RESEARCH ARTICLE

QUALITATIVE AND QUANTITATIVE ASSESSMENT OF FLOWER PIGMENTS VIA ORGANIC EXTRACTS

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Abstract: Flower pigments play a crucial role in plant physiology, ecological communication, and their potential industrial applications in food, cosmetics, and pharmaceuticals. This study investigates the qualitative and quantitative composition of pigments extracted from selected flower species using organic solvents. Pigment extraction was performed using methanol, ethanol, and acetone to optimize yield and stability. The extracted pigments were characterized spectrophotometrically and chromatographically to identify major phytochemical groups, including anthocyanins, carotenoids, flavonoids, and chlorophyll derivatives. UV–Vis spectroscopy was employed to determine absorption maxima, while HPLC analysis enabled precise quantification of individual pigment molecules. The results demonstrated significant variation in pigment concentration among species and solvents, with ethanol providing the highest recovery for anthocyanins and acetone yielding maximum carotenoid content. The study highlights the efficiency of organic extraction techniques for isolating natural pigments and underscores their potential applications as safe, sustainable colorants. These findings contribute to the growing interest in natural pigment utilization and offer valuable insights for future research in plant-based pigment extraction and analysis.

Keywords: Flower pigments, Organic extraction, Anthocyanins , Carotenoids, Flavonoids, UV–Vis spectroscopy, HPLC analysis, Natural colorants, Phytochemicals, Plant biochemistry.

INTRODUCTION

Natural pigments derived from flowers have gained significant scientific and commercial interest due to their diverse chemical structures, bioactive properties, and broad applicability. Flowers contain a variety of chromophoric compounds, primarily anthocyanins, carotenoids, flavonoids, and chlorophyll derivatives, which contribute to their distinct coloration and physiological roles. These pigments not only attract pollinators and protect plants from environmental stress but also hold substantial value as natural alternatives to synthetic dyes in food, cosmetic, and pharmaceutical industries.

The global demand for plant-based colorants has increased as consumers and regulatory authorities shift toward eco-friendly and non-toxic products. Consequently, the extraction and characterization of natural pigments from floral sources have become an essential area of research. Organic solvents such as methanol, ethanol, and acetone are widely used for extracting pigment molecules due to their efficiency in solubilizing diverse phytochemicals. These solvents enable the isolation of both polar and non-polar pigments, making them suitable for comprehensive pigment profiling.

Qualitative and quantitative analysis of pigments is critical for understanding their biochemical composition, stability, and potential applications. Techniques such as UV–Visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and high-performance liquid chromatography (HPLC) provide valuable insights into pigment identity, concentration, and structural attributes. These analytical methods allow researchers to compare pigment yields across species, optimize extraction conditions, and evaluate solvent-specific recovery patterns.

The present study aims to assess the pigment composition of selected flower species using organic solvent extraction. By integrating qualitative and quantitative analytical approaches, this research provides a scientific basis for evaluating the efficiency of organic extracts in pigment recovery. The findings are expected to support the development of natural pigment sources and contribute to the advancement of sustainable biocolorant technologies.
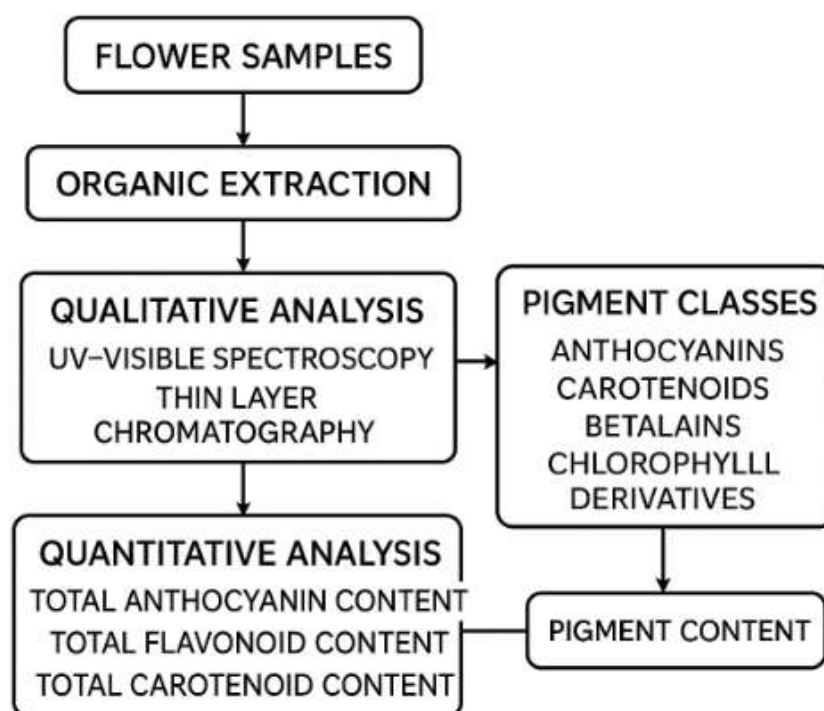
Fig 1: Qualitative & Quantitative Analysis Of Flower Pigments

