JPharmSci
Journal of Pharmaceutical Sciences
Open access peer-reviewed Journal
www.jpsjournal.com [ISSN: 1520-601x]
JPHARMSCI

Analysis of Phytochemicals, Pigment Composition, Isatin Quantification, and Antioxidant Potential in *Couroupita guianensis* Flowers

Rashmi V[1*], Tejaswini S J[1], Prakash Kumar Jha[2]

[1]*Department of Biotechnology, JSS Science and Technology University, Mysuru, Karnataka, India*
[2]*Greenenviro Biosciences (GEBS), Mysuru, Karnataka, India.*

ABSTRACT

This study provides a comprehensive investigation on the extraction and quantification of bioactive compounds from *Couroupita guianensis* (CG) scared flowers. The phytochemical profiling using different solvents confirmed the presence of diverse bioactive secondary metabolites - alkaloids, flavonoids, glycosides, phenols, tannins, and steroids, which contribute to the plant's medicinal properties. Chromatographic separation facilitated the isolation of natural pigments such as indigo, indirubin, and isatin which were identified by UV–Vis, FTIR, and Raman spectroscopy. HPLC analysis revealed an average isatin concentration of 5.99 ppm. Antioxidant assays demonstrated that isolated isatin exhibited greater free radical scavenging activity than crude flower extracts, highlighting its therapeutic potential, including for drug delivery applications. The presence of natural pigments validates the traditional medicinal use of CG and highlights its potential as a natural dye source. Overall, CG is a versatile species with significant potential for applications in pharmaceuticals, natural product research, and sustainable technology development. These results lay the groundwork for future studies aimed at exploring its bioactive constituents in greater depth, optimizing extraction methods, and assessing their suitability for pharmaceutical and environmental applications.

Keywords: *Couroupita guianensis,* isatin, indigo, indirubin, HPLC.

INTRODUCTION

Couroupita guianensis Aubl. (CG), is also called as the cannonball tree, belong to the family Lecythidaceae. CG is native to the Amazonian forests of South America, and in India, its flowers are offered to Lord Shiva, where the plant is valued for its religious, ornamental, cultural, and therapeutic significance. Its distinctive large spherical fruits resembling cannonballs, along with fragrant and structurally elaborate flowers (Fig. 1a), contribute to its unique identity [1].

Ethnobotanically the leaves, flowers, fruit pulp, and bark of CG have been traditionally employed in treatment of wounds, skin diseases, inflammatory conditions, respiratory issues, and digestive disorders [2]. These therapeutic effects are attributed to diverse bioactive compounds including isatin, tryptanthrin, eugenol, linoleic acid, terpenoids, flavonoids, and alkaloids[1,2]. Recent studies have experimentally validated the utilization of CG. For example, bark extracts containing high levels of sulfonated ellagic acid derivatives promote wound healing by triggering the activation of the ERK and AKT signaling pathways and methanolic fruit pulp extracts demonstrate antioxidant, antimicrobial, and anti-inflammatory activities was recently proved [3,4].

Among the bioactive constituents, isatin (1H-indole-2,3-dione) is predominantly noteworthy. The varied chemical nature and pharmacological significance of isatin make it a valuable biosynthetic precursor and an attractive framework for drug design. The methanolic extracts of its flowers exhibit anticancer activity against HL60 leukemia cells, primarily attributed to isatin and its derivatives, which influence cell proliferation and induce apoptosis. [5,6]. Synthetic derivatives of isatin have been investigated for their antiviral, antimicrobial, anticonvulsant,

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

antitubercular, and anti-inflammatory potential, with structure–activity studies revealing that modifications at the C-3 and N-1 positions considerably influence their biological activity[7]. Although CG has been widely used in traditional medicine, comprehensive scientific confirmation of its bioactive constituents is still lacking.

Even though preliminary phytochemical analyses have been conducted, extraction, purification, and structural characterization of isatin from the CG flowers using advanced techniques remain limited. Furthermore, few studies have investigated the relationship between isatin content and the antioxidant or therapeutic properties of the extracts, indicating an important research gap that this study seeks to fill.

MATERIALS AND METHODS

Preparation of CG Flower Dried Powder:



Figure 1: a) CG flower b) and c) Hot-air oven drying of separated CG flower samples, (d Dried CG powder

Fresh flowers of CG were collected from the garden of JSS Science and Technology University, Mysuru, India. To get rid of dust and debris, the flowers were rinsed with distilled water after being properly cleaned with tap water. To prevent microbial contamination and preserve sensitive metabolites, the flowers parts were separated and shade-dried for 48 to 72 hours at room temperature (25 to 30 °C). After that, the partially dried material was moved to a hot-air oven that was set to 50 °C. It was incubated for 24 to 48 hours to dry until its weight remained constant. Using a mechanical grinder, the dried flowers were crushed into a fine powder and kept at 4 °C in airtight amber glass containers until further studies. [8] (Figure 1).

