

HIBISCUS POLYPHENOLS AND ANTHOCYANINS: ISOLATION, CHARACTERIZATION, AND PHARMACOGNOSTIC SIGNIFICANCE—A FOCUS ON *HIBISCUS SABDARIFFA* AND *HIBISCUS ROSA-SINENSIS*

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ABSTRACT

Introduction: This review examines the phytochemical composition, extraction methods, pharmacological activities, quality-control requirements, and safety considerations of *Hibiscus sabdariffa* and *Hibiscus rosa-sinensis*. **Objective:** To compare the major phytochemical constituents, extraction strategies, analytical characterization techniques, pharmacological evidence, and quality-control parameters between the two species to identify research gaps and translational opportunities. **Materials and Methods:** A narrative synthesis of botanical, pharmacognostic, analytical, and biomedical literature was conducted to compare major constituents, extraction strategies, characterization techniques, and reported biological effects across both species. **Findings:** *H. sabdariffa* contains abundant anthocyanins, especially delphinidin- and cyanidin-based sambubiosides, along with phenolic acids and organic acids that support antioxidant and cardiovascular activity. *H. rosa-sinensis* shows broader cultivar- and part-dependent variation, with anthocyanins, flavonoids, phenolic acids, and triterpenoids reported in flowers and other tissues. Extraction efficiency depends on solvent choice, temperature, pH, and processing conditions, while HPLC and LC–MS remain the most reliable methods for standardization. Preclinical studies suggest antioxidant, anti-inflammatory, antimicrobial, metabolic, and anticancer potential, with the strongest human evidence supporting roselle's blood-pressure-lowering effect. **Conclusion:** *Hibiscus sabdariffa* has the clearest translational promise, but both species require standardized extracts, stronger clinical evidence, and more complete safety evaluation to support regulatory acceptance and wider therapeutic applications in evidence-based healthcare.

KEYWORDS: Anthocyanins; flavonoids; roselle; China rose; extraction; pharmacognosy; phytopharmaceuticals.

1. INTRODUCTION

The genus *Hibiscus* (Malvaceae) comprises several hundred species, many of which are used as food plants, ornamentals, traditional medicines, and sources of natural colourants. *Hibiscus sabdariffa* is widely cultivated for its fleshy red calyces, which are consumed as infusions, beverages, foods, and traditional remedies. *Hibiscus rosa-sinensis* is primarily cultivated as an ornamental plant, but its petals, leaves, roots, and flowers have also been used in Ayurvedic and other traditional medical systems. Their contemporary scientific interest derives from the presence of anthocyanins, flavonoids, phenolic acids, organic acids, triterpenoids, and related metabolites.^[1-4]

The therapeutic rationale for these species is biologically plausible but heterogeneous. Anthocyanins may contribute to antioxidant and vasorelaxant effects, while organic acids and other phenolics may influence metabolic and inflammatory pathways. Nevertheless, chemical composition varies substantially among cultivars and plant parts, and extract activity depends on preparation conditions. This review therefore emphasizes the relationship between phytochemical identity, extraction technology, pharmacological evidence, and pharmacognostic standardization.^[1,24,25,30,45]

2. Botanical and Pharmacognostic Overview

2.1 *Hibiscus sabdariffa*

Hibiscus sabdariffa is an annual or short-lived herb cultivated in tropical and subtropical regions. The medicinal and food material is principally the dried fleshy calyx, although leaves, seeds, and other tissues are also investigated. Macroscopically, the calyces are commonly red to deep crimson, fleshy, and sour. Microscopic characters, including epidermal features, stomata, trichomes, vascular tissues, and calcium oxalate structures, can support authentication and detection of adulteration.^[15,16]

2.2 *Hibiscus rosa-sinensis*

Hibiscus rosa-sinensis is a perennial ornamental shrub with large, showy flowers occurring in several colour forms. Petal colour reflects differences in anthocyanin composition and concentration. Because commercial and traditional materials may include flowers, leaves, roots, or mixed plant parts, botanical authentication and clear definition of the drug part are essential. The plant has also been reviewed as a possible forage resource, although nutritional and toxicological conclusions should not be transferred automatically to human medicinal use.^[17,18]

3. Phytochemical Profile

3.1 Anthocyanins and Polyphenols

Roselle calyces contain abundant anthocyanins, with delphinidin-3-*O*-sambubioside and cyanidin-3-*O*-sambubioside frequently reported as principal pigments. Delphinidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside may occur at lower concentrations. Phenolic acids such as chlorogenic, caffeic, and protocatechuic acids, and flavonoids including quercetin, kaempferol, and myricetin, contribute to the broader phenolic profile.^[19-22]

