Postprint: Research on Traditional Botanical Knowledge of Indigo Plants in China

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Abstract

Indigo plants were historically significant economic crops in China. The plant indigo derived from these species exhibits health-promoting properties, including antibacterial, anti-inflammatory, and ultraviolet-resistant effects. However, the craftsmanship of indigo production from indigo plants has nearly disappeared under the commercial impact of synthetic indigo. In recent years, growing public attention toward environmental protection and biodiversity has led to continuously increasing demand for plant indigo, consequently drawing greater research interest toward indigo plants. Based on a review of existing literature, this study briefly introduces the species and distribution of indigo plants traditionally utilized in China, systematically examines the developmental trajectory of China' s indigo production processes, elaborates on the principles underlying these processes and the current status of craftsmanship inheritance, and concludes with a discussion of existing challenges. The analysis reveals that historical records document a total of 11 indigo plant species in China, classified within 6 families and 6 genera, while only 5 species remain in current use. China' s indigo production processes have evolved from the immersion-rubbing dyeing method to solid-state fermentation indigo production and subsequently to liquid-state fermentation indigo production, with only the latter method still employed today. The indigo production process involves the conversion of precursor substances present in indigo plants into indigo, accompanied by the generation of by-products such as indirubin. Factors influencing indigo purity include the selection of raw plant materials, temperature, soaking duration, pH value, dissolved oxygen content, among others. Although research on indigo plants in China has achieved certain accomplishments, further investigation is warranted in areas including the textual verification of indigo plant species in ancient literature, the excavation of indigo plant germplasm resources, and the restoration and reproduction of traditional indigo production craftsmanship.

Full Text

1. Species of Chinese Indigo Plants

The earliest recorded mention of indigo plants in China appears in the "Da Dai Li Ji • Xia Xiao Zheng" from the Xia Dynasty: "In the fifth month, begin irrigating the indigo and knotweed." References become abundant after the Zhou Dynasty. Indigo roots can be processed into the traditional Chinese medicine Banlangen (Isatis root), the fruit is known as Lan Shi (indigo fruit), and the foam from indigo processing becomes Qing Dai (indigo naturalis)—all possessing antimicrobial, anti-inflammatory, and heat-clearing medicinal properties. Consequently, historical records of indigo plants frequently appear in successive "bencao" (materia medica) texts. Su Jing' s "Xin Xiu Bencao" from the Tang Dynasty listed "three types of indigo fruit" : wood indigo (Mu Lan), Chinese woad (Song Lan), and knotweed indigo (Liao Lan). Su Song' s "Bencao Tujing" from the Song Dynasty noted "according to records, there are several types of indigo," enumerating five: wood indigo, Chinese woad, knotweed indigo, true indigo (Ma Lan), and Wu Lan. Li Shizhen' s "Bencao Gangmu" from the Ming Dynasty stated "there are five types of indigo," listing various wood indigos, Chinese woad, knotweed indigo, true indigo, and Wu Lan. Song Yingxing' s "Tian Gong Kai Wu" from the Ming Dynasty also listed five indigo types: tea indigo (Cha Lan), knotweed indigo, true indigo, Wu Lan, and amaranth indigo (Xian Lan) (Rong, 1990).

Regarding the botanical identity of these historically documented indigo plants, Zhao (1987) conducted textual research on dye plants in "Tian Gong Kai Wu," identifying tea indigo as Chinese woad (*Isatis indigotica*), knotweed indigo as *Polygonum tinctorium*, true indigo as *Strobilanthes cusia* (Ban Lan), Wu Lan as wood indigo (*Indigofera tinctoria*), while noting that amaranth indigo requires further verification. Subsequent studies by Li et al. (2013) systematically analyzed 50 local gazetteers from the Ming to Republican period, and Han (2015) conducted interdisciplinary research on four historical documents from the Ming and Qing dynasties—both confirming Zhao' s four identified species. Li (2017) compiled and analyzed 73 ancient texts on indigo dye plants, adding *Indigofera suffruticosa* (Ye Qing Shu) to this list.