Extraction of Bioactive Compounds:

Extraction was performed using the Soxhlet method, a continuous hot-percolation process considered reliable for isolating both polar and non-polar phytoconstituents. 10 g of CG flower powder sample was loaded into a thimble and placed in a Soxhlet extractor. Solvents of varying polarity—ethanol, ethyl acetate, chloroform, and distilled water—were used sequentially to ensure comprehensive extraction of compounds. Each extraction was continued for 6–8 hours until the solvent in the siphon tube became

colourless. These crude extracts were stored at 4 °C in sterile glass vials for further analysis [9].

Phytochemical Profiling:

Preliminary phytochemical screening of crude extracts prepared using different solvents—chloroform, ethyl acetate, ethanol, and water—was conducted to detect the presence of major secondary metabolites through standard qualitative assays. Alkaloids were detected with Wagner's and Mayer's reagents, flavonoids by NaOH and Shinoda tests, total polyphenols and tannins by ferric chloride test, glycosides by Keller–Killiani and Liebermann's tests, and saponins by observing persistent foam formation [10].

Quantitative estimation of antioxidant-related metabolites was carried out by determining total polyphenol content using the Folin–Ciocalteu (FC) assay with gallic acid as a reference standard, and absorbance was read at 660 nm. Tannin content was assessed using the FC reagent with tannic acid as the standard, measured at 700 nm. Reducing sugar levels were quantified through the DNS method, with absorbance recorded at 540 nm using a glucose calibration curve [10].

Isolation and Purification of Isatin:

For specific isolation of isatin, 10 g of dried CG flower powder was subjected to Soxhlet extraction using 100 mL of chloroform and Soxhlet was operated for 8 hours. After 8 hours of extraction, the chloroform was filtered. This CG crude extract was then passed through silica gel column chromatography, using methanol as the mobile phase at a flow rate of 3 mL/min. Fractions were collected, and a distinct violet-blue band corresponding to indole derivatives and reddish-purple color indicating indirubin was observed. This fraction was pooled, concentrated, and subjected to analytical confirmation. Subsequently, the violet-blue pigment was converted to isatin by mild acid treatment [5].

Characterization of indole derivatives and isatin:

To confirm the structural identity of the isolated isatin and its derivatives, multiple complementary analytical techniques were employed:

- **UV–Vis Spectroscopy**: Absorption spectra (200–800 nm) was recorded to identify characteristic chromophoric bands associated with indole derivatives (Shimadzu UV 1800).
- **FTIR Spectroscopy**: Functional groups were analyzed by recording infrared spectra from 4000 to 400 cm^{-1} to identify key bond vibrations such as carbonyl and aromatic groups (JASCO 4100 spectrometer, Japan).
- **Raman Spectroscopy**: Molecular vibrations were examined to further confirm the presence of characteristic functional groups in isatin from 3000 to 0 cm^{-1} (HR800-UV confocal micro-Raman spectrometer, France).
- **High-Performance Liquid Chromatography (HPLC):** A reverse-phase C18 column was used to quantitatively determine isatin using HPLC. The analysis utilized a mobile phase comprising acetonitrile and 0.1% phosphoric acid in water (10:90, v/v) at a flow rate of 1.0 mL/min. Detection was performed at a wavelength of 241 nm. To generate the calibration curve, standard isatin solutions ranging from 20 to 100 µg/mL were prepared. The peak area and retention time obtained from the sample chromatograms were compared with those of the standards to verify compound identity and estimate the isatin concentration. (Shimadzu HPLC system).

Antioxidant activity by DPPH method:

CG flower extracts and CG isatin was used for calculating % RSA (Radical Scavenging activity) using DPPH assay and the absorbance at 517 nm was noted in Shimadzu UV 1800 spectrophotometer. Ascorbic acid acid was used as the reference standard. Data analysis was performed using one-way ANOVA in IBM SPSS Statistics version 24. All experiments were carried out in triplicate, and the results are expressed as the mean ± standard deviation (SD). Statistical significance was determined at a p-value below 0.05.

RESULTS:

Phytochemical Profiling of CG Extracts using Various Solvents

Table 1: Phytochemical profiling of CG crude extracts

S. No	Phytochemical	Test	100%Ethanol	50% Ethanol	Dist. Water	Ethyl acetate
1.	Test for phenol	a) Ellagic Test	+	+	+	-
		b) Phenol Test	+	+	-	+
		c) Ferric Test	+	+	+	+
2.	Detection of Flavonoids	a) NaoH Test	+	+	+	+
		b) H2SO4 Test	-	+	+	+
3.	Test for Glycosides	a) Keller-Kilani Test	+	+	+	+
		b) Glycosidic Test	+	+	+	+
		c) Liberman's Test	+	+	-	+
		d) Salkowski's Test	+	+	+	+
4.	Detection of Alkaloids	a) Iodine Test	+	+	+	+
		b) Wanger Test	+	+	+	+
		c) Mayer's Test	-	+	+	+
5.	Detection of steroids	a) Steroid Test	+	+	+	+
6.	Detection of Tannins	a) Ferric Test	+	+	-	+
7.	Detection of saponins	a) Foam Test	-	-	-	-

