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Physicochemical Characterization and Evaluation of Optimal Application Conditions of Black Catechu (*Acacia catechu* (L.f.) Willd.) for Hair Coloration

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Abstract

This study evaluates the physicochemical characteristics and hair-dyeing potential of black catechu (*Acacia catechu* (L.f.) Willd.) as a natural alternative to synthetic oxidative dyes. Physicochemical analysis confirmed high quality and stability, with a low moisture content of 1.01±0.01% and a high water-soluble extractive yield of 46.26±3.76%. Quantitative evaluation identified a significant tannin content (24.44±3.47%), while thin-layer chromatography (TLC) confirmed catechin ($R_f = 0.75$) as the principal marker compound. The aqueous dispersions of black catechu extract exhibited a brown coloration, whereas its combination with ferric chloride produced a deep black color. These differences resulted in distinct color appearances when applied to bleached hair samples. It was found that aqueous preparations of black catechu extract alone or black catechu–ferric chloride, at a total concentration of 20% w/w, provided visually and optically satisfactory color on treated hair samples after 60 min under the investigated dyeing conditions. In addition, a closed patch test conducted on 20 healthy volunteers revealed only a mild irritation response to the black catechu–ferric chloride preparation at this concentration on the inner forearm test site, observed within 1 h after patch removal, whereas black catechu alone was considered non-irritating.

Keywords: *Acacia catechu* (L.f.) Willd, Black catechu, Tannin-based dye, Natural hair dye

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Introduction

The increasing demand for natural and safe alternatives to synthetic hair dyes has catalyzed an interest in plant-based coloring agents, particularly those with historical and ethnobotanical significance. Semi-permanent plant-based hair dyes differ fundamentally from permanent oxidative systems in terms of mechanism, concentration requirements, and safety profile. Plant-based dyes function primarily through direct adsorption and binding of active compounds (e.g., tannins, quinones) to keratin, resulting in surface-localized coloration on the hair cuticle with minimal penetration, which inherently limits systemic exposure. In contrast, permanent oxidative dyes, involving compounds such as p-phenylenediamine, operate via penetration into the hair cortex followed by oxidative polymerization, allowing effective coloration at lower concentrations but with increased risks of skin sensitization, systemic exposure, and hair damage due to their reactive intermediates (Cui et al., 2022). These differences indicate that, although plant-based systems primarily function through surface-binding mechanisms, they exhibit a comparatively favorable safety profile due to their localized action and limited systemic bioavailability.

Black catechu, a solid extract derived from the heartwood of *Acacia catechu* (L.f.) Willd., has been an integral component of Thai primary healthcare due to its pronounced astringent and antimicrobial properties. Traditionally, it is prepared by boiling heartwood chips in water until a concentrated, dark solution is obtained, which is subsequently dried into a resinous mass known locally as “Kon Si Siet” (Bruneton, 1999; Sittichai & Picheansoonthon, 2014). This traditional remedy is employed for the management of oral ulcers, sore throats, diarrhea, and minor skin infections. In oral health applications, approximately 0.5 g is dissolved in warm water and used as a gargle two to three times daily. For gastrointestinal ailments, a decoction prepared from 0.5–1 g in 150 mL of boiled water is administered twice daily after meals. For topical treatment, the extract is ground and mixed with sterile water to form a paste applied directly to affected areas. Owing to its high tannin content, prolonged use may result in mucosal dryness; thus, its use is recommended for short durations under appropriate supervision (Sittichai & Picheansoonthon, 2014; Tewari, 1995). The continued presence of black catechu in

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community-based Thai herbal medicine underscores the enduring significance of ethnobotanical knowledge in public health.

Phytochemically, black catechu is a well-characterized natural product rich in condensed tannins—predominantly catechin and epicatechin (Yaneva et al., 2020)—along with flavonoids, gallic acid, protocatechuic acid, and trace alkaloids (Adhikari et al., 2021). Its high polyphenolic content accounts for its strong astringent activity, which forms the basis for its traditional use in treating mucosal inflammation and diarrhea (Lahare et al., 2025). Catechin, the principal bioactive constituent, exhibits potent antioxidant activity by scavenging free radicals and inhibiting lipid peroxidation, thereby conferring cytoprotective benefits (Duangyod et al., 2014). Experimental studies have demonstrated broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as antifungal effects, attributed to membrane-disruptive and enzyme-inhibitory mechanisms (Negi & Dave, 2010). Furthermore, its anti-inflammatory effects involve downregulation of pro-inflammatory cytokines such as TNF-α and IL-6, in addition to suppression of COX-2 expression (Yimam et al., 2013). Evidence also supports its wound-healing potential via stimulation of fibroblast proliferation and collagen synthesis (Tiwari et al., 2023), as well as its antidiabetic potential through α-glucosidase inhibition and enhancement of insulin sensitivity (Zhang et al., 2022). Nevertheless, the high tannin content necessitates cautious use, as excessive consumption may lead to gastrointestinal irritation or hepatotoxicity (Ozogul et al., 2025).

Black catechu has long been utilized as a natural dyeing agent in traditional hair coloring practices across South and Southeast Asia. It is rich in tannins, notably catechin, epicatechin, and catechu-tannic acid, which impart a deep brown to black coloration to keratinous substrates such as human hair (Jamagondi et al., 2019; Saranraj et al., 2021). Often used in combination with other botanical dyes like *Lawsonia inermis* (henna) and *Indigofera tinctoria*, black catechu contributes to the modulation of shade and enhancement of color fastness, while also offering antimicrobial and astringent properties that support scalp health (Henna, n.d.). The hair-darkening efficacy of black catechu is fundamentally rooted in its high concentration of condensed tannins—specifically catechin, epicatechin, and catechu-tannic acid. These polyphenolic compounds are characterized by an abundance of phenolic hydroxyl groups. In natural hair-dye systems, these groups act as ligands that readily form hydrogen bonds with the amino, carboxyl, or sulfhydryl moieties within the keratin matrix of the hair cuticle, facilitating dye adherence (Sargsyan et al., 2020). The introduction of transition metal ions, such as ferric ions (Fe^{3+}), triggers the formation of iron–tannate coordination complexes. These complexes exhibit a shift in light absorption across the visible spectrum, resulting in a transition from the natural brownish hues of the extract to an intense, durable deep-black pigmentation. This chemical synergy not only enhances color intensity but also improves wash resistance by creating stable iron–tannate–keratin bridges (Han et al., 2019; Sargsyan et al., 2021). Unlike synthetic dyes containing para-phenylenediamine (PPD) and other irritants associated with allergic reactions and long-term toxicity, herbal dyes from black catechu present a promising alternative with favorable primary skin compatibility under tested conditions (Saranraj et al., 2021).

This study aims to investigate the hair-dyeing potential of black catechu through comprehensive physicochemical characterization, optimization of application conditions, *in vitro* evaluation of dyeing efficacy and color retention, and assessment of primary dermal irritation potential.