Additionally, *Flora Reipublicae Popularis Sinicae* documents six more species used for blue dye: *Wrightia laevis* (Lan Shu) from Apocynaceae (Chinese Academy of Sciences, 1977a); *Marsdenia tinctoria* var. *tinctoria* (Lan Ye Teng), *Marsdenia tinctoria* var. *tomentosa* (Rong Mao Lan Ye Teng), *Marsdenia hainanensis* var. *hainanensis* (Hai Nan Niu Nai Cai), and *Marsdenia hainanensis* var. *alata* (Chi Ye Niu Nai Cai) from Asclepiadaceae (Chinese Academy of Sciences, 1977b, 1977c); and *Indigofera galegoides* (Jia Da Qing Lan) from Leguminosae (Chinese Academy of Sciences, 1994). These species and their distribution ranges are compiled in Table 1 "Species and Distribution Areas of Indigo Plants Used in China," revealing that historical records document 11 indigo species belonging to 6 families and 6 genera.

2. Development of Indigo Processing Techniques

Traditional Chinese indigo processing represents a continuously evolving craft. Before the Qin and Han dynasties, direct leaf-rubbing dyeing predominated. Solid-state fermentation began in the Eastern Han dynasty and was gradually replaced by liquid fermentation after the Ming dynasty. The earliest detailed record of liquid fermentation appears in Jia Sixie' s "Qi Min Yao Shu" from the Northern Wei dynasty, a method still employed today.

The direct-rubbing method involves crushing fresh indigo leaves with fabric or first extracting leaf juice, assisted by plant ash as a mordant, then soaking the fabric (Zhang, 2010). Constrained by indigo' s growing season and harvest timing, ancient practitioners developed methods to first ferment indigo plants into natural indigo through traditional processes, then reduce the indigo for dyeing—thus overcoming temporal and spatial limitations. Based on the physical form of the produced indigo, processing techniques can be categorized as solid-state fermentation (producing "indigo balls") or liquid fermentation (producing indigo paste).

The solid-state fermentation process is described as: "After harvesting, spread the leaves on the floor, soak with water to induce fermentation and heating. When dry, stir thoroughly, then soak again for fermentation. Repeat this process multiple times until fermentation is complete, resulting in a dark blue-black substance called indigo"(Li, 2017). From the Eastern Han through Sui dynasties, knotweed indigo was processed exclusively by this method into solid "indigo balls." Similar descriptions appear in the Sui dynasty' s "Yu Zhu Bao Dian," Tang dynasty' s "Chu Xue Ji," and Song dynasty' s "Tai Ping Yu Lan." Only after the Ming dynasty did liquid fermentation for knotweed indigo become widespread (Zhang & Zhang, 2015). In Japan, solid-state fermentation remains in use (Chavan, 2015).

"Qi Min Yao Shu" provides the first detailed account of liquid fermentation: "Cut indigo and place it inverted in a pit, add water, and weigh down with wood and stones to submerge completely. In hot weather, soak overnight; in cold weather, soak for two nights. Strain to remove stems, pour the liquid into a vat. For every ten dan of vat capacity, add one dan five sheng of lime, stir vigorously for the duration of a meal. When clear, pour off the water. Prepare a separate small pit, store the indigo paste in it. When it reaches the consistency of thick porridge, transfer back to the vat—the indigo is complete" (Yu, 2013). Song Yingxing' s "Tian Gong Kai Wu" from the Ming dynasty describes the same method, differing only in extended soaking times and increased lime quantities. Liquid fermentation produces indigo paste known as "indigo mud" or "indigo ointment" due to its thick, porridge-like consistency. By the mid-to-late Ming dynasty, blue had become the "national color," making indigo plants important cash crops and indigo production a distinct handicraft industry. However, during the late Qing and Republican periods, foreign synthetic dyes' market impact gradually eroded the prosperity of traditional indigo cultivation and processing (Liu et al., 2014). Today, traditional indigo processing persists only in some ethnic minority regions.

3.1.1 Precursor Conversion Mechanism

Indigo processing transforms precursors in indigo plants into indigo, accompanied by byproducts such as indirubin. Precursor research began in the 19th century. Most indigo plants (e.g., knotweed indigo, wood indigo, and true indigo) contain only indican (a) as their precursor, while Chinese woad additionally contains isatan B (b), also known as woad glucoside. These precursors are water-soluble, colorless compounds, explaining why indigo plant sap appears completely clear (Mohd & Shahid, 2017).

