Research Article

Comparative In-Vitro Evaluation of Antioxidant and Anti-Inflammatory Activities of Clitoria Ternatea (Sangu Poo) and Jasminum Spp. (Jasmine) Flower Extracts

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Abstract

Background:
Oxidative stress and inflammation are interrelated processes implicated in the pathogenesis of multiple chronic disorders. Medicinal flowers represent promising natural sources of bioactive phytochemicals with antioxidant and anti-inflammatory potential. Clitoria ternatea (Sangu poo) and Jasminum species (Jasmine) are traditionally used for inflammatory and systemic conditions; however, direct comparative pharmacological evaluation using standardized in-vitro models remains limited.
Objective:
To comparatively evaluate the antioxidant and anti-inflammatory activities of methanolic flower extracts of Clitoria ternatea and Jasminum species using established in-vitro assay systems.
Methods:
Methanolic extracts of authenticated flowers were assessed for antioxidant activity by DPPH radical scavenging and ferric-reducing (reducing power) assays, and for anti-inflammatory activity by inhibition of bovine serum albumin (BSA) denaturation and human red blood cell (HRBC) membrane stabilization. Percentage inhibition, absorbance changes, and IC_{50} values were determined from concentration–response relationships.
Results:
Clitoria ternatea demonstrated markedly stronger antioxidant activity, with a DPPH IC_{50} of 143.85 µg/mL compared with 660.34 µg/mL for Jasminum, and higher reducing power across concentrations. In the BSA denaturation assay, Jasminum showed slightly greater inhibition (IC_{50} = 842.64 µg/mL) than C. ternatea (IC_{50} = 929.19 µg/mL). Conversely, HRBC membrane stabilization was higher for C. ternatea (96.96 % at 1000 µg/mL), approaching the diclofenac standard and exceeding Jasminum (90.94 %).
Conclusion:
Clitoria ternatea exhibits superior antioxidant and membrane-stabilizing anti-inflammatory activity, whereas Jasminum shows comparatively stronger inhibition of protein denaturation, indicating distinct yet complementary mechanisms of action. These findings support medicinal flowers as valuable natural sources of bioactive compounds and highlight the need for further phytochemical, mechanistic, and in-vivo investigations to establish clinical relevance.

Keywords: Clitoria ternatea; Jasminum; antioxidant; anti-inflammatory; DPPH; HRBC membrane stabilization; medicinal flowers.

Introduction

Oxidative stress and inflammation are interrelated biological processes implicated in the pathogenesis of several chronic disorders, including cardiovascular diseases, metabolic syndromes, neurodegenerative conditions, and immune-mediated pathologies. Continuous production of reactive oxygen species and activation of inflammatory mediators contribute to cellular injury and tissue dysfunction, thereby highlighting the need for safe therapeutic agents capable of modulating these pathways [1,2].

Natural products derived from medicinal plants continue to serve as an important source of bioactive molecules with antioxidant and anti-inflammatory potential [3,4]. Among them, medicinal flowers represent a comparatively underexplored reservoir of phenolics, flavonoids, terpenoids, and volatile constituents capable of exerting free-radical scavenging, reducing, and membrane-protective effects [5,6].

Clitoria ternatea (Sangu poo) and Jasminum species (Jasmine) are widely used in traditional medicine for the management of inflammatory disorders, skin diseases, and systemic ailments [7,8]. Although individual pharmacological properties of these flowers have been reported, direct comparative evaluation of their antioxidant and anti-inflammatory efficacy using standardized in-vitro models remains limited [7,9].

Therefore, a comparative in-vitro assessment of methanolic flower extracts of Clitoria ternatea and Jasmine was undertaken using DPPH radical scavenging, FRAP reducing power, BSA protein denaturation inhibition, and HRBC membrane stabilization assays.

Materials And Methods

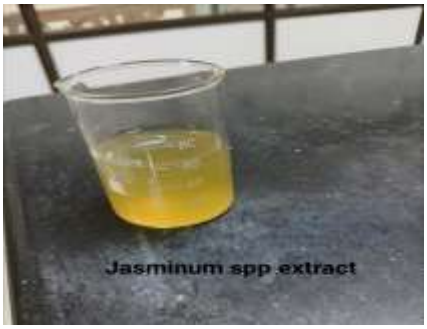
Study design

A laboratory-based in-vitro experimental comparative study was carried out to evaluate and compare the antioxidant and anti-inflammatory activities of methanolic flower extracts of Clitoria ternatea (Sangu poo) and Jasminum species (Jasmine). All experiments were performed under controlled laboratory conditions using validated spectrophotometric assay models.