Materials and methods

1. Black catechu

"Kon Si Siet" was procured from a Thai traditional drug store in Bangkok. The raw material was identified and authenticated as black catechu by Associate Professor Chanchai Sardsaengjun, a pharmacognosist at the Faculty of Pharmaceutical Sciences, Huachiew Chalermprakiet University. The resinous blocks were cleaned using ethanol to remove surface impurities, air-dried at ambient temperature, and subsequently ground into a fine powder to facilitate use in subsequent experimental procedures.

2. Chemical and reagents

The catechin reference standard, (+)–catechin hydrate (purity≥98.0%; CAS No. 225937-10-0), was obtained from Sigma-Aldrich (Merck KGaA, Germany). Vanillin (purity 99%; CAS No. 121-33-5) and iron (III) chloride hexahydrate (purity≥98.0%; CAS No. 10025-77-1) were purchased from Sigma-Aldrich (Merck KGaA, Germany). Additional analytical-grade reagents, including chloroform, ethyl acetate, formic acid, sulfuric acid, hydrogen peroxide, and absolute ethanol, were procured from the same supplier.

3. Physicochemical evaluation

Macroscopic evaluation, loss on drying, and determinations of extractive matters, and chemical compositions by thin-layer chromatography parameters of black catechu were performed according to WHO guidelines for quality control methods for medicinal plant materials as briefly described below (World Health Organization, 2011; Department of Thai Traditional and Alternative Medicine, 2021):

3.1 Macroscopic evaluation

Black catechu was identified by visual examination of physical properties such as size, color, texture, and other visual inspections.

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3.2 Loss on drying

The moisture content of the raw material was quantitatively assessed using the loss on drying method, in accordance with standard pharmacognostic procedures to ensure compliance with quality control specifications. Approximately 2 g of the powdered sample was accurately weighed into a pre-dried, glass-stoppered weighing bottle that had been conditioned at 105°C for 30 min. The sample was evenly distributed within the bottle, and the stopper was removed prior to placing the bottle in a hot-air oven maintained at 105°C for 5 h.

Upon completion of the initial 5-h drying period, the bottle was transferred to a desiccator and allowed to cool to room temperature before weighing. The sample was then returned to the oven and dried for additional 1-h interval until the difference between 2 consecutive weighing did not exceed 5 mg, indicating that a constant weight had been achieved. The final weight used for calculation of the loss on drying was the constant weight obtained after the repeated drying cycles, rather than the weight recorded after the initial 5-h drying period. The percentage loss on drying was calculated using the following equation:

$$\% \text{ Loss on drying} = [(\text{Initial weight} - \text{Constant weight})/\text{Initial weight}] \times 100$$

Each determination was performed in triplicate, and the mean and standard deviation value was reported.

3.3 Determination of extractive values

The extractive values of the raw material were determined to evaluate their quality, consistency, and solubility characteristics, expressed as ethanol-soluble and water-soluble fractions. This classical pharmacopeial approach was selected for its high reproducibility and suitability for the quality control of resinous plant materials like black catechu. Furthermore, the selection of water and ethanol as solvents is scientifically consistent with the phytochemical profile of *Acacia catechu* (L.f.) Willd, as condensed tannins and related polyphenolic compounds possess a high affinity for polar aqueous and hydroalcoholic systems. Reflux extraction was employed to ensure the exhaustive recovery of these hydrophilic constituents, providing a preliminary evaluation of the tannin-rich matrix required for effective hair-dyeing.

Approximately 4 g of coarsely powdered air-dried sample was accurately weighed into a glass-stoppered conical flask containing 100 mL of solvent (ethanol or chloroform water). The flask was weighed, shaken, and left to stand for 1 h, then fitted with a reflux condenser and gently boiled for 1 h. After cooling, the flask was reweighed and adjusted to the original weight with solvent, shaken, and filtered rapidly through a dry filter. An aliquot of 25 mL of the filtrate was evaporated to dryness in a tared flat-bottomed dish on a water bath, dried at 105°C for 6 h, cooled in a desiccator for 30 min, and immediately weighed. The percentage of ethanol- or water-soluble extractive was calculated with reference to the air-dried crude drug. All measurements were performed in triplicate, and results were expressed as mean±standard deviation.

3.4 Determination of tannin content

The total tannin content was determined using the gravimetric method as described in the Thai Herbal Pharmacopoeia (Department of Thai Traditional and Alternative Medicine, 2021) and WHO guidelines on quality control methods for herbal materials (World Health Organization, 2011). This method involves the precipitation of tannins using hide powder, followed by summing the difference in weight of the sample lost, which equals the weight of the tannins.

For sample preparation, 3 g of black catechu, previously ground and accurately weighed, was placed in a 250 mL conical flask. A total of 150 mL of distilled water was added, and the mixture was heated on a water bath for 30 min with frequent swirling to facilitate extraction. After cooling under running water, the extract was quantitatively transferred to a 250 mL volumetric flask and adjusted to volume with distilled water. The solution was allowed to settle, and the supernatant was filtered to obtain a clear aqueous extract, which was used for subsequent determinations.

To determine the total water-extractives, 50 mL of the filtrate was evaporated toward dryness, and the residue was further dried at 105°C for 4 h and weighed (T_1). For the determination of water-extractives not bound with hide powder, 80 mL of the filtrate was mixed with 6 g of hide powder, shaken thoroughly for 60 min to allow tannin adsorption, and filtered. A 50 mL portion of the resulting filtrate was evaporated toward dryness, further dried at 105°C for 4 h, and weighed (T_2). To account for the water-soluble components of hide powder, 6.0 g of hide powder was suspended in 80 mL of distilled water, shaken for 60 min, and filtered. A 50 mL aliquot of this filtrate was evaporated toward dryness, further dried at 105°C for 4 h, and weighed (T_3).

The percentage of tannins was calculated using the following formula: % Tannins = $[(T_1 - T_2 + T_3) \times 5 \times 100]/3$, where T_1 is the weight of total water-extractives, T_2 is the weight of water-extractives not bound with hide powder, and T_3 is the weight of water-soluble of hide powder. All weights were recorded in grams and calculated on a dried basis. Each determination was performed in triplicate, and the results were expressed as mean±standard deviation.

3.5 Thin-layer chromatographic identification

Fingerprint analysis was performed using TLC to identify catechins as the primary chemical. The fixed

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phase consisted of an aluminum silica gel plate 60 F_{254} (Merck, Merck KGaA, Germany), and the mobile phase was a mixture of chloroform, ethanol, and formic acid (3:6:1, v/v/v). This specific solvent system was selected by optimizing the parameters for black catechin analysis (Wagner & Bladt, 1996; Duangyod et al., 2014).