During processing, indican hydrolyzes into glucose and indoxyl (c) under endogenous enzyme action, while isatan B hydrolyzes under acidic or alkaline conditions. Indigo (d) forms when two indoxyl molecules condense under alkaline conditions and oxygen. In oxygen-rich environments, indoxyl also spontaneously reacts with isatin (e) to produce the byproduct indirubin (f) (Figure 1 [Figure 1: see original paper]) (Yang et al., 2010). In practice, once precursors hydrolyze, side reactions become inevitable. Natural indigo thus contains not only the primary pigment indigo but also indirubin, isoindirubin, and indigo brown pigments, with indirubin comprising approximately 5-20% of total pigments and exhibiting stronger antioxidant activity than indigo (Liu et al., 2014). Consequently, indirubin serves as an indicator for distinguishing plant-dyed from synthetic indigo-dyed fabrics.

3.1.2 Factors Affecting Indigo Content

Natural indigo has long been used in ethnic clothing and handmade textiles, but its purity and quality have proven difficult to standardize for large-scale promotion like synthetic indigo. Factors affecting indigo purity include plant material selection, temperature, soaking duration, pH, and dissolved oxygen concentration (Dutta et al., 2017).

(1) Material Selection
Kokubun et al. (1998) found that precursor content in Chinese woad was 24% of dry weight in young leaves versus 14% in old leaves, with no significant difference between leaf sections. Yoshiko Minami et al. (2000) confirmed that indican exists only in the vacuoles of fresh leaves, with higher concentrations in young versus mature leaves, explaining why traditional processing uses fresh aerial parts rather than roots. Indican in Chinese woad becomes detectable by April (Minami et al., 2000), and both indican and isatan B content increase from June to September (Oberthür et al., 2004), corroborating the ancient practice of "harvesting in the seventh month for indigo production" (Zhao, 1987). Timely harvesting when precursor content peaks thus maximizes indigo yield.

(2) Temperature
While indican is stable, isatan B disappears after conventional drying at 40°C

(Oberthür et al., 2004). Purnama et al. (2017) analyzed pretreatment effects on wood indigo processing, finding that cold water soaking at 30°C yielded the highest indigo production, with microbial inactivation occurring above 48°C. This validates the scientific basis of traditional practices using fresh leaves soaked in cold water to enhance indigo content.

(3) Soaking Duration

Indican, a glucoside, hydrolyzes into indoxyl under endogenous enzyme action, enabling direct fabric rubbing with knotweed indigo. Isatan B in Chinese woad, however, is a lipid requiring alkaline conditions to release indoxyl. Thus, the "Qi Min Yao Shu" instruction to soak "overnight in hot weather, two nights in cold weather" specifically addresses Chinese woad. Without microbial or nutrient addition, knotweed indigo containing only indican cannot produce indigo in such short periods. Therefore, historical records stating "knotweed indigo is unsuitable for indigo production" refer to short-term fermentation methods, not that knotweed indigo cannot produce indigo at all (Dai & Mei, 2016). The Ming dynasty' s "Tian Gong Kai Wu" specifies "seven days of water immersion," a duration accommodating all indigo species (Rong, 1991).

(4) pH and Dissolved Oxygen

Indigo production requires alkaline conditions and oxygen. Traditional processors commonly add slaked lime, lime powder, or ash from burned shells (primarily calcium carbonate) to create alkaline environments. The resulting calcium carbonate precipitation adsorbs suspended indigo, accelerating sedimentation (Rong, 1991). After lime addition, rapid stirring of the fermentation liquid —known as "beating the indigo" —introduces oxygen to generate indigo. Excessive oxygen drives reactions toward indirubin formation, so "rapid" stirring controls oxygen concentration to favor indigo production. Thus, ancient practitioners empirically recognized pH and dissolved oxygen as critical control points for enhancing indigo purity.