Qualitative phytochemical analysis of CG extracts (ethanol, ethyl acetate, chloroform, and aqueous) revealed the presence of diverse classes of bioactive compounds (Table 1). Alkaloids, flavonoids, phenols, tannins, and glycosides were consistently detected across solvents, while saponins were absent. The results confirm the chemical richness of CG, aligning with previous reports that linked its pharmacological activities to multiple secondary metabolites.

Quantitative estimation of Polyphenols, Tannins, and Reducing Sugars:

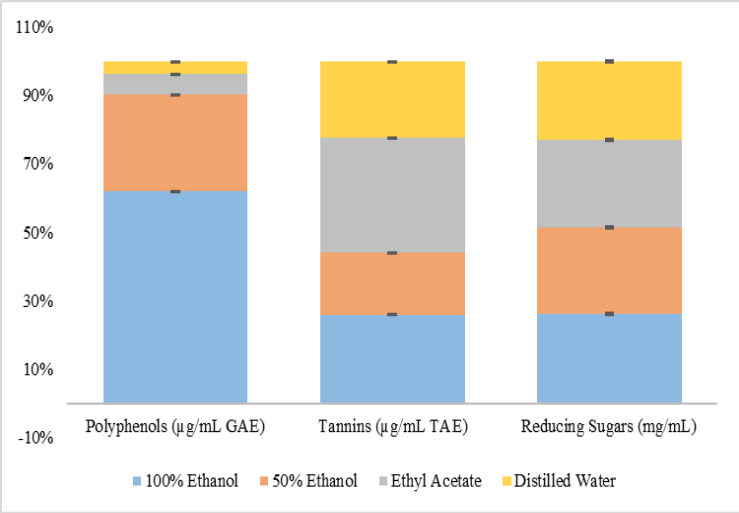


Figure. 2: Quantitative estimation of phytochemicals using CG extract

The quantitative assays provided further insights into the antioxidant potential of CG extracts. Ethanol (100%) yielded the highest polyphenol content (937.5 ± 0.047µg/mL gallic acid equivalent, GAE), while tannins were most abundant in ethyl acetate (81.75 ± 0.051µg/mL tannic acid equivalent, TAE). Reducing sugars were relatively consistent across solvents, with ethanol extract showing the highest concentration (8.86 ± 0.016 mg/mL) (Figure 2). These findings suggest that the best solvent for phenolic compound extraction is ethanol, while ethyl acetate preferentially extracts tannins. Such solvent-dependent variations reflect the polarity of metabolites. Since polyphenols and tannins are strong antioxidants, their presence supports the therapeutic use of CG in traditional medicine [8-10].

Characterization of indigo-type compound from CG flower extract:

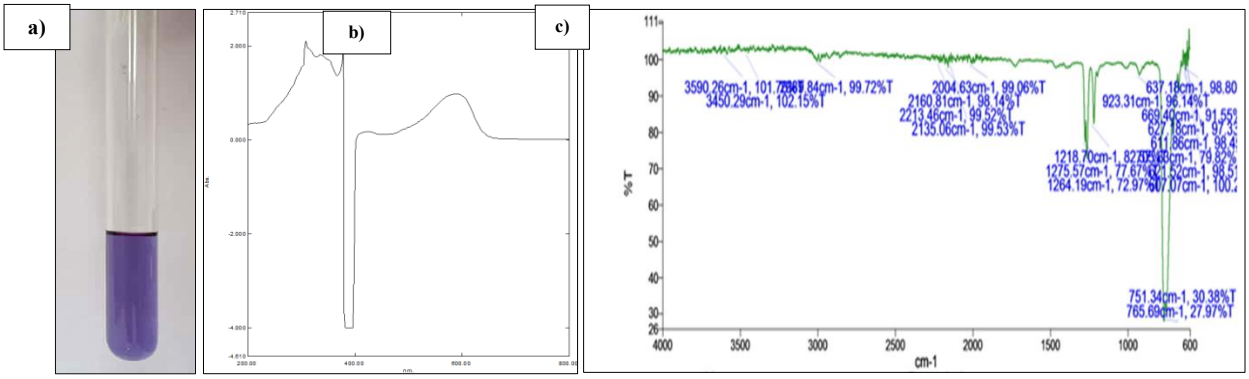


Figure 3: a) Indigo-type compounds are indicated by a distinct blue in the test tube, b) UV - Vis spectrum, and c) FTIR spectrum of Indigo from CG flower extract

. Following chromatography, the pigment molecules were successfully extracted and partially separated from the CG flower, as evidenced by the clear blue coloring that suggests the existence of indigo-type chemicals [12] (Figure 3a).