Thin-layer chromatography (TLC) was conducted to identify phytochemical constituents in black catechu and to compare tannins and other active compounds with a catechin reference standard. Sample preparation involved extracting 0.1 g of powdered raw material with 10 mL of 95% ethanol, followed by filtration, evaporation to dryness, and reconstitution in 1 mL of ethanol. Standard catechin solutions (0.1% w/v) and sample aliquots (3 µL and 5 µL each) were applied onto silica gel GF_{254} TLC plates (10×10 cm) with a band spacing of 5 mm. The plates were developed in a saturated chamber using a mobile phase composed of chloroform, ethanol, and formic acid (3:6:1, v/v/v). After development, chromatograms were examined under UV light at 254 nm to detect dark bands against a green background, and at 366 nm to observe fluorescent bands. Visualization was enhanced by sequential spraying with vanillin reagent (1 g vanillin dissolved in 100 mL ethanol) and sulfuric acid reagent (10 mL concentrated H_2SO_4 in 100 mL ethanol), followed by heating at 100°C for 5–10 min to reveal characteristic chromatographic spots.

4. Preparation of hair samples prior to the dyeing process

4.1 Cleansing process

The obtained hair sample used in this study was healthy and naturally black with approximate 20 cm in length. It exhibited no visible signs of damage, such as breakage or split ends. The hair was sectioned into 1-g bundles, each approximately 10 cm long, with the ends secured using transparent adhesive tape. The prepared hair bundles were rinsed with tap water, gently towel-dried, and then completely dried using a hair dryer. The dried samples were subsequently stored in plastic bags, protected from light, and maintained at room temperature until use in the experimental procedures

4.2 Bleaching process

To facilitate dye deposition and ensure a visibly detectable color change after the dyeing process, the hair samples were pre-bleached using Lolane Pixxel Hair Bleaching Powder with a 12% hydrogen peroxide (S.C. Sereechai Beauty Co., Ltd., Thailand). Bundles of hair were immersed in the bleaching mixture for 15 min and subsequently rinsed with tap water. This procedure was repeated until the samples reached a very light blond shade (Lueadnakrob et al., 2025). After the bleaching process was completed, the hair samples were rinsed thoroughly and dried. Following the bleaching process, the treated hair samples were stored in well-sealed plastic bags at room temperature under dry conditions until further experimental use.

5. Preparation of hair dye samples containing black catechu extract

The experiments were designed to evaluate the efficacy of color deposition on hair cuticles using black catechu alone and its complex with ferric ions. The test hair dye samples included aqueous preparations of black catechu extract and a mixture of black catechu extract with ferric chloride (Sigma-Aldrich, Merck KGaA, Germany) in appropriate proportions. The hair dye samples were evaluated for their physical properties, including color, homogeneity, and consistency. Viscosity changes were qualitatively assessed based on the fluid-to-paste transition relative to water content to determine the suitability for topical application.

5.1 Black catechu–water

Hair dye samples were prepared by mixing black catechu powder with deionized water at various weight-by-weight (w/w) ratios. The mixing ratios of black catechu to deionized water ranged from 5 to 90% by weight, as detailed in Table 1.

Table 1 The mixing ratios between black catechu extract and deionized water used for hair dying process

Compositions	**Amount (g)**									
Water	95.00	45.00	28.33	20.00	15.00	7.50	3.33	2.14	1.25	0.56
Black catechu powder	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Concentrations of black catechu (%w/w)	5	10	15	20	25	40	60	70	80	90

5.2 Black catechu–ferric chloride

The complex between black catechu and ferric chloride formed most effectively at a ratio of 2.7:2.3 (w/w). This weight ratio was selected based on preliminary optimization studies which identified this proportion as the stoichiometric equilibrium point for maximum color density. At this ratio, the coordination between the Fe^{3+} ions and the polyphenolic ligands in the catechu extract reaches a saturation point, yielding a stable, deep-black iron–tannate complex without an excess of unreacted metal salts. The black catechu-ferric chloride mixture was subsequently diluted with deionized water to produce final hair dye samples containing 5–90% w/w of the mixture, as summarized in Table 2.

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Table 2 The mixing ratios between black catechu-ferric chloride and deionized water used for hair dying process

Compositions	Amount (g)									
Water	95.00	45.00	28.33	20.00	15.00	7.50	3.33	2.14	1.25	0.56
Black Catechu/ ferric chloride	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Concentrations of black catechu–ferric chloride (%w/w)	5	10	15	20	25	40	60	70	80	90

6. Effect of black catechu concentration on hair color development

A series of black catechu alone and black catechu-ferric chloride prepared at varying concentrations in deionized water, as detailed in Table 1 and 2, were evaluated to determine the optimal concentration for hair dye coloration. For each test condition, 1 g of bleached hair samples was fully immersed into the test solution and allowed to soak for 1 h at room temperature. Following the soaking period, the hair samples were subjected to a standardized cleansing procedure involving rinsing with tap water and subsequent drying using a hair dryer at a controlled temperature. The resulting hair coloration was evaluated by visual inspection and optical microscopy at 40×magnification using a camera-equipped microscope (Eclipse 50i, Nikon Corp., Japan). Microscopy was utilized to evaluate the uniformity of dye deposition on the hair cuticle and the depth of pigment penetration into the cortex. The optimal concentration was defined as the formulation yielding the deepest and most homogeneously distributed color on the hair fibers.

7. Effect of dyeing duration on hair color development

The effect of varying incubation times on hair color development was evaluated using test preparations containing 20% active content, either black catechu alone or in combination with the ferric chloride. The hair samples were divided into equal portions of approximately 1 g each. Subsequently, 5 g of each test sample was applied to thoroughly soak the entire length of the hair portions. After incubation periods of 15, 30, 45, and 60 min, a one-fifth of the treated hair samples was collected at each time point. These collected samples were then subjected to a cleaning process involving rinsing with tap water, followed by drying with a hair blower. The intensity of hair color deposition was evaluated using visual inspection and optical microscopy (40x), as described above.

8. Evaluation of hair color retention properties

The color retention of hair treated with the test samples containing black catechu alone or with black catechu–ferric chloride was evaluated after repeated shampooing. A 1-g sample of the dyed hair was subjected to a standardized washing procedure: each sample was washed with a commercial shampoo for 10 min, thoroughly rinsed under running tap water for 2 min, and subsequently dried using a hairdryer set to speed level 2, positioned at a fixed distance of 14 cm from the hair surface, until completely dry. The residual hair color was assessed both visually and using an optical microscope, and the results were compared to those of the dyed hair samples that had not undergone washing. This cleansing and evaluation cycle was repeated eight times to simulate repeated usage and to observe progressive color fading.

9. Skin irritation assessment of hair dye samples

The aim of this study was to evaluate the acute irritation potential of the plant-based dyestuff following short-term exposure in human volunteers. The dermal safety study was conducted in strict accordance with the ethical principles of the Declaration of Helsinki. The study protocol was reviewed and authorized by the Ethics Committee of Huachiew Chalermprakiet University (Study Code: HCU-EC1571/2567). All participants provided written informed consent prior to enrollment, and the study was conducted under institutional oversight to ensure participant safety and data integrity.

A total of 20 healthy volunteers—comprising 10 males and 10 females—were enrolled in the study based on the following inclusion criteria: age over 18 years, willingness to participate, healthy skin with no pre-existing dermatological conditions, absence of known hypersensitivity to cosmetic or topical products, and no visible skin abnormalities such as wounds, scars, itching, or inflammation at the test site. Exclusion criteria included current use of systemic corticosteroids or immunosuppressive agents, as well as pregnancy or breastfeeding.

