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Extraction and characterisation of *Caesalpinia sappan* L. heartwood: A potential pH-responsive colorant for natural cosmetics

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Abstract
Caesalpinia sappan L., commonly known as sappanwood, is a natural source of phenolic pigments exhibiting pH-dependent chromatic transitions. This study focuses on the optimised extraction and characterisation of its heartwood pigment for potential use as a pH-responsive, natural colorant in adaptive cosmetics. Soxhlet extraction using 95% ethanol was employed to isolate the pigment from authenticated, coarsely ground heartwood, followed by solvent evaporation and lyophilization to obtain a powdered extract. The extract demonstrated a characteristic deep red color and underwent distinct hue transitions from yellow-orange to deep redwhen exposed to varying pH conditions. Organoleptic assessment and solubility profiling indicated moderate compatibility with both aqueous and lipid phases, supporting its potential for broad formulation use. Total phenolic content (TPC) and total flavonoid content (TFC) were determined spectrophotometrically, yielding values of 373 mg GAE/g and 7.4 mg QE/g of extract, respectively, suggesting significant bioactive potential. The developed extraction and stabilisation protocol resulted in a reproducible yield of 3.6% w/w. These findings validate the feasibility of C. sappan extract as a bio-derived pigment with tunable color response, laying the foundation for further investigations into its formulation behaviour, stability, and regulatory viability for commercial application. Given its historical acceptance in traditional medicine and food dyeing, this botanical source aligns with the modern demand for sustainable and multifunctional ingredients.

Keywords: *Caesalpinia sappan*, sappanwood, natural pigment, pH-sensitive colorant, Brazilin, Soxhlet extraction

1. Introduction
Natural plant-derived pigments have gained increasing interest as sustainable and safe alternatives to synthetic dyes due to their biocompatibility, low toxicity, and environmental compatibility [1, 2]. Among such botanicals, *Caesalpinia sappan* L., commonly known as sappanwood, is a well-documented dye-yielding and medicinal plant belonging to the family Fabaceae.Native to Southeast Asia and widely distributed across India, China, and Indonesia, this small thorny tree has been traditionally used for its deep red heartwood, which contains a range of phytochemical with colouring, antioxidant, and therapeutic benefits [3, 4].

Fig 1: Sliced heartwood of *Caesalpinia sappan L.*

The heartwood of C. sappan is particularly rich in brazilin, a compound that oxidises to brazilein, producing a red hue under alkaline conditions [5]. The extract has found applications in traditional medicine, textile dyeing, and more recently, natural cosmetic colorants due to its pH-responsive behaviour and bioactive profile [6-8].

This study focuses on the ethanolic extraction of C. sappan heartwood using a Soxhlet apparatus, followed by lyophilization and preliminary characterization of the extract. Special emphasis is placed on the extract's color-shifting properties under varying pH, as well as estimation of its Total Phenolic Content (TPC) and Total Flavonoid Content (TFC), laying the foundation for its future application in multifunctional cosmetic formulations, particularly those with color-changing or antioxidant properties.

2. Materials and Method

2.1 Materials

The heartwood of *Caesalpinia sappan* was purchased online from a verified source. The plant material was taxonomically verified by the Department of Botany, RTM Nagpur University, where it was noted that *Caesalpinia sappan* is now updated to *Biancaea sappan* under current nomenclature [9]. However, due to its widespread traditional and scientific recognition, the term *Caesalpinia sappan* is retained throughout this study for consistency.
All reagents used were of analytical grade.

Preparation of plant material: The dried heartwood was thoroughly cleaned and coarsely ground using a mechanical grinder. The powder was stored in an airtight container at room temperature until further use.

2.2 Extraction by Soxhlet Apparatus: [10]

A total of 100 g of the coarsely ground C. sappan heartwood was subjected to Soxhlet extraction using 300 mL of 95% ethanol. The process continued for approximately 15 hours until the siphoning cycle completed and the extract appeared colourless. The ethanolic extract was then concentrated under reduced pressure using a rotary evaporator to remove the solvent.
The concentrated extract was freeze-dried to obtain a stable powdered pigment. The extract was stored in a desiccator until further use.

3. Evaluation and Results of Extract

3.1 The lyophilised extract obtained from *Caesalpinia sappan* appeared as a reddish-brown powder with a characteristic woody odor and free-flowing consistency. These organoleptic traits are typical of heartwood-derived extracts rich in brazilin [11].