The chromophore's π–π* electronic transitions are shown by the broad UV absorption band in the UV-Vis spectra, which has peaks at about 300–350 nm. Baseline correction or scattering artifacts are reflected by a noticeable negative trough about 400 nm. The primary electronic transition that gives the compound its color is represented by a clear absorption peak in the visible spectrum that occurs between 580 and 620 nm [13] (Figure 3b).

The infrared (IR) spectrum serves as a molecular fingerprint, revealing the functional groups of the compound by their characteristic absorption bands. 4 sharp signals between 2004.63 cm^{-1} and 2213.06 cm^{-1} indicate the presence of a terminal alkyne group, corresponding to the stretching vibration of C≡C bond. This interpretation is supported by a signal near 627.13 cm^{-1}, attributed to the bending vibration of the alkyne's C-H bond. Additionally, a broad absorption peak near 3450.29 cm^{-1} suggests the presence of an O-H group. Moderate bands between 1264.19 cm^{-1} and 1275.57 cm^{-1} indicate a C–O bond, typical of esters or ethers. Two sharp valleys - 751.35 cm^{-1} and 765.69 cm^{-1}, where a benzene ring with two nearby hydrogen atoms is evident from the dramatic drop in IR transmission, which is a pattern common to ortho-disubstitution on an aromatic ring [12] (Figure 3c).

Characterization of Indirubin from CG flower extract:

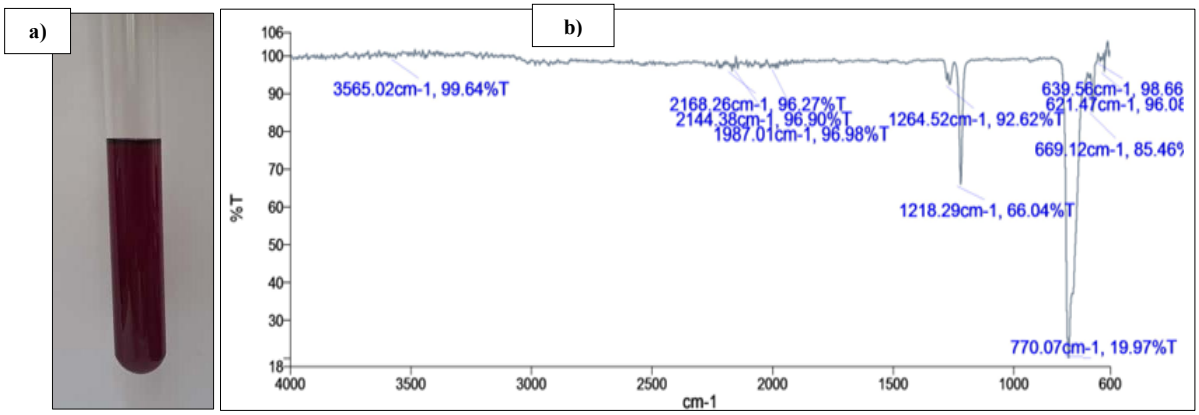
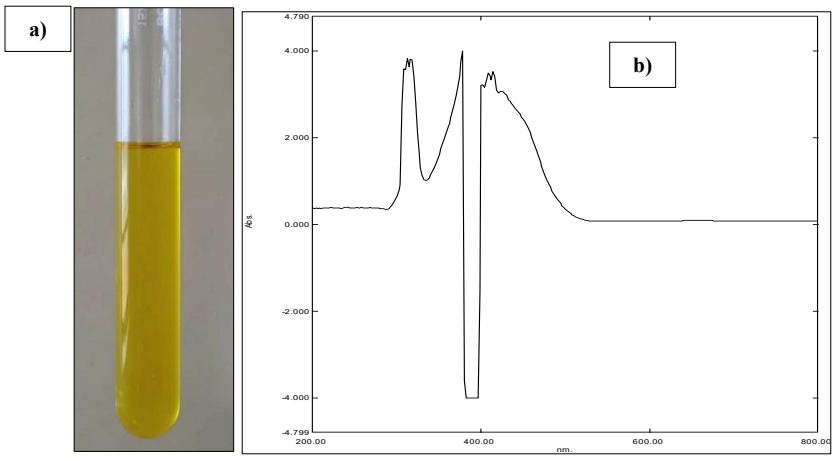


Figure 4: a) Deep reddish-purple fraction indicates the presence of Indirubin and b) FTIR spectrum of Indirubin from CG flower extract

. Indirubin, an isomer of indigo, is the deep reddish-purple portion found in the test tube. Indirubin, a recognized bioactive pigment found in CG flower extracts, is distinguished from indigo's blue-violet hue by its characteristic red-violet tint with absorbance maximum between 550–600 nm, indicating that it was adequately isolated [12,13] (Figure 4a).