Each volunteer was instructed to arrive at the designated testing location at a specified time and to remain there until the completion of the test procedure. Prior to sample application, the test site—located on the inner forearm—was cleansed using 0.9% sodium chloride solution and allowed to air dry naturally. The test hair dye samples included either black catechu alone or black catechu mixed with ferric chloride, each prepared at a concentration of 20% w/w in deionized water. The 20% w/w concentration was selected as it represented the optimal balance between pigment availability and sample fluidity, producing the deepest and most uniform color distribution in the efficacy trials described above. Purified water served as the negative control.

For each volunteer, the test samples were applied in duplicate, while the purified water control was applied only once. A volume of 0.2 mL of each sample was dispensed onto a 1×1 cm gauze patch. These

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patches were then placed on marked areas of the inner forearm skin and secured using transparent surgical tape (Transpore™). To prevent cross-interference, each gauze patch was spaced 1 cm apart from adjacent patches. After an exposure period of four h, the occlusive patches were removed, and the tested areas were gently cleaned with purified water using a cotton ball. Visual inspections of the skin were conducted immediately after patch removal and subsequently at 1, 24, 48, and 72 h post-exposure.

The inspections were focused on signs of cutaneous irritation, such as erythema, eschar formation, and edema, which were evaluated by comparison with the control sites, including water control and blank control (untreated skin). According to the CTFA Safety Testing Guidelines, skin responses in human patch tests are typically graded on a 5-point scale (0–4) to measure primary irritation, with scores ranging from 0 to 4; 0: no visible reaction, 1: slight erythema (either spotty or diffuse), 2: moderate uniform erythema, 3: intense erythema with edema, 4: intense erythema with edema and vesicles. Skin irritation scores were calculated using the following formula, adapted from human patch test–based irritation assessment methods described in cosmetic safety studies (An et al., 2014).

$$\text{Score} = \frac{\sum(\text{Grade} \times \text{n of response})}{4\ (\text{maximum grade}) \times \text{N (total subjects)}} \times 100 \times 1/2$$

Results and discussion

1. Physicochemical evaluation

As there is currently no established standard for black catechu in the Thai Herbal Pharmacopoeia, it was not possible to definitively assess whether the samples used in this study conformed to any official quality criteria. Therefore, the findings presented herein are reported solely as observed physico-chemical characteristics obtained from the evaluation process, without reference to regulatory benchmarks.

1.1 Macroscopic evaluation

Macroscopic evaluation of black catechu revealed that the raw material typically occurs as hard, nearly spherical blocks with a diameter of approximately 4–5 cm (Fig. 1). The external surface appeared dark brown to black, varying from smooth to slightly rough with fine porosity, while the inner texture was dense, compact, and glossy. The material lacked any distinctive odor in its dry form; however, upon contact with water, a faint woody scent was perceptible. These findings are consistent with the general descriptions of black catechu reported in pharmacognostic references, where physical characteristics such as color, hardness, and surface features are recognized as diagnostic attributes of authenticity and quality. Notably, while the shape and size of blocks may vary across countries depending on manufacturing and drying processes, the characteristic dark coloration and solid, lustrous interior remain common features. Such morphological observations are important for preliminary quality assessment, as they provide a rapid and reliable means of distinguishing genuine black catechu from potential adulterants or substitutes in the raw drug market (Shah & Seth, 2020).



Fig. 1 Black catechu raw material

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1.2 Loss on drying

Moisture determination using the loss on drying method showed a mean value of 1.01±0.01% (Table 3), which is within acceptable limits for raw materials to prevent microbial growth and degradation (Department of Thai Traditional and Alternative Medicine, 2021). This low moisture content suggests good storage stability and aligns with WHO recommendations that emphasize the importance of controlling volatile matter to ensure product quality (World Health Organization, 2011).

Table 3 Loss on drying of black catechu

Sample	Initial weight (g.)	Final weight (g.)	Loss weight (g.)	% Loss on drying
N1	1.99	1.97	0.02	1.01
N2	1.97	1.95	0.02	1.02
N3	1.99	1.97	0.02	1.01
Mean±SD (%w/w)				1.01±0.01%

1.3 Determination of extractive values

The extractive values of the raw material were determined to evaluate the quality, consistency, and solubility characteristics of the raw material, expressed as ethanol-soluble and water-soluble fractions. The ethanol-soluble extractives obtained from 3 independent trials exhibited a dark brown coloration, viscous consistency, and a smooth, glossy surface, indicating the presence of non-polar to moderate polar phytoconstituents. The extractive yields were 30.00, 31.26, and 32.52%, respectively, with a calculated mean value of 31.26% and a standard deviation of ±1.03%, as shown in Table 4.

Table 4 Ethanol-soluble extractive values of black catechu

Sample	Weight of sample (g.)	Weight of extract (g.)	% Ethanol-soluble extractive
N1	4.00	1.20	30.00
N2	4.00	1.25	31.25
N3	3.99	1.30	32.58
Mean±SD (%w/w)			31.28±1.29%

In contrast, the water-soluble extractives from the same 3 trials were light brown in color, similarly viscous, and exhibited a smooth, lustrous surface. The extractive yields were notably higher, measured at 42.50, 46.27, and 50.01%, with a mean value of 46.26% and a standard deviation of ±3.76%, as presented in Table 5. The higher yield of water-soluble extractives may reflect the abundance of hydrophilic constituents such as polysaccharides, glycosides, or organic acids. These findings highlight the differential solubility profiles of the raw material and provide a basis for selecting appropriate solvents in future phytochemical or pharmacological investigations.

Table 5 Water-soluble extractive values of black catechu

Sample	Weight of sample (g.)	Weight of extract (g.)	% Water-soluble extractive
N1	4.00	1.7	42.50
N2	4.00	1.85	46.27
N3	4.00	2.00	50.01
Mean±SD (%w/w)			46.26±3.76%

The extractive values obtained from black catechu revealed distinct solubility characteristics, with ethanol-soluble fractions yielding an average of 31.28±1.29% and water-soluble fractions yielding a notably higher average of 46.26±3.76%. This disparity suggests a predominance of polar phytoconstituents within the raw material matrix, particularly tannins, flavonoids, and other hydrophilic polyphenolic compounds known for their potent astringent and therapeutic properties (Adhikari et al., 2021; Yaneva et al., 2020). The elevated water-soluble extractive content aligns with the traditional medicinal applications of black catechu in aqueous formulations—such as decoctions, infusions, and gargles—commonly employed for the management of mucosal inflammation, oral ulcers, and gastrointestinal disturbances (Sittichai & Picheansoonthon, 2014). These results not only affirm the pharmacognostic

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relevance of extractive value analysis in quality control but also underscore the importance of solvent selection in downstream phytochemical profiling and bioactivity-guided fractionation. Importantly, this solubility behavior also holds practical implications for formulation science, particularly in the development of natural hair dye agents. The high yield of water-soluble polyphenols supports the feasibility of incorporating black catechu into aqueous-based hair dye formulations, which may enhance dye penetration, color stability, and scalp compatibility. Thus, the extractive value data not only validates the traditional uses of black catechu but also informs modern strategies for optimizing its application in cosmetic and dermatological preparations.