3.2 Current Status of Indigo Processing

Based on field survey literature, traditional indigo processing is currently reported in only seven provinces, all employing liquid fermentation methods comprising five steps: soaking, removing impurities, adding alkali, beating, and settling. Variations exist in soaking duration and alkali type/quantity across regions. The indigo species used are: (1) Guizhou: true indigo, wood indigo, knotweed indigo, and Chinese woad (Chun & Li, 2014); (2) Yunnan: true indigo, wood indigo, Chinese woad, and *Indigofera suffruticosa* (Chai et al., 2017; Li, 2017); (3) Zhejiang: true indigo (Tian, 2012; Li, 2015); (4) Hunan: true indigo (Liu et al., 2012); (5) Guangxi: true indigo (Su et al., 2013; He, 2015); (6) Fujian: true indigo (Lin & Lin, 2015); (7) Hainan: wood indigo (Zhan et al., 2013). True indigo is used in six provinces, knotweed indigo persists only in a few Miao villages in Guizhou, and *Indigofera suffruticosa* processing is reported only in Xishuangbanna, Yunnan.

Additionally, Zhang (2007) identified *Peristrophe baphica* and *Marsdenia tinctoria* as potential blue dyes used locally for coloring chopsticks and cotton/linen threads, though their use for indigo production remains unconfirmed. Due to inconsistent local nomenclature, some literature fails to strictly identify and name plants according to taxonomic principles, leading to misidentifications—for example, judging from photographs that a plant is true indigo rather than the "knotweed indigo" claimed in the text (Zhang & Zhang, 2015). Therefore, comprehensive systematic surveys of China' s indigo plant germplasm resources are urgently needed.

4. Problems and Discussion

4.1 Textual Research on Ancient Indigo Plants

Correct identification of historical indigo plants is fundamental for chemical analysis of textile dyes and research on dyeing history. Ancient texts used the term "lan" (蓝) for indigo plants, but from the Tang dynasty onward, "lan" expanded from specifically referring to blue-dyeing plants to generally denoting all dye plants, complicating botanical origins (Yang, 2017). When identifying indigo plants in historical records, ancient scholars could state "there are five types of indigo,"and modern researchers also list 4-5 species, but how to correlate these with regional common names across different classics remains challenging. Historical documents often refer to one or two species or their varieties, exhibiting the phenomenon of multiple names for the same plant. The lack of unified nomenclature rules and conversion between different taxonomic systems constitutes the primary obstacle to indigo plant identification. Therefore, species determination must remain faithful to original texts, integrating period classics with local gazetteers while accounting for differences between ancient Chinese plant classification and modern systematic botany.

4.2 Indigo Production Mechanism

The precursor conversion mechanism discussed herein specifically addresses *Isatis indigotica* (Chinese woad). European woad (*I. tinctoria*) used abroad contains additional precursors isatan A and isatan C besides indican and isatan B (Oberthür et al., 2004; Maugard et al., 2001). Gilbert et al. (2015) confirmed that both Chinese and European woad contain isatan B, and Oberthür et al. (2015) detected isatan C in Chinese woad. However, no studies have demonstrated whether conversion mechanisms are identical between Chinese and European woad. Therefore, this paper does not discuss isatan A and C conversion mechanisms. Historically, China introduced European woad from Kyoto, Japan (Zhou et al., 1994), raising questions about whether both species were used in folk practice and whether their conversion mechanisms are identical—issues requiring further investigation.

4.3 Optimization of Indigo Processing

Optimizing indigo processing aims to increase indigo purity in the final product. Current research primarily examines factors affecting indigo yield, yet different studies yield contradictory results. For instance, Mo et al. (2015) identified optimal conditions as a 1:10 fresh leaf-to-water ratio, 36-hour soaking at 30°C, and pH 4.0, whereas Dutta et al. (2017) achieved maximum indigo yield with a 1:10 ratio, 12-hour soaking at 40°C. These discrepancies suggest that methodological improvements alone cannot achieve standardization. Comprehensive field surveys of traditional indigo processing are essential to document folk techniques, identify critical control points and conditions, and reconstruct traditional methods. Additionally, microorganisms involved in fermentation require attention to establish standardized traditional indigo preparation and quality control protocols.

China’ s folk traditions have accumulated rich botanical knowledge of indigo plants. Natural indigo offers irreplaceable biodegradability and environmental compatibility, aligning with contemporary green values and possessing significant market potential. The exploration of China’ s indigo plant germplasm resources and reconstruction of traditional processing techniques hold practical value for promoting green, healthy transformation in textile dyeing, while carrying vital significance for preserving national cultural heritage, maintaining ethnic identity, and strengthening cultural confidence.

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