Fig 2 and 3: Lyophilised extract of *Caesalpinia sappan*

3.2 The ethanolic extraction of 100 grams of coarsely ground *Caesalpinia sappan* heartwood resulted in 3.6 grams of lyophilised extract[12].
The percent yield was calculated using the formula:

$$\frac{actual\ weight}{theoretical\ weight}\ x\ 100$$

$$\frac{3.6}{100}\ x\ 100 = 3.6\%$$

3.3 The solubility of the lyophilised extract was quantitatively assessed in water and paraffin oil using a gravimetric method [13].

- In water: 7.6 mg/mL
- In oil: 5.7 mg/mL

3.4 The extract of *Caesalpinia sappan* wood was screened for the presence of various biomolecules and secondary metabolites such as tannins, saponins, phenols, flavonoids, and terpenoids using standard qualitative methods [14, 15].

Table 1: Phytochemical Analysis of Sappanwood Extract

Sr. No.	Phytoconstituent Tested	Name of the Test / Short Procedure	Observation	Result
1	Tannins	Lead Acetate Test: Extract + 10% lead acetate	Bulky white precipitate	Present
2	Saponins	Foam Test: Extract shaken with distilled water	Persistent foam with 2 cm thick layer	Present
3	Phenols	Ferric Chloride Test: Extract + 5% ferric chloride	Dark green coloration	Present
4	Flavonoids	Alkaline Reagent Test: Extract + NaOH + HCl	Yellow color turned colorless on acid addition	Present
5	Terpenoids	Salkowski Test: Extract + chloroform + conc. H2SO4	Reddish-brown coloration at interface	Present
6	Alkaloids	Hager's Test: Extract + Hager's reagent (picric acid)	No yellow precipitate	Absent
7	Coumarins	Extract + 10% NaOH	Yellow coloration	Present
8	Steroids	Extract + chloroform + conc. H2SO4	No red color or fluorescence	Absent
9	Glycosides	Extract + glacial acetic acid (FeCl traces) + conc. H2SO4	No blue color	Absent
10	Quinones	Extract + conc. H2SO4	Red coloration	Present
11	Proteins	Biuret Test: Extract + 4% NaOH + 1% CuSO4	No violet or purple coloration	Absent

3.5 To determine the pH, 0.5 g of the lyophilised extract was dispersed in 100 mL of distilled water. Due to partial solubility in water, the mixture was filtered using Whatman filter paper to eliminate undissolved residues. The clear filtrate was then subjected to pH measurement using a calibrated digital pH meter at room temperature[13]. The extract exhibited a pH of 4.2, and the resulting solution appeared yellow in color.

3.6 Influence of pH on Color of *Caesalpinia sappan* Extract [11, 16]

A 0.5% w/v aqueous solution of *Caesalpinia sappan* extract was prepared by dissolving 0.5 g of the powdered extract in 100 mL of distilled water. As the extract was only partially soluble, the solution was filtered before use. From this filtered stock, 5 mL aliquots were transferred into clean test tubes. The pH of each aliquot was adjusted by the drop wise addition of 0.05% NaOH solution, and color changes were observed visually.

Table 2: pH dependent Color Response of *Caesalpinia sappan* Extract

Sr. No.	Volume of Extract Used	Estimated Volume of 0.05% NaOH Added (mL)	Shade Observed	Final pH Value
1	5 mL	-	Yellow (Oil-like)	4.2
2	5 mL	~0.05	Light Peach-Orange	4.9
3	5 mL	+ ~0.03 (Total ~0.08)	Orange	5.2
4	5 mL	+ ~0.04 (Total ~0.12)	Orange to Pink Transition	5.6
5	5 mL	+ ~0.02 (Total ~0.14)	Pink	5.9
6	5 mL	+ ~0.05 (Total ~0.19)	Darker Pink	6.4
7	5 mL	+ ~0.06 (Total ~0.25)	Bright Pink	7.2
8	5 mL	+ ~0.08 (Total ~0.33)	Light Red	8.5
9	5 mL	+ ~0.05 (Total ~0.38)	Dark Red	8.9