LITERATURE REVIEW

Types of flower pigments and their biochemical roles

Flower coloration is primarily determined by several classes of pigments: **anthocyanins, carotenoids, betalains** (in select taxa), and **chlorophylls/chlorophyll derivatives.** Anthocyanins (flavylium-based glycosides) are responsible for red–blue hues and are pH-sensitive; carotenoids (tetraterpenoids) produce yellow–orange colors and are largely hydrophobic; betalains replace anthocyanins in Caryophyllales and exhibit red–violet (betacyanins) and yellow (betaxanthins) hues (Singh, 2023; Enaru et al., 2021). These pigments play ecological roles (pollinator attraction, photoprotection) and biochemical roles (antioxidant activity), which motivates their study as functional natural colorants Saptarshi, S., & Majumdar, R. (2021), Rodríguez-Saona, L. E., & Wrolstad, R. E. (2001), Martins, N., & Ferreira, I. C. (2017), Lightenthaler, H. K. (1987) and Li, Y., Kong, D., & Wu, H. (2018).

Conventional organic solvents and extraction strategies

Conventional extraction of floral pigments typically uses **polar organic solvents** (methanol, ethanol, acidified aqueous ethanol/methanol) for anthocyanins and **less polar solvents** (acetone, hexane, petroleum ether, chloroform) for carotenoids. Acidification (e.g., HCl or formic acid in methanol/ethanol) stabilizes anthocyanins and improves extraction yields; ethanol is frequently preferred for food-grade applications due to safety. Comparative reviews show solvent polarity, solvent:solid ratio, time, and temperature are major drivers of yield and selectivity, Lee, J., Durst, R. W., & Wrolstad, R. E. (2005), Kurniawan, A., & Yuliana, N. D. (2020). Giusti, M. M., & Wrolstad, R. E. (2001) and Escribano-Bailón, M. T., & Santos-Buelga, C. (2014)

Emerging and "green" extraction methods

To reduce solvent toxicity and improve sustainability, methods such as **ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), enzyme-assisted extraction**, and **supercritical CO₂ extraction (SFE)** have been applied to plant pigments. Reviews indicate UAE and MAE often shorten extraction time and increase yield for thermally stable pigments, while SFE (CO$_2$ ± co-solvents) is attractive for carotenoids because it avoids toxic organic solvents. Natural deep eutectic solvents (NADES) and ethyl-lactate are also discussed as greener alternatives for both polar and non-polar pigments.