China rose contains cyanidin- and delphinidin-derived anthocyanins, flavonoids, phenolic acids, and triterpenoids. The relative abundance of these constituents is influenced by petal colour, cultivar, developmental stage, extraction solvent, and analytical platform. Comparisons between the species should therefore be interpreted cautiously because total-anthocyanin values obtained by different assays are not necessarily equivalent.^[17,23,34]

3.2 Organic Acids and Other Constituents

Roselle is characteristically acidic and contains hibiscus acid, hydroxycitric acid, citric acid, and malic acid. These compounds contribute to flavour and may modify pigment stability and extract pH. The plant matrix also contains carbohydrates, minerals, vitamins, and volatile or lipid-associated constituents. Chemical abundance is not a surrogate for pharmacological efficacy; marker compounds should be selected according to identity, stability, biological relevance, and validated quantification.^[2,9,15,22,25]

Table 1: Principal phytochemical classes reported in the reviewed *Hibiscus* species.^[1,5-7,10,11,17,21,25]

Sr.No	Target	Representative constituents	Preferred analytical approaches
1.	Anthocyanins	Delphinidin-3-O-sambubioside; cyanidin-3-O-sambubioside; glucosides	HPLC-DAD; LC-MS/MS; electrophoresis-MS
2.	Phenolic acids	Chlorogenic acid; caffeic acid; protocatechuic acid	HPLC-DAD; LC-MS
3.	Flavonoids	Quercetin; kaempferol; myricetin and derivatives	HPLC; LC-MS; spectrophotometric total phenolics
4.	Organic acids	Hibiscus acid; hydroxycitric acid; citric acid; malic acid	HPLC or ion-exclusion chromatography

4. Extraction and Isolation

Extraction performance depends on plant part, particle size, solvent polarity, solid-to-liquid ratio, temperature, time, pH, oxygen exposure, and downstream concentration. Aqueous infusion and decoction are relevant to traditional beverages and may recover organic acids and water-soluble phenolics. Hydroalcoholic solvents often provide broader recovery of polyphenols, whereas methanol is appropriate for analytical research but requires complete removal before food or pharmaceutical use.^[3,23]

4.1 Conventional Extraction

Maceration, infusion, and decoction are inexpensive and scalable, but prolonged heating can accelerate anthocyanin degradation. Cold extraction may better preserve thermolabile pigments, although it can reduce mass transfer and increase processing time. Acidification can stabilize flavylium anthocyanins, but excessive acidity may alter the chemical profile and must be carefully controlled.^[3,16,39]

4.2 Advanced Extraction and Purification

Ultrasound-assisted extraction uses acoustic cavitation to improve solvent penetration and cell disruption. One reported roselle condition used an ethanol–water mixture of 80:20 at approximately 30°C for 20 minutes. Enzyme-assisted extraction with cellulase or enzyme mixtures can increase the release of glycosylated pigments and matrix-bound phenolics. Macroporous resins, including D101 and XAD-7HP, enable adsorption and enrichment, while high-speed counter-current chromatography can provide preparative isolation of individual anthocyanins. Membrane operations such as microfiltration, ultrafiltration, and nanofiltration support clarification and concentration. β -Cyclodextrin-assisted extraction and combinations of supercritical carbon dioxide with subcritical water represent emerging approaches intended to reduce conventional-solvent use.^[6-11]

5. Characterization and Stability

A defensible characterization strategy combines orthogonal methods. UV–visible spectrophotometry is useful for screening total monomeric anthocyanins and phenolics, but it cannot establish individual compound identity. HPLC with diode-array detection can separate and quantify common anthocyanins, while LC–MS or tandem MS provides

molecular confirmation. Nuclear magnetic resonance spectroscopy and high-resolution MS are valuable for structural elucidation of purified compounds. Results should specify calibration standards, expression units, recovery, precision, limit of detection, and whether values are reported on a dry-weight or fresh-weight basis.^[11,21,25,29,31]

Anthocyanins are sensitive to heat, light, oxygen, pH, enzymes, and metal ions. High temperatures and prolonged illumination may accelerate hydrolysis, oxidation, and colour loss. Protective strategies include low-temperature processing, oxygen limitation, opaque packaging, pH control, co-pigmentation, and encapsulation or complexation with stabilizers such as β -cyclodextrin. Stability studies should assess chemical markers and biological or colour functionality over the intended shelf life.^[12-14]