1.4 Determination of tannin content

The tannin content of black catechu was quantitatively determined using the gravimetric hide-powder adsorption method, which selectively measures condensed tannins through their affinity for collagenous substrates (World Health Organization, 2011). The experimental results showed that the percentage of tannins in the raw material was 24.44±3.47% (Table 6). This value reflects the predominance of polyphenolic constituents, confirming that black catechu is a tannin-rich extract derived from the heartwood of *Acacia catechu* (L.f.) Willd, consistent with previous phytochemical studies reporting high levels of catechin, epicatechin, and catechu-tannic acid (Adhikari et al., 2021; Yaneva et al., 2020).

Table 6 The tannin content of black catechu

Sample	Weight of sample (g)	Water-extractives (g)	Water-extractive not bound with hide powder (g)	Water-soluble of hide powder (g)	Tannin content (g)	% tannin content/dry weights of sample
N1	3.0	0.25	0.45	0.33	0.65	21.67
N2	3.0	0.27	0.45	0.35	0.85	28.33
N3	3.0	0.26	0.44	0.32	0.70	23.33
Mean±SD (%w/w)						24.44±3.47%

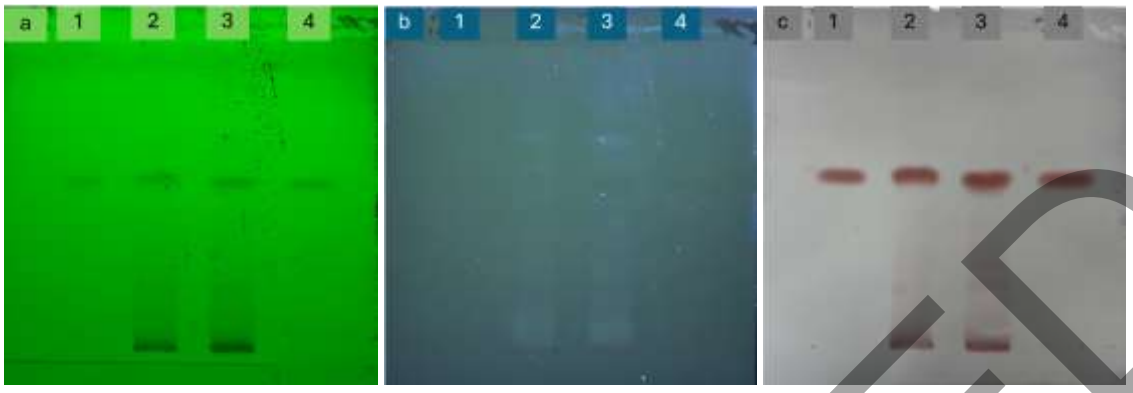
Tannins are water-soluble polyphenols capable of forming strong hydrogen bonds with proteins, alkaloids, and polysaccharides. Their high concentration in black catechu contributes to its pronounced astringency, antimicrobial efficacy, and dyeing ability. The obtained tannin level agrees with earlier reports by Duangyod et al. (2014), who quantified catechin and epicatechin as the dominant constituents of commercial black catechu samples and linked their presence to potent antioxidant activity. Likewise, Lahare et al. (2025) highlighted that the tannin-rich nature of black catechu underpins its pharmacological and cosmetic relevance, particularly in formulations such as herbal toothpowders and topical astringents.

From a pharmacognostic standpoint, the tannin content serves as a key quality indicator for *Acacia catechu* (L.f.) Willd. Variations in tannin percentage may arise from differences in geographic origin, extraction conditions, or storage stability. The value obtained in this study falls within the general range reported for authenticated black catechu samples and therefore substantiates the identity and purity of the tested material. Overall, the data affirms that tannins are the predominant phytochemical group responsible for both the therapeutic and functional hair-dyeing properties of black catechu, underscoring its potential for incorporation into standardized herbal cosmetic formulations.

1.5 Thin-layer chromatographic identification

Thin-layer chromatographic (TLC) analysis provided compelling evidence for the presence of catechin as a major phytochemical constituent in black catechu (Fig. 2). A distinct chromatographic spot observed at R_f 0.75 exhibited consistent characteristics across multiple detection modalities: a prominent dark band under UV light at 254 nm (Fig. 2a), absence of fluorescence under UV 366 nm (Fig. 2b), and a reddish-violet coloration following vanillin–sulfuric acid derivatization (Fig. 2c). These findings confirm the presence of catechin as a principal bioactive compound in the test black catechu sample, which is consistent with its documented antioxidant, antimicrobial, and anti-inflammatory properties (Negi & Dave, 2010; Yimam et al., 2013; Duangyod et al., 2014). While previous reports often identify both catechin and epicatechin as major constituents of *Acacia catechu* (L.f.) Willd, only catechin was definitively detected under the specific TLC conditions utilized in this study.

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(a) (b) (c)

Fig. 2 Thin-layer chromatogram of black catechu extract and catechin reference standard, showing catechin standard at 3 µL and 5 µL (tracks 1 and 4, respectively) and black catechu extract at 3 µL and 5 µL (tracks 2 and 3, respectively). a) UV 254 nm: Shows dark quenching bands, indicating the presence of UV-absorbing catechin. b) UV 366 nm: Confirms the absence of fluorescence for the catechin marker. c) Vanillin-Sulfuric Acid: Reveals the definitive reddish-violet coloration, which is the diagnostic reaction for flavonoids like catechin.

The clarity and intensity of the catechin band, particularly in comparison to other chromatographic spots, suggest its predominance within the extract and reinforce its pharmacological relevance. According to previous phytochemical reports, black catechu contains high concentrations of tannins—including catechin, epicatechin, and catechu-tannic acid—which reinforces its potential for therapeutic and cosmetic applications (Adhikari et al., 2021; Yaneva et al., 2020). The current study empirically confirmed the presence of high tannin content and the catechin marker, thereby aligning the tested sample with the documented phytochemical profile of *Acacia catechu* (L.f.) Willd. The TLC data not only confirms the phytochemical identity of key constituents but also provides a foundation for quality control and standardization in plant-based raw materials.

These polyphenolic compounds are known to impart a deep brown to black coloration when applied to keratinous substrates such as human hair, thereby validating the potential of black catechu as a natural hair dyeing agent (Jamagondi et al., 2019; Saranraj et al., 2021).

2. Hair samples for dyeing process

The original hair samples were healthy, naturally black, and exhibited a straight morphology (Fig. 3a). To facilitate dye uptake and enhance the visibility of color deposition, the hair samples underwent a bleaching process, which resulted in a transformation to a super light blond shade. This treatment also produced a slightly rougher texture. The coarse texture observed in bleached hair arises from the detachment of cuticle cells and the adherence of fragmented endocuticle material to the exposed surface of the underlying cuticle layers (Kim et al., 2024). In natural hair, the presence of melanin granules within the cortex is responsible for the characteristic black coloration. During bleaching, oxidative reactions under strongly alkaline conditions degrade these melanin granules. As the chromophoric structures of melanin are destroyed, the hair undergoes a progressive color shift, with black hair first appearing reddish and eventually turning yellow as the pigments are further decomposed (Fig. 3b).