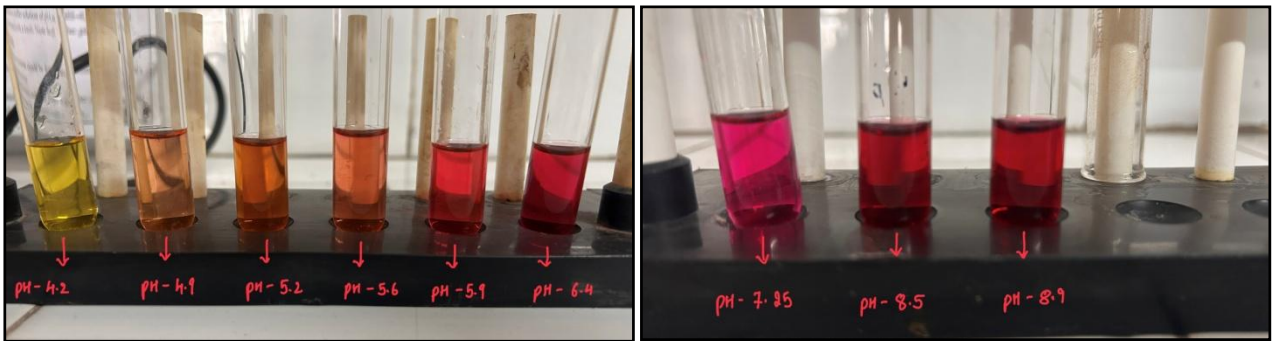

Fig 4 and 5: Color variation of *Caesalpinia sappan* extract at different pH levels.

3.7 Total Phenolic Content Test

The total phenolic content of *Caesalpinia sappan* extract was determined using the Folin-Ciocalteu method[17, 18], a widely accepted colorimetric technique based on the oxidation of phenolic compounds. In this assay, gallic acid was used as the standard, and a calibration curve was prepared by reacting different concentrations of gallic acid with Folin-Ciocalteu reagent and sodium carbonate, followed by absorbance measurement at 765 nm using a UV-visible spectrophotometer. The lyophilised extract was dissolved in ethanol to prepare the test solution, which underwent the same reaction process. After incubation, the intensity of the blue coloration correlated with the phenolic concentration. Based on the standard calibration curve, the total phenolic content of the extract was calculated and found to be 206.67 mg GAE/g of extract, indicating a substantial presence of polyphenolic constituents contributing to antioxidant potential.

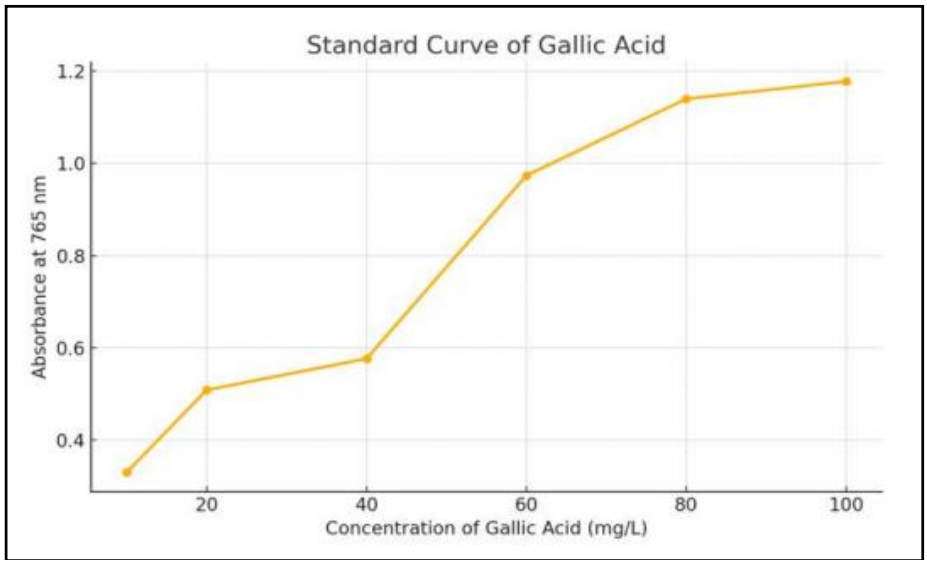

Fig 6: Standard calibration curve of gallic acid used for determining total phenolic content (TPC)

3.8 Total Flavonoid Content Test

The total flavonoid content was estimated using the aluminum chloride colorimetric method, wherein flavonoids present in the sample form a stable complex with aluminum ions, resulting in a yellow coloration. Quercetin was used as the standard, and a calibration curve was generated by measuring the absorbance of quercetin solutions at 415 nm [19]. The ethanolic extract was reacted with aluminum chloride and potassium acetate, and after incubation, absorbance was recorded. The absorbance intensity was directly proportional to the flavonoid concentration. Using the quercetin calibration curve, the total flavonoid content of the extract was quantified as 70 mg QE/g of extract, suggesting a significant level of flavonoids with potential biological activity.

