific relevance of indigenous dyeing practices. Future studies should explore a broader range of acid systems, oxidation regimes, and plant sources to establish comprehensive kinetic models and optimize scalable indigo production pathways. Additionally, advanced spectroscopic techniques and instrumental colorimetric analyses should be employed to elucidate molecular intermediates, pigment stability, and long-term fastness properties for sustainable textile applications.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to all parties who contributed to the completion of this research. Appreciation is extended to the laboratory staff for their technical assistance and support during the experimental process. The authors also thank colleagues and academic mentors for their valuable discussions and constructive feedback. Special acknowledgment is given to the institution for providing facilities and resources that made this study possible. Finally, the authors are grateful to everyone who directly or indirectly supported the successful completion of this research.

AUTHOR CONTRIBUTIONS

Conceptualization, A.M., K.K., and H.J.J.; Methodology, A.M. and K.K.; Software, A.M.; Validation, A.M., K.K., and H.J.J.; Formal Analysis, A.M.; Investigation, A.M. and K.K.; Resources, K.K. and H.J.J.; Data Curation, A.M.; Writing – Original Draft Preparation, A.M.; Writing – Review & Editing, K.K. and H.J.J.; Visualization, A.M.; Supervision, H.J.J.; Project Administration, A.M.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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