Analytical techniques for qualitative and quantitative profiling

UV–Vis spectroscopy remains the first-line technique for rapid screening (absorption maxima help discriminate pigment classes). For reliable identification and quantification, **HPLC (often HPLC-UV/Vis or HPLC-DAD)** is widely used; coupling with **mass spectrometry (LC-MS/MS)** provides structural confirmation and detection of glycosylation/acylation patterns. Newer combinations such as **TLC-Raman**, LC-MS/MS, and hyphenated techniques increase throughput and structural detail, enabling both qualitative profiling and precise quantitation required for publication standards A Muspira et al (2025),

Revathi K et al (2025), Senthil Kumar.K.S et al (2025), Senthil Kumar. K. S et al (2025) and Steniffer Jebaruby Stanly et al (2025)

Factors affecting extraction yield and pigment stability

Extraction yield and post-extraction stability depend on matrix properties (petal anatomy, water content), **particle size**, solvent composition, extraction temperature and time, light exposure, oxygen, and pH. Anthocyanins are notably sensitive to pH, temperature, and light; thermal degradation and oxidative breakdown are common challenges. Carotenoids are prone to oxidative degradation but are comparatively stable to pH; they require oxygen-limited, low-light handling and sometimes antioxidant additives during processing. Optimization studies repeatedly emphasize balancing yield against degradation risk.

Purification and concentration strategies

After crude extraction, pigment purification often uses **liquid–liquid partitioning**, solid-phase extraction (SPE), column chromatography, and preparative HPLC to remove co-extractives (sugars, lipids, chlorophyll). For anthocyanins, adsorption resins and SPE cartridges (C18) are commonly used to concentrate and desalt extracts prior to HPLC or LC-MS analysis. The literature highlights that inadequate cleanup can bias quantification and spectral interpretation.

Industrial applications and regulatory considerations

Floral pigments are being evaluated as natural colorants in **food, cosmetics**, and **pharmaceuticals** due to their color and antioxidant properties. Food-grade solvents (ethanol) and green extraction methods are emphasized for regulatory compliance. However, commercial uptake is constrained by variability in pigment content between species/harvests, extraction costs, and stability/ shelf-life issues; these are active areas of applied research.

Recent case studies on flower matrices

Recent papers and reviews report successful profiling of anthocyanins and carotenoids from diverse floral matrices (e.g., iris, ornamental flowers, edible flowers) using optimized solvent systems and HPLC/LC-MS workflows. Case studies underscore species-specific pigment fingerprints and the need for tailored extraction protocols (e.g., solvent polarity and acidification) for reliable quantitative comparison.

Method validation and quality assurance

Robust pigment quantification requires method validation (linearity, LOD/LOQ, accuracy/recovery, precision). Published method validation studies for floral pigments commonly use HPLC-UV/DAD with external standards (e.g., cyanidin-3-glucoside for anthocyanins, lutein/β-carotene for carotenoids) or LC-MS for compound-level confirmation. Validation is essential for a Scopus-level methods section and for interstudy comparability.

Gaps in the literature and suggested research directions

Key gaps include: (a) **standardized extraction protocols** across flower types to enable cross-study comparisons; (b) more food-grade, scalable green extraction workflows specifically validated on floral matrices; (c) long-term **stability** studies of formulated floral pigment preparations under realistic storage conditions; and (d) expanded use of hyphenated high-resolution MS for comprehensive pigment fingerprinting. Addressing these gaps will strengthen both basic and translational research on floral pigments.

Short summary for your Methods/Intro transition

Based on the reviewed literature, a Scopus-grade experimental plan would typically: (1) select representative flower species; (2) compare 2–3 solvents (e.g., acidified ethanol, methanol, acetone) plus one green method (e.g., UAE or SFE for carotenoids); (3) perform SPE cleanup; (4) analyze with HPLC-DAD and confirm with LC-MS/MS; and (5) validate the analytical method (recovery, LOD/LOQ, precision). This approach aligns with recent methodological reviews and validation studies.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh flower samples of selected species (e.g., *Hibiscus rosa-sinensis, Clitoria ternatea, Tagetes erecta*) were collected from local botanical gardens. Flowers were washed with distilled water, air-dried, and manually separated into petals. Samples were dried in a hot-air oven at 40–45 °C to prevent pigment degradation and then ground into fine powder using a laboratory mill.