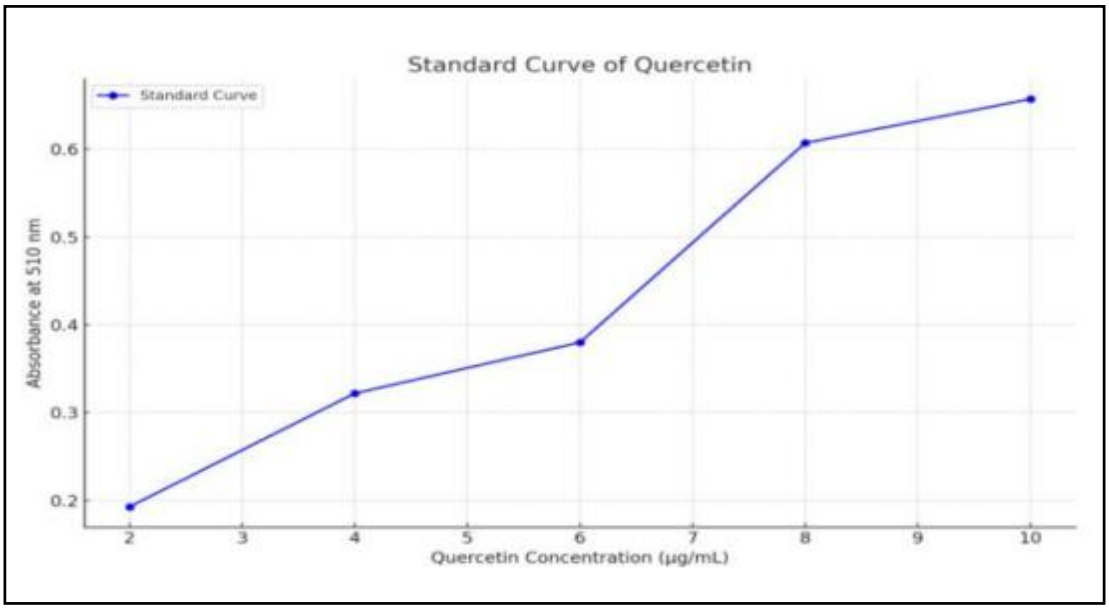

Fig 7: Standard calibration curve of quercetin used for determining total flavonoid content (TFC).

4. Discussion and Conclusion

The present study highlights the potential of *Caesalpinia sappan* extract as a multifunctional natural pigment, with a specific focus on its pH-responsive color transformation and phytochemical profile. The extract exhibited a gradual color transition from yellow to red as pH increased from acidic to alkaline, demonstrating its suitability as a dynamic, pH-sensitive colouring agent. This chromatic behaviour is attributed to the oxidative transformation of brazilin into brazilein, a well-documented reaction that alters the pigment's molecular structure and enhances its color intensity in basic environments [20].

The pH-dependent response observed in this study suggests that C. sappan extract can be effectively utilised in cosmetic formulations that respond to skin's natural pH variations or are intended to visually signal product activation or application. Such properties are particularly relevant in products like lip and cheek tints, facial cleansers, or serums, where subtle color shifts can enhance user experience and aesthetic value [21-23].

Phytochemical screening confirmed the presence of key bioactive constituents such as flavonoids, phenols, tannins, and quinones, which are known for their antioxidant and mild anti-inflammatory potential. Quantitative evaluation revealed a Total Phenolic Content of 206.67 mg GAE/g and Total Flavonoid Content of 70 mg QE/g, reinforcing the extract's functional value beyond pigmentation. These compounds may support overall skin wellness by helping neutralise oxidative stress, a benefit desirable in both decorative and therapeutic cosmetic applications.

Moreover, C. sappan has a long-standing history of use in food and traditional medicine across various Asian cultures, supporting its perceived safety [24]. Its use in herbal beverages, food colouring, and natural dyes [25] suggests a favourable biocompatibility profile, although formal safety evaluations would still be necessary for commercial cosmetic application.

In conclusion, the extract of Caesalpinia sappan demonstrates strong potential as a natural, pH-responsive pigment with added antioxidant benefits. Its dual functionality as a colorant and active botanical ingredient offers a promising avenue for clean-label, sustainable product innovation. Further studies exploring formulation compatibility, pigment stabilisation, and sensory acceptance will support its translation into market-ready cosmetic products.

5. Statements and Declarations

Competing Interests

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 manuscript.

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7. Author Contributions

Anuja Huddar conceptualized the study, carried out the experimental work, data analysis, and manuscript writing. Dr. Sana Ahmed supervised the research work, reviewed, and contributed to the final editing of the manuscript. Both authors approved the final version of the manuscript.

8. Data Availability

All data generated or analyzed during this study are included in this published article. Additional data can be made available upon reasonable request from the corresponding author.

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