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(a) (b)

Fig. 3 Studied hair samples a) pre-bleached hair samples and b) bleached hair sample

3. Preparation of hair dye samples containing black catechu extract

The test samples investigated in this study were prepared in purified water using 2 different active-contents: (1) black catechu extract alone and (2) a combination of black catechu extract and ferric chloride. The test samples prepared by mixing different amount of black catechu extract with purified water, as described in Table 1, produced a brownish suspension, characteristic of the natural color of catechin and tannin-rich plant extracts. In contrast, the test samples incorporating black catechu and ferric chloride at a weight ratio of 2.7:2.3, prepared at different concentrations as shown in Table 2, resulted in deep black-colored samples. This pronounced color change is attributed to the formation of iron–tannin complexes.

The test samples exhibited marked differences in appearance due to variations in water content. The preparations covered a wide range of fluidity, from liquid suspensions to paste-like systems (approximately 10–95% water content). Samples containing less than 30% purified water appeared paste-like, whereas increasing the water content beyond 30% progressively increased fluidity. At 95% water content, the samples became non-uniform suspensions. Consequently, standard viscometric measurements were not feasible for accurately determining their viscosity values.

4. Effect of black catechu concentration on hair color development

All test samples were applied to pre-bleached hair samples at a ratio of 2 mL of the samples/1 g of hair. After a 1-h incubation period, the treated hair samples were evaluated through both macroscopic (visual) inspection and microscopic analysis to determine the intensity and uniformity of the coloration. As illustrated in Fig. 4, hair treated with the samples containing black catechu extract alone produced a dark brown coloration. In contrast, the samples combining black catechu extract with ferric chloride resulted in shades ranging from dark green to black, indicating the formation of more complex pigment structures during the dyeing process. Tannins, a major constituent of catechu extract, is a phenolic compound rich in hydroxyl groups. These hydroxyl functionalities readily form hydrogen bonds with the terminal groups of amino acids within the keratin structure of hair cuticles, facilitating strong interactions and promoting dye adherence (Cui et al., 2022). Under optical microscopy (Fig. 5), dye particles existing in catechu were scarcely observed within the cortex of the hair shafts. This limited penetration is likely due to the macromolecular nature of the natural colorants, which are water-soluble compounds. Their hydrophilic characteristics hinder their ability to traverse the hydrophobic cuticle layer of the hair, thereby restricting their diffusion into deeper hair structures (Prathumtet et al., 2019). Polyphenolic compounds exhibit low substantivity to hair fibers; however, the incorporation of metal salts such as iron as mordants has been widely reported to enhance their affinity through the formation of stable, colored complexes (Sargsyan et al., 2020; Geng et al., 2020; Sargsyan et al., 2021). This complexation leads to the formation of iron–tannate complexes, which are poorly soluble and strongly absorbed across the visible spectrum, thereby reflecting minimal light and producing an intense deep-black coloration (Han et al., 2019; Espina et al., 2022). In accordance with this phenomenon, distinct differences were observed in the microscopic images of hair samples treated with black catechu extract alone compared to those treated with the black catechu–ferric chloride samples (Fig. 5c vs. 5d). The hair treated with the black catechu–ferric chloride samples exhibited a black coloration that extended throughout the hair shaft.

Among the various concentrations tested, samples containing 20% active ingredients—whether comprising

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black catechu extract alone (Fig. 4b) or in combination with ferric chloride (Fig. 4f)—were identified as the optimal threshold, based on the maximum homogeneity of color distribution observed under 40x optical microscopy. No visible or microscopic differences in color intensity were observed compared with the higher concentrations (40, 60, and 80%). This finding likely reflects saturation of the available pigment-binding sites and/or limitations in pigment diffusion, suggesting that the maximum dyeing capacity had already been achieved under the experimental conditions of the study. Therefore, concentrations higher than 20% did not provide additional observable benefits. Quantitatively, while higher concentrations (up to 80%) provided a greater total pigment load, they simultaneously reduced the available aqueous medium required for efficient dye diffusion. As a result, 20% represents the saturation point where the solvent-to-solute ratio optimally facilitates the transfer of dye molecules from the formulation to the hair-water interface.

The hair dyeing process generally involves two fundamental steps: (i) the diffusion of dye molecules from the formulation into the keratin matrix of the hair shaft; and (ii) the formation of chemical interactions, particularly hydrogen bonds, between functional groups in the dye molecules such as hydroxyl or carboxyl groups and the amino, carboxyl or sulfhydryl moieties in hair keratin (Schäfer et al., 2021). Effective diffusion involves the transfer of dye molecules from the formulation to the hair–water interface, followed by their subsequent penetration into the inner regions of the hair shaft. Thus, both the concentration of dye molecules and the presence of an adequate solvent medium are critical parameters influencing the dyeing outcome. The present study found that a 20% active ingredient concentration provided an optimal balance between pigment availability and formulation fluidity, thereby enabling efficient hair dyeing. These results are consistent with prior findings emphasizing the crucial role of dye concentration and solvent composition in maximizing the affinity of colorants on keratin-based substrates (Li & Qin, 2023). Consistent with the study's findings, black catechu has been reported to exhibit effective dyeing performance at concentrations up to 15% w/w when formulated in an emulsion system, without evidence of instability or acute toxicity in hair applications (Prathumtet et al., 2019). This behavior can be attributed to the intrinsic mechanism of natural dyes, which act as non-oxidative, surface-adsorbing colorants with limited chromatic intensity and penetration compared to synthetic oxidative systems (Cui et al., 2022). Consequently, high concentrations or the use of combined extract systems is generally required to achieve adequate color strength (Lueadnakrob et al., 2025; Su et al., 2026).

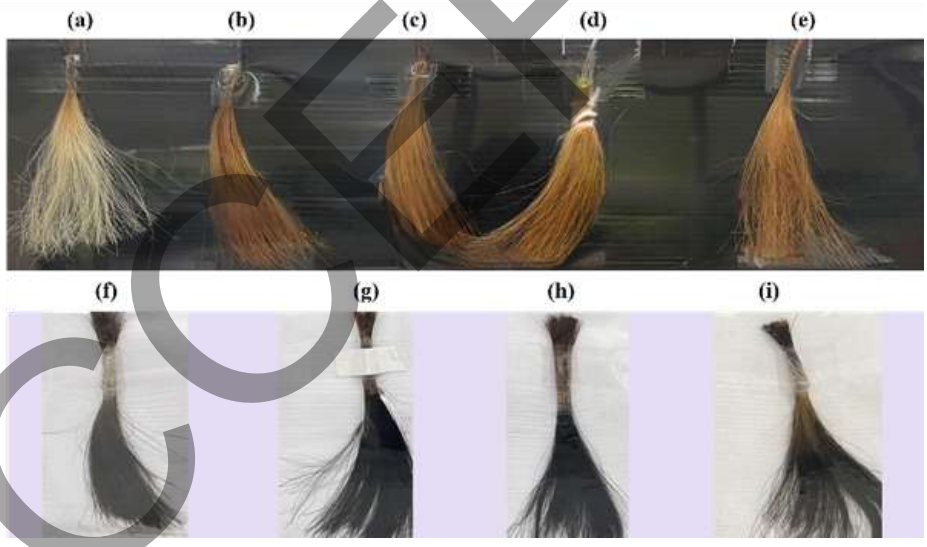


Fig. 4 Pictures of hair samples after the dyeing process with various concentrations of active ingredients compared to untreated hair (a); Catechu: (b) 20%, (c) 40%, (d) 60%, (e) 80% Catechu – ferric chloride: (f) 20%, (g) 40%, (h) 60%, (i) 80%