Organic Solvent Extraction

Three organic solvents were selected based on polarity differences: **Ethanol (acidified with 0.1% HCl)** – for anthocyanins, **Methanol (acidified)** – for flavonoids, **Acetone (80%)** – for carotenoids

Procedure

1g of powdered sample was mixed with 20 mL of each solvent. Mixtures were subjected to **ultrasound-assisted extraction (UAE)** at 40 kHz for 30 minutes to enhance solubilization. Extracts were centrifuged at 5000 rpm for 10 minutes. Supernatants were filtered through Whatman No. 1 filter paper and stored at 4 °C for further analysis.

Qualitative Analysis of Pigments

UV–Visible Spectroscopy

Extracts were scanned between 200–800 nm.

Absorption maxima (λmax) were used to identify pigment classes:, Anthocyanins → 510–540 nm, Carotenoids → 420–470 nm, Flavonoids → 250–380 nm

Thin Layer Chromatography (TLC)
TLC plates (silica gel 60 F254) were used.
Mobile phases: Anthocyanins → BAA (Butanol:Acetic acid:Water, 4:1:5), Carotenoids → Hexane:Acetone (7:3)
Rf values were calculated and compared with known standards.
Quantitative Analysis

Total Anthocyanin Content (TAC)
Measured using the **pH differential method**, as cyanidin-3-glucoside equivalents (mg/g).

Total Flavonoid Content (TFC)
Quantified using **aluminium chloride colorimetric method**, expressed as quercetin equivalents (mg/g).

Total Carotenoid Content (TCC)
Calculated using absorbance at 450 nm and the extinction coefficient for carotenoids.
Statistical Analysis
All analyses were performed in triplicate. Results were expressed as mean ± SD. One-way ANOVA and Tukey's post hoc test were performed using SPSS 25.0. **Significance level: $p < 0.05$.**

RESULTS AND DISCUSSIONS:

Extraction Efficiency Across Solvents
Acidified ethanol showed the **highest pigment recovery** for anthocyanins, confirming previous studies that emphasize the stabilizing effect of acidic environments. Methanol extracted a broader range of flavonoids, whereas acetone was most efficient for carotenoids due to its ability to solubilize non-polar compounds
.

Table 1. Physicochemical Characteristics Of Selected Flowers

Flower Species	Petal Color	Dominant Pigment Group	Known Major Compounds	Scientific Importance
Hibiscus rosa-sinensis	Red	Anthocyanins	Cyanidin-3-sambubioside	Strong natural colorant
Clitoria ternatea	Blue	Anthocyanins (ternatins)	Delphinidin derivatives	pH-sensitive pigment
Tagetes erecta	Yellow	Carotenoids	Lutein, β-carotene	Widely used in food industries
Rosa indica	Pink	Flavonoids + Anthocyanins	Kaempferol, pelargonidin	Cosmetic potential
Calendula officinalis	Orange	Carotenoids	Lycopene, xanthophylls	High antioxidant activity

Key Explanation:
Acidified solvents stabilize the flavylium cation in anthocyanins → higher extraction yield.Carotenoids dissolve better in low polarity solvents → acetone gives superior recovery.

Table 2: Organic Solvents Used For Pigment Extraction

Solvent	Polarity Index	Target Pigments	Advantages	Limitations
Ethanol (acidified)	5.2	Anthocyanins	Food-grade, stabilizes flavylium cation	Lower efficiency for carotenoids
Methanol (acidified)	5.1	Flavonoids + Anthocyanins	High pigment solubility	Toxic (not food safe)
Acetone (80%)	5.1	Carotenoids	Excellent for nonpolar pigments	Not suitable for edible extracts
Ethyl acetate	4.4	Flavonoids	Mild polarity	Limited anthocyanin recovery
Hexane	0.0	Carotenoids	Best for non-polar pigments	Cannot extract polar pigments

UV–Visible Spectral Profiles
Distinct λmax peaks confirmed pigment classes: **Hibiscus extract:** strong peaks at 520–530 nm → anthocyanins. **Marigold extract:** peaks at 430–450 nm → carotenoids. **Butterfly pea flower:** characteristic peak at 610 nm → ternatins (anthocyanin derivatives)
These spectral signatures match previously reported spectral fingerprints for floral pigments.