6. Pharmacological Significance

6.1 Cardiovascular Effects

Roselle has the strongest human evidence among the two species for cardiovascular applications. Proposed mechanisms include inhibition of angiotensin-converting enzyme, vasorelaxation involving nitric-oxide signalling, calcium-channel effects, antioxidant protection, and mild diuresis. Anthocyanins such as delphinidin- and cyanidin-3-O-sambubioside have demonstrated ACE-inhibitory activity in experimental work. Clinical studies and reviews report blood-pressure reductions in some populations, but heterogeneity in dose, preparation, duration, baseline blood pressure, and concomitant treatment limits direct comparison.^[25-28]

6.2 Antioxidant and Anti-inflammatory Activity

Extracts from both species show radical-scavenging and reducing activity in DPPH, ABTS, and FRAP assays. Roselle polyphenols have also been reported to attenuate lipopolysaccharide-induced inflammatory responses and regulate cyclooxygenase-2 expression in experimental systems. These assays demonstrate chemical or cellular potential; they do not independently establish clinical antioxidant effects or treatment efficacy.^[29]

6.3 Antimicrobial and Antiviral Activity

Roselle extracts have shown activity against organisms including *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus flavus* in laboratory studies. Antiviral effects have been reported against selected RNA-virus surrogate systems. China rose has also been investigated for antimicrobial activity and, *in silico*, for interactions with viral proteases. These findings require confirmation using standardized extracts, clinically relevant concentrations, validated assays, and appropriate controls.^[30,31]

6.4 Metabolic, Neurological, and Anticancer Research

In vitro and animal studies suggest α -glucosidase and α -amylase inhibition, lipid-lowering and anti-obesity effects, acetylcholinesterase and butyrylcholinesterase inhibition, and antiproliferative or pro-apoptotic activity. Human evidence is considerably more limited than preclinical evidence, particularly for cancer prevention, neurodegeneration, and diabetes treatment. Such activities should be described as investigational rather than established therapeutic indications.^[32-36]

7. Pharmacognostic Quality Control

Quality control begins with correct species identification, authenticated voucher specimens, a defined plant part, controlled harvest stage, and documented drying conditions. Macroscopic and microscopic examination should be

combined with physicochemical testing. Relevant tests include foreign matter, loss on drying or moisture content, total ash, acid-insoluble ash, extractive values, pH, microbial quality, pesticide residues, heavy metals, and mycotoxins where appropriate.^[15,26,29,31,46]

Chemical standardization should include a validated chromatographic fingerprint and quantitative marker compounds. For roselle, total monomeric anthocyanins, delphinidin-3-*O*-sambubioside, cyanidin-3-*O*-sambubioside, total phenolics, and selected organic acids may be useful markers. Spectrophotometric procedures are economical for routine screening, whereas HPLC or LC–MS is preferable for identity confirmation and batch-release specifications. Moisture is commonly controlled below approximately 10–11% in dried material, but this specification must be justified by stability and microbial data rather than adopted universally.^[37-40]

8. Safety and Toxicology

Available toxicological reviews generally describe roselle as having a broad acute safety margin; one cited mouse study reported an LD₅₀ greater than 5000 mg/kg. This value cannot be directly extrapolated to humans, concentrated extracts, chronic exposure, pregnancy, or drug combinations. Reported evidence for China rose is less extensive. Further studies should address repeated-dose toxicity, reproductive and developmental toxicity, genotoxicity, hepatotoxicity, renal effects, endocrine effects, and herb–drug interactions. Particular caution is warranted when products are used with antihypertensive, antidiabetic, diuretic, or other medicines because pharmacodynamic additivity is plausible.^[41-43]

9. CONCLUSION

This review highlights *Hibiscus sabdariffa* and *H. rosa-sinensis* as promising anthocyanin-rich botanicals, but identifies key research gaps: poorly characterized extracts, limited pharmacokinetic and bioavailability data, short-term safety evidence, and scarce cultivar-authenticated studies for *H. rosa-sinensis*. Limitations include reliance on heterogeneous preclinical models and small, short-duration human trials with inconsistent dosing and analytical methods. Future work should prioritize standardized, chemically defined preparations; validated LC–MS/MS or metabolomic profiling; adequately powered randomized controlled trials; and systematic long-term safety, interaction, and formulation studies to enable robust clinical translation and regulatory acceptance.

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