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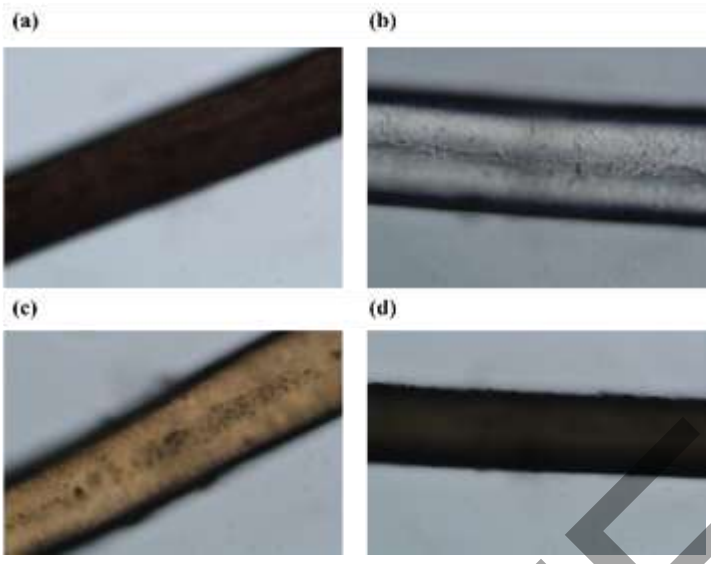


Fig. 5 Microscopic images (40x) of hair samples (a) Natural black hair, (b) Bleached hair, (c) 20% catechu dyed hair and (d) 20% catechu-ferric chloride dyed hair

The effect of ferric chloride content on the hair-dyeing performance of phenolic-rich extracts from *Trapa quadrispinosa* was investigated by Liu et al. (2023), who reported that the incorporation of 4% ferric chloride produced darker coloration while maintaining acceptable tensile strength. Lower Fe^{3+} concentrations were associated with the formation of lighter chromophores, whereas higher Fe^{3+} levels may promote the formation of larger metal–phenolic complexes that limit penetration into the hair cuticle and reduce the tensile strength of treated hair. Consequently, dyestuff deposition is governed by appropriate Fe^{3+}–phenolic coordination, which facilitates binding to the hair fiber through hydrogen bonding (Sargsyan et al., 2021). Consistent with these findings, this study's results indicate that the hair coloration was achieved with an Fe^{3+}–tannate system containing 20% total active ingredients, consisting of black catechu extract (providing 24.44±3.47% tannin) and ferric chloride, with ferric chloride representing 5% of the overall sample. These findings highlight that both the tannin content and the corresponding Fe^{3+} level are critical factors governing dyeing efficacy.

5. Effect of dyeing duration on hair color development

The effect of dyeing time for hair coloration was investigated using test samples containing 20% w/w active ingredients, consisting of either black catechu alone or black catechu in combination with ferric chloride. Bleached hair samples were treated with dye samples for varying durations—15, 30, 45, and 60 min—followed by standardized washing and drying procedures. The intensity and uniformity of the resulting hair color were then assessed and compared across the different treatment times. As illustrated in Fig. 6, the test samples containing 20% black catechu alone and in combination with ferric chloride exhibited the highest color intensity, uniformity and adequacy after 60 min of incubation. These findings suggest that prolonged exposure time enhances the dyeing performance, with a 60-minute application yielding the most effective and homogeneous color deposition. The incubation facilitated the diffusion of hydroxyl-rich compounds presented in the formulation, allowing them to penetrate the cuticle layer and interact with functional groups within hair keratin through hydrogen bonding and other non-covalent interactions. This effect is attributed to rapid diffusion of dye molecules into the accessible pores and cuticular pathways of the hair shaft, after which the rate of penetration declines as saturation and binding equilibrium are gradually approached. This finding is consistent with the results reported by Chandrashekara and Rangnathaiah (2009), who demonstrated that the first hour of the dyeing process represented the most effective period for dye molecule deposition within the hair structure. Furthermore, plant-based dyeing systems typically require longer incubation times (often 60–120 min or more) compared with oxidative dyes, which can achieve effective coloration within a shorter period (commonly 20–60 min) (Gutierrez-Ninachoque et al., 2025). These differences highlight the importance of optimizing the application time in natural dye formulations to ensure adequate color development and performance.

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Fig. 6 Pictures of bleached hair samples after the dyeing process for various times compared to bleached hair sample (a) Catechu: (b) 15 min, (c) 30 min, (d) 45 min, (e) 60 min Catechu – ferric chloride: (f) 15 min, (g) 30 min, (h) 45 min and (i) 60 min

6. Hair color retention properties

Color retention following repeated shampooing (1 to 8 washes) was evaluated using hair samples treated with the dye preparations containing 20% active ingredients in purified water and incubated for 60 min. The results were compared to freshly dyed hair samples to assess the durability of the color. As observed in the microscopic images (Fig. 7), the hair shafts retained a brownish color when dyed with black catechu alone, while a darker black coloration was evident in samples treated with the black catechu–ferric chloride combination. A progressive decrease in color intensity was observed with increasing shampoo cycles. Notably, after eight shampoo washes, the majority of the surface-deposited color was visibly diminished or nearly eliminated in both test samples. However, hair treated with the black catechu–ferric chloride formulation retained a deeper residual coloration compared to hair samples dyed with black catechu alone. This finding aligns with Sargsyan et al. (2021), who demonstrated that ferric ions (Fe^{3+}) bridge the phenolic hydroxyl groups of tannins to amino side chains and carboxylic acid groups in keratin, generating iron–tannate–keratin coordination complexes that enhance dye–keratin binding and improve wash resistance. This study indicated that the developed natural dye-based preparations exhibited characteristics consistent with traditional semi-permanent hair dyes, wherein the color gradually fades with washing rather than providing permanent coloration.