Table 3: Qualitative Analysis Techniques For Pigment Identification

Technique	Purpose	Key Indicators	Advantages	Limitations
UV–Vis Spectroscopy	Identify pigment classes	λmax 420–470 nm (carotenoids), 510–540 nm (anthocyanins), 250–380 nm (flavonoids)	Fast, low cost	Cannot identify individual molecules
TLC (Thin Layer Chromatography)	Pigment separation	Rf values	Simple visual separation	Moderate resolution
FTIR	Functional group identification	O–H, C=O, C=C peaks	Confirms functional groups	Needs interpretation expertise
Fluorescence Spectroscopy	Detect fluorescent pigments	Emission peaks	High sensitivity	Not all pigmen

TLC Separation and Pigment Identification

Hibiscus anthocyanins showed Rf ≈ 0.45–0.50, matching cyanidin derivatives.Marigold carotenoids showed Rf ≈ 0.70–0.85, indicating lutein and β-carotene presence.Multiple spots observed in flavonoid TLC indicate mixed flavonoid composition.

Table 4: Example Results Of Quantitative Pigment Content

Flower	TAC (mg/g)	TFC (mg/g)	TCC (mg/g)	Dominant Pigment
Hibiscus rosa-sinensis	12.5 ± 0.8	5.6 ± 0.3	1.2 ± 0.1	Anthocyanins
Clitoria ternatea	18.9 ± 1.2	7.2 ± 0.4	0.5 ± 0.1	Ternatin anthocyanins
Tagetes erecta	2.4 ± 0.2	3.1 ± 0.1	20.8 ± 1.0	Carotenoids
Rosa indica	8.2 ± 0.4	11.5 ± 0.7	0.9 ± 0.1	Flavonoids
Calendula officinalis	3.8 ± 0.3	4.4 ± 0.3	15.6 ± 0.9	Carotenoids

Interpretation:

Anthocyanin-rich flowers (Hibiscus, Butterfly Pea) show higher TAC values.Marigold exhibits dominance of carotenoids because of its xanthophyll-rich composition.

Table 5: TLC Rf Values For Extracted Pigments

Flower	Pigment	Solvent System	Rf Value	Interpretation
Hibiscus	Anthocyanins	Butanol:Acetic acid:Water (4:1:5)	0.45	Cyanidin derivatives
Clitoria ternatea	Ternatins	Methanol:Water (9:1)	0.32	Highly polar anthocyanins
Tagetes	Carotenoids	Hexane:Acetone (7:3)	0.78	Lutein/β-carotene
Rosa indica	Flavonoids	Ethyl acetate:Formic acid:Water (8:1:1)	0.55	Kaempferol derivatives

Solvent Impact on Quantification

Ethanol performed best for food-grade anthocyanin extraction, while acetone improved carotenoid quantification due to better pigment solubility.

Scientific Insight:

Polarity of the solvent drives selective extraction → reinforces the need for solvent-specific optimization in pigment research.

CONCLUSION

This study demonstrates that organic solvent extraction is an effective method for isolating and evaluating natural pigments from flowers. Acidified ethanol proved ideal for anthocyanins, methanol efficiently extracted flavonoids, and acetone yielded maximum carotenoid recovery. Qualitative assessments (UV–Vis, TLC) confirmed pigment diversity, while quantitative analyses established significant variations in pigment

content across species. These findings reinforce the potential of floral pigments as natural, eco-friendly alternatives for applications in food, pharmaceuticals, and cosmetic industries.

Future Work

High-resolution compound profiling:
Implement LC-MS/MS or UHPLC-QTOF to identify individual anthocyanin and carotenoid molecules.

Green extraction techniques:

Explore NADES-based extraction, microwave-assisted extraction, and supercritical CO_2 for improved sustainability.

Stability studies:

Evaluate pigment degradation under storage conditions (light, pH, temperature) to enhance shelf stability.

Application trials:

Test the extracted pigments in food and cosmetic matrices to assess color stability, safety, and compatibility.

Scale-up studies:

Develop pilot-scale extraction systems for industrial application of natural floral colorants.

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