The FTIR spectroscopic analysis of indirubin dye reveals characteristic vibrational peaks that correlate with its unique molecular structure and functional groups. The peak at 3565.02 cm^{-1} corresponds to the O–H stretching vibrations from the chelation structure involving hydrogen bonding between the carbonyl groups and NH groups, which is typical for indirubin's conjugated indole system. The absorptions at 2168.26 cm^{-1}, 2144.38 cm^{-1}, and 1987.01 cm^{-1} are associated with the stretching vibrations of the conjugated C=C, C=O, and N–H systems that form the extended π-conjugated framework characteristic of indirubin's bisindole structure. The peaks at 1264.52 cm^{-1} and 1218.29 cm^{-1} represent C–O stretching vibrations from the lactam rings and deformation vibrations of the aromatic C–H bonds within the indole moieties. Finally, the lower-wavenumber absorptions at 770.07 cm^{-1}, 669.12 cm^{-1}, 639.56 cm^{-1}, and 621.47 cm^{-1}correspond to out-of-plane bending of aromatic C–H bonds and skeletal vibrations of the substituted indole ring systems, confirming the presence of the characteristic bisindole backbone that distinguishes indirubin from its isomer indigo [12,14] (Figure 4b).

Characterization of Isatin from CG flower extract:



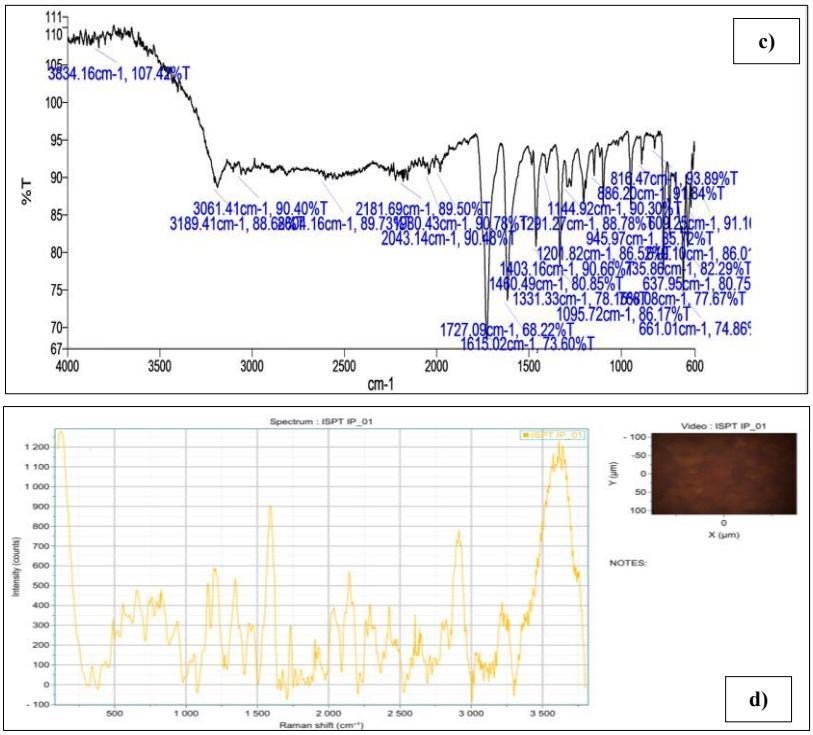


Figure 4: a) Yellow pigment indicates the presence of Isatin, b) UV - Vis spectrum, and c) FTIR spectrum of Isatin from CG flower extract.

The UV–Vis spectrum of cannonball tree fruit extract shows strong absorption peaks in the 280–350 nm range, characteristic of the indole and keto functional groups of isatin. A pronounced absorbance band near 410–440 nm in the visible region corresponds to π–π* and n–π* electronic transitions, confirming the presence of isatin and distinguishing it from related bioactive compounds [15, 16] (Figure 5a and Figure 5b).

The high-frequency N–H stretch at 3534 cm^{-1} is in line with known IR data for isatin's indole nitrogen. Peaks found at 3189 and 3061 cm^{-1} are consistently reported as aromatic and N–H stretching—key markers for isatin's fused ring and amide functionalities. The strong 1727 cm^{-1} absorption directly corresponds to the lactam/keto carbonyl stretch, typically documented between 1740–1620 cm^{-1} in canonical IR reports for isatin. Aromatic C=C stretches in the 1615–1400 cm^{-1} region and out-of-plane C–H bending at 886 cm^{-1} are likewise standard for indole and benzene ring systems, matching textbook and research assignments. Thus, the detected fingerprint peaks—N–H, aromatic C–H, lactam C=O, aromatic C=C, and C–H bending—are all justified and match the known vibrational assignments for isatin, unequivocally confirming its indole-2,3-dione identity[17] (Figure 5c).

The Raman spectrum reveals molecular vibrations through Raman shifts (cm^{-1}), with intensity indicating the strength of light scattering by bonds. Key observations include: signals at 500–1000 cm^{-1} arising from aromatic ring vibrations; peaks between 1200–1700 cm^{-1} corresponding to C=C stretching in aromatic rings and C=O groups, indicating isatin-like structures; C–H stretching vibrations from aromatic and aliphatic hydrogens appearing between 2800–3100 cm^{-1}; and a broad strong peak at 3200–3500 cm^{-1} attributed to –NH or –OH groups, confirming amine or hydroxyl functionalities. These features collectively support the presence of isatin extracted from CG flowers [17, 18] (Fig 5 Figure).

High-performance chromatography (HPLC) Quantification of Isatin:

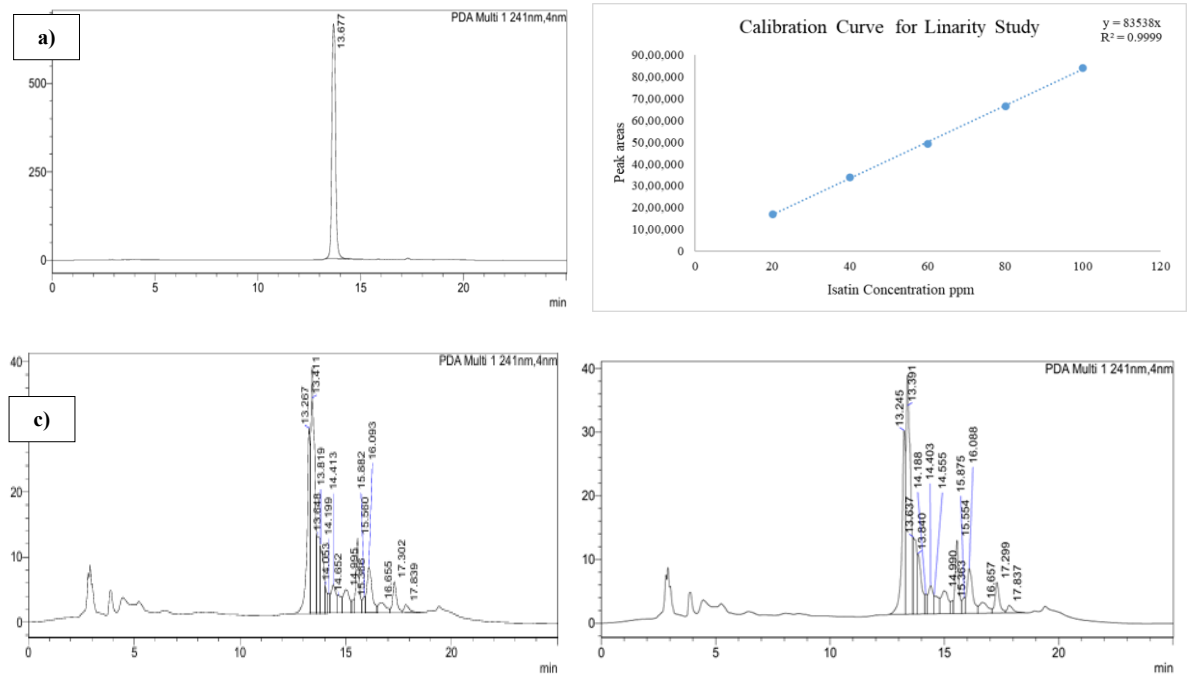


Fig.5: HPLC Chromatogram Showing a) Standard isatin at 100 ppm, b) Calibration curve of isatin showing linearity, c) and d) isatin from CG Extract at two different concentrations

. HPLC analysis of the analyte at concentrations of 20-100 ppm showed sharp, symmetrical peaks at a consistent retention time of 13.6-13.7 minutes, demonstrating good method precision and reproducibility (Figure 5 a and b). The isatin obtained from CG extract releaved 16 distinct peaks with retention times between 13.2-17.8 minutes. The most prominent peak appeared at 13.419 min (29.59% of total area), followed by peaks at 13.267 min (17.91%) and 13.731 min (7.25%). Additional significant peaks were observed at 15.306 min (7.58%) and 16.093 min (7.49%), indicating higher concentrations of these compounds. Minor constituents were detected at 14.052 min (1.42%) and 17.839 min (1.21%) (Fig 5 c and d). The retention time similarity to the standard suggests potential identification of isatin, though confirmation requires direct comparison. The average concentration of isatin obtained from CG extract was 5.99 ppm. This complex phytochemical profile reflects the natural diversity of bioactive compounds successfully resolved at 241 nm [19-20].

Antioxidant activity:

The DPPH assay was used to assess the scavenging activity of free radicals, utilizing DPPH as a stable free radical. The IC50 value of CG flower extract was found to be 255 ± 0.36 µg/mL, whereas CG isatin exhibited an IC50 of 101.7 ± 0.1 µg/mL. For standard ascorbic acid, the IC50 was 203.58 µg/mL [4, 5, 15].

DISCUSSION:

By evaluating the phytochemical profile and pharmaceutical properties of Couroupita guianensis Aubl. supports its traditional applications and underscores its substantial ethnobotanical significance. For ages, people in tropical areas have utilized CG to treat a range of illnesses, such as wounds, inflammatory problems, and skin infections. Modern scientific research, which is methodically investigating the bioactive substances responsible for these effects, is now supporting this vast body of traditional knowledge [2, 10].

Overall, the phytochemical profile of CG and its assortment of pigments, including isatin, indirubin, and indigo, are highlighted in this work. Isatin, a derivative of indole, is found in trace levels in certain microbes, and plants like CG and Isatis tinctoria.

Its existence in animals is associated with the metabolism of tryptophan, where it serves as a

signaling molecule and possible disease biomarker [7]. The molecule has carbonyl groups at positions two and three and is made up of a pyrrole ring fused with a benzene ring. Because of its high reactivity, this 1,2-dicarbonyl system allows condensation processes and makes it easier to synthesize colored compounds like indigo and indirubin [17]. In this investigation, the indigo fractions derived from the crude extract were used to create the isatin molecules.

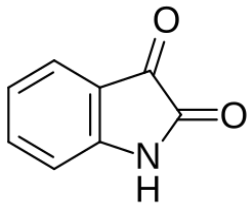


Fig 6: Chemical structure of Isatin (1H-indole-2,3-dione, $C_8H_5NO_2$)

Also, the diverse pharmacological and biomedical potentials of Couroupita guianensis, ranging from anthelmintic, antiulcer, CNS depressant, to green synthesis of silver nanoparticles (AgNPs). For example, Elumalai et al. (2012) reported the ethanolic leaf extract's antiulcer activity in rat models, with marked reductions in ulcer index, gastric volume, and acidity, suggesting antisecretory mechanisms confirmed by pyloric ligation and ethanol-induced ulcer models[21,22]. Furthermore, Rumzhum et al. (2012) provided pharmacological evidence of CNS depressant and analgesic activities of the crude ethanolic flower extract in mice, with dose-dependent sedative effects and significant writhing inhibition, supporting traditional claims of central nervous system involvement [23]. In a green chemistry approach, Inbathamizh et al. (2021) successfully synthesized and characterized spherical AgNPs (4.44–40.20 nm) using leaf extract as a reductant and stabilizing agent; the biosynthesized AgNPs showed promising properties favoring eco-friendly scalable production [24].Across these studies, traditional uses of Couroupita guianensis for ailments like intestinal worms, inflammation, and gastric disorders find scientific validation through phytochemical activity and advanced biotechnological applications, promising potential for future therapeutic and nanotechnological developments.

Insight response to the development of edible pigments. In this study, we attempted to obtain results with respect to indigo, indirubin, and isatin standards. The comparative analysis of these compounds provided valuable insights into their stability, color intensity, and potential as natural pigment candidates. The findings indicate that optimized extraction and purification parameters can enhance pigment yield suitable for drug delivery applications [12 -14].

ACKNOWLEDGEMENT:

The authors gratefully acknowledge the infrastructure support provided by JSS Science and Technology University (JSS STU), Mysuru, which greatly facilitated this work.

REFERENCES

1. Jagannatha S, Maity S, Santra A, Kuruvalli G, Reddy VD and Golla R: An Updated Review on Ethnomedicinal Uses Phytochemistry and Pharmacological Activities of Couroupita guianensis, Pharmacognosy Reviews (2024), 18(36):111-116.
2. Sundararajan R and Koduru R: A complete profile on Couroupita guianensis-traditional uses, pharmacological activities and phytoconstituents, Pharmacophore (2014), 5(1):147-159.
3. Sheba LA, Anuradha V, Ali MS and Yogananth N: Wound healing potential of Couroupita guianensis Aubl. Fruit pulp investigated on excision wound model, Applied Biochemistry and Biotechnology (2023), 195(11):6516-6536.
4. Kekuda TP: Antimicrobial, radical scavenging, and insecticidal activity of leaf and flower extracts of Couroupita guianensis Aubl, International Journal of Green Pharmacy (2017), 11(03):1-10.
5. Premanathan M, Radhakrishnan S, Kulangiappar K, Singaravelu G, Thirumalaiarasu V, Sivakumar T and Kathiresan K: Antioxidant & anticancer activities of isatin (1H-indole-2, 3-dione), isolated from the flowers of Couroupita guianensis Aubl, Indian Journal of Medical Research (2012), 136(5):822-826.
6. Sathishkumar G, Jha PK, Vignesh V, Rajkuberan C, Jeyaraj M, Selvakumar M, Jha R and Sivaramakrishnan S: Cannonball fruit (Couroupita guianensis, Aubl.) extract mediated synthesis of gold nanoparticles and evaluation of its antioxidant activity, Journal of Molecular Liquids (2016), 215:229-236.

7. Kakkar R: Isatin and its derivatives: a survey of recent syntheses, reactions, and applications, MedChemComm (2019), 10(3):351-368.
8. Liu Z and Fang Y: Wound healing and signaling pathways, Open Life Sciences (2025), 20(1):20251166.
9. Augusco MA, Sarri DA, Panontin JF, Rodrigues MA, Fernandes RD, Silva JF, Cardoso CA, Rambo MK and Scapin E: Extracts from the leaf of Couroupita guianensis (Aubl.): phytochemical, toxicological analysis and evaluation of antioxidant and antimicrobial activities against oral microorganisms, Plants (2023), 12(12):2327.
10. More A and Chaulkar P: Physicochemical and phytochemical studies on leaves of Couroupita guianensis Aubl, Journal of Pharmacognosy and Phytochemistry (2023), 12(6):125-129.
11. Venkatasubbaiah R, Jha PK and Sanjay KR: Centella asiatica crop residue fabricated silver nanoparticles as potent antioxidant agents in photo-catalytic degradation of hazardous dyes, Chemical Engineering Communications (2022), 209(7):938-956.
12. Ju Z, Sun J and Liu Y: Molecular structures and spectral properties of natural indigo and indirubin: experimental and DFT studies, Molecules (2019), 24(21):3831.
13. Laitonjam WS and Wangkheirakpam SD: Comparative study of the major components of the indigo dye obtained from Strobilanthes flaccidifolius Nees. and Indigofera tinctoria Linn, International Journal of Plant Physiology and Biochemistry (2011), 3(7):108-116.
14. Mahltig B: Overview on natural dyes and their IR-spectra–Part II: Indigo containing plant dyes, Communications in Development and Assembling of Textile Products (2024), 5(1):66-80.
15. Fikriya SH and Cahyana AH: Study of antioxidant activity of the derivatives of quinoline-4-carboxylic acids by the modification of isatin via pfitzinger reaction, Makara Journal of Science (2023), 27(2):9-15.
16. Tisovský P, Horváth M, Csicsai K, Donovalová J, Filo J, Cigáň M, Sokolík R, Addová G and Gáplovský A: Isatin-1, 8-naphthalimide hydrazones: A study of their sensor and on/off functionality, Molecules (2019), 24(3):397.
17. Tisovský P, Šandrik R, Horváth M, Donovalová J, Filo J, Gáplovský M, Jakusová K, Cigáň M, Sokolík R and Gáplovský A: Effect of structure on charge distribution in the isatin anions in aprotic environment: spectral study, Molecules (2017), 22(11):1961.
18. Kumar BR: Application of HPLC and ESI-MS techniques in the analysis of phenolic acids and flavonoids from green leafy vegetables (GLVs), Journal of Pharmaceutical Analysis (2017), 7(6):349-364.
19. Skaikar R, Younes O, Arous B and Al-Mardini A: Synthesis and identification of a novel N-Mannich base from Isatin using a Sonicator by adding DCM as catalyst, Research Journal of Pharmacy and Technology (2024), 17(10):4851-4855.
20. de A Cavalcante M, dos S Oliveira J, da S Barreto MS, Pinheiro LP, de C Cantuária P, Borges WL, da Silva GA and de Souza TM: An HPLC method to determine phenolic compounds of plant extracts: application to Byrsonima crassifolia and Senna alata leaves, Pharmacognosy Research (2022), 14(4):340-348.
21. Uppala PK, Krishna M, Kumar BK and Ramji DJ: Evaluation of anthelmintic activity of the chloroform and aqueous extracts of leaves of Couroupita guianensis on Pheretima posthuma by worm motility assay method, Research Journal of Pharmacology and Pharmacodynamics (2016), 8(3):118-122.
22. Rumzhum NN, Rahman M and Islam AF: CNS Depressant Effect of Crude Ethanolic Extract of Flowers of Couroupita guianensis Aubl. in Swiss-albino Mice, Research Journal of Pharmacy and Technology (2012), 5(5):615-618.
23. Inbathamizh L, Kalpana V and Yadav KR: Synthesis and Characterization of Silver Nanoparticles from Couroupita guianensis leaf extract–A Simple Green Route, Research Journal of Pharmacy and Technology (2021), 14(5):2796-2800.

HOW TO CITE: Rashmi V, Tejaswini S J, Prakash Kumar Jha, Analysis of Phytochemicals, Pigment Composition, Isatin Quantification, and Antioxidant Potential in Couroupita guianensis Flowers, J. Pharm. Sci., 2026, 2 (3), 591-599. https://doi.org/10.5281/zenodo.19309374