Collection and preparation of plant material

Fresh flowers of Clitoria ternatea and Jasminum spp. were collected from local sources and authenticated by a qualified botanist. The plant materials were washed with distilled water to remove surface impurities, shade-dried at ambient temperature, and pulverized into coarse powder using a mechanical grinder.

Methanolic extraction was performed by macerating the powdered material in analytical-grade methanol for an adequate duration with intermittent shaking. The extract was filtered and concentrated under reduced pressure to obtain a semi-solid residue, which was stored at 4 °C until further use. Stock solutions were prepared in methanol, and working concentrations of 200, 400, 600, 800, and 1000 µg/mL were used for all experimental assays.



Evaluation of antioxidant activity

DPPH radical scavenging assay

Free-radical scavenging activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. A freshly prepared DPPH solution in methanol was protected from light. Briefly, 900 µL of DPPH solution was mixed with 100 µL of test extract at different concentrations and incubated in the dark for 30 minutes at room temperature.

Absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Methanol served as the blank, DPPH solution without extract served as the control, and ascorbic acid was used as the reference antioxidant standard [10].

Percentage inhibition of DPPH radicals was calculated using:

$$\% \text{ inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

The half-maximal inhibitory concentration (IC_{50}) was obtained from concentration–response curves.

Ferric reducing antioxidant power (FRAP) assay

The reducing capacity of the extracts was assessed using a modified FRAP method. One millilitre of test extract or standard was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1 % potassium ferricyanide. The mixture was incubated at 50 °C for 20 minutes, followed by addition of 2.5 mL of 10 % trichloroacetic acid and centrifugation at 3000 rpm for 10 minutes [11].

An aliquot of the supernatant (2.5 mL) was combined with 2.5 mL distilled water and 0.5 mL of 0.1 % ferric chloride, and absorbance was recorded at 700 nm. Increased absorbance indicated greater reducing antioxidant power. All determinations were performed in triplicate.

Evaluation of anti-inflammatory activity

BSA protein denaturation assay

Anti-inflammatory activity was evaluated by measuring inhibition of heat-induced denaturation of bovine serum albumin (BSA). A 0.4 % (w/v) BSA solution was prepared in phosphate-buffered saline. Test extracts at concentrations ranging from 200 to 1000 µg/mL were added to 1 mL of BSA solution and incubated at 72 °C for 10 minutes, followed by cooling at room temperature for 30 minutes [12].

Absorbance was measured at 660 nm, with distilled water as blank. Methanol served as the negative control, and aspirin was used as the positive control.

Percentage inhibition of protein denaturation was calculated as:

$$\% \text{ inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

IC_{50} values were derived from concentration–response relationships.

HRBC membrane stabilization assay

The human red blood cell (HRBC) membrane stabilization method was employed to assess anti-inflammatory potential. Fresh human blood collected in a heparinized container was mixed with Alsever's solution and

centrifuged at 3000 rpm for 10 minutes. The packed erythrocytes were washed three times with phosphate-buffered saline (PBS, pH 7.4), and a 10 % HRBC suspension was prepared in normal saline.
Reaction mixtures containing 0.5 mL HRBC suspension and 5 mL of extract solution at various concentrations were incubated at 37 °C for 1 hour, followed by centrifugation at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured at 540 nm [13].

Controls included:

- Hypotonic control (distilled water) for complete haemolysis
- Negative control (PBS)
- Positive control (diclofenac sodium)

Percentage membrane stabilization was calculated using:

$$\% \text{ stabilization} = \frac{A_{sample}}{A_{control}} \times 100$$

Statistical analysis

All experiments were performed in replicate, and results were expressed as mean ± standard deviation (SD). IC_{50} values were calculated from dose-response curves using regression analysis. Comparative interpretation between extracts was performed descriptively across corresponding concentrations.

Results

Antioxidant Activity

DPPH Radical Scavenging Assay

Both flower extracts demonstrated concentration-dependent free-radical scavenging activity.
Sangu poo exhibited markedly stronger antioxidant potency, with an IC_{50} of 143.85 µg/mL, whereas Jasmine showed moderate activity with an IC_{50} of 660.34 µg/mL. At the highest tested concentration (1000 µg/mL), Sangu poo achieved 73.29 % inhibition, compared