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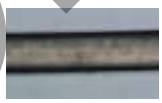
Shampoo (Time)	20% black catechu	20% black catechu – ferric chloride
Bleached Hair		
0		
1		
2		
3		
4		
5		
6		
7		
8		

Fig. 7 Optical microscopic images (x40) of the bleached hair strands treated with 20% black catechu and 20% black catechu-ferric chloride for 60 min after 1 to 8 shampoo cycles

To further enhance color retention and wash fastness against repeated shampooing, future development should focus on strategies that either physically lock the pigment within the hair structure or protect it from surfactant-induced leaching. Implementing a post-dyeing acidic rinse (e.g., citric or acetic acid) can effectively close the hair cuticles, thereby trapping the tannin within the cortex. Additionally, the incorporation of film-forming biopolymers, such as chitosan or hydrolyzed silk proteins, may provide a protective shield over the hair fiber to resist mechanical and chemical stripping. Additionally, utilizing synergistic herbal blends, such as *Terminalia chebula* or *Punica granatum*, could create a more stable, multi-anchored pigment matrix with the iron mordant, thereby potentially reducing dyeing time and enhancing the long-term durability of the natural hair coloration.

7. Skin irritation assessment of hair dye samples

The skin irritation assessment was evaluated in human participants using closed patch tests. Acute irritation is characterized as a localized, non-immunological response (e.g., erythema or edema) that occurs rapidly after a single exposure. This approach is consistent with established human studies using patch testing, which is widely employed to assess dermal safety of cosmetic and botanical products *in vivo* (Wattanakrai et al., 2007, An et al., 2014, Hafner et al., 2020). The skin responses were classified into 4 levels based on the intensity of the skin reaction determined by the z-score as follows: slight, mild, moderate and severe irritation. Slight irritation was defined as $0.0 \leq z < 0.5$; mild irritation, $0.5 \leq z < 2.0$; moderate irritation, $2.0 \leq z < 3.0$; and severe irritation, $3.0 \leq z$ (An et al., 2014).

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In the acute skin irritation test in 20 human volunteers, black catechu alone and purified water produced no visible irritation, whereas black catechu combined with ferric chloride induced a grade 1 reaction at 3 of 40 test sites (7.5%), corresponding to 3 of 20 subjects (15%). No higher-grade reactions such as edema, vesiculation, or intense erythema were observed. The calculated z-score of 1.875 falls within the mild irritation range. These results indicate that the catechu–ferric chloride sample produces mild acute irritation under occlusive test conditions, while black catechu alone and purified water are considered non-irritating under identical conditions.

The mild reactions observed in a subset of participants may arise from several hypothetical mechanisms. For instance, these reactions may be related to the relatively high concentration of bioactive tannins identified in this study (24.44±3.47%). Tannins are recognized for their potent astringent properties, characterized by the ability to precipitate proteins through complex hydrogen bonding and hydrophobic interactions with peptide backbones (Scalbert et al., 2005; Haslam, 2007). When applied to the skin, this protein-precipitating effect can reduce surface lipid availability, potentially inducing a tightening or drying sensation. Individuals with sensitive skin may therefore experience mild irritation during extended exposure, such as the 60-min incubation period used in this study, as the possibility of irritant contact dermatitis cannot be entirely excluded in more vulnerable populations (Draelos, 2015).

Beyond the extract itself, the safety profile is influenced by the coordination chemistry between tannins and the ferric chloride mordant. In the presence of ferric ions (Fe^{3+}), catechu tannins facilitate the formation of iron–tannate complexes through chelation with the *ortho*-dihydroxyl groups of catechol-type polyphenols (Han et al., 2019). While this synergy is essential for achieving intense, durable deep-black pigmentation, the resulting complexes exhibit distinct physicochemical properties—such as increased molecular aggregation and heightened substantivity to keratin—that differ significantly from the raw extract. Consequently, these complexes may adhere more strongly to both hair fibers and the skin surface, potentially leading to temporary staining of the scalp or surrounding areas during application (Sargsyan et al., 2020).

The selection of ferric chloride was predicated on its ability to form stable polyphenolic ligands; however, its characterization as a strong Lewis acid and corrosive agent in concentrated forms necessitates strict toxicological oversight. While the specific coordination complexes evaluated in this study demonstrated mild skin irritation, these findings provide only preliminary evidence of acute safety. Given that the application of iron–tannin chemistry to human hair remains largely experimental despite its historical roots, these results cannot be extrapolated to confirm long-term safety, non-sensitization, or the absence of systemic absorption. Future iterations of this system should investigate more biocompatible iron sources, such as ferrous gluconate or iron oxides, to improve safety profiles and ensure alignment with international cosmetic regulatory standards.

Overall, these proposed mechanisms remain hypothetical, and further mechanistic studies are needed to clarify their respective contributions to the observed skin reactions. Although the present findings demonstrate acute cutaneous compatibility, they represent only a preliminary assessment. Consequently, additional evaluations, including the Human repeated insult patch test (HRIPT), are warranted to characterize the potential for long-term sensitization and allergic contact dermatitis. To further strengthen the sensitivity and predictive value of the safety assessment, future studies should also incorporate standardized mild irritants as positive controls, validated in vitro skin irritation models such as Reconstructed Human Epidermis (RhE), and larger-scale clinical investigations. Together, these approaches would provide a more comprehensive toxicological characterization and support the long-term safety evaluation of the black catechu dyeing system.

Finally, the overall safety and reproducibility of catechu-based products are inextricably linked to the quality and standardization of the raw botanical material. In the present study, the crude material, locally known as "Kon Si Siet," was authenticated as *Acacia catechu* (L.f.) Willd. However, traditional raw materials may contain variable levels of impurities, such as heavy metals or pesticide residues, arising from environmental exposure during harvesting and storage. For commercial development and regulatory compliance, the transition to standardized, cosmetic-grade extracts are essential. These materials must comply with rigorous quality specifications regarding purity and microbial limits in accordance with international safety guidelines (Association of Southeast Asian Nations, 2008; European Parliament & Council of the European Union, 2009; World Health Organization, 2011).

Conclusion

This study successfully established a framework for the physicochemical characterization and optimized hair-dyeing application of black catechu (*Acacia catechu* (L.f.) Willd.) as a raw material. Standardization of the heartwood extract revealed a high tannin content (24.44±3.47%) and the presence of catechin (R_f = 0.75) as a characteristic chemical marker. These findings provide useful physicochemical parameters that may serve as potential quality-control markers for future standardization of black catechu raw materials intended for cosmetic applications.

The investigation into optimal application conditions showed that a 20% w/w extract concentration, coordinated with a ferric chloride mordant over 60 min, produces a uniform, deep-black pigmentation on human hair fibers. Mechanistically, this coloration is attributed to the formation of stable iron–tannate complexes that bind to the hair keratin matrix. Primary irritation assessment using a 4-h closed patch test revealed that the samples

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containing black catechu alone was classified as non-irritant, whereas the samples containing black catechu in combination with ferric chloride demonstrated mild irritation.

These findings suggest that black catechu has potential as a natural alternative for hair coloration systems. However, to fully validate its broader application, further research is required. Specific future directions should include comprehensive scalp safety evaluations, batch-to-batch standardization of the botanical raw material, and quantitative color fastness analysis using a CIELAB colorimetry to compare its performance against established commercial synthetic dyes.

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Author contributions

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used ChatGPT for enhancing language clarity or improving grammar. The final content was reviewed and edited by the authors, who take full responsibility for the accuracy and integrity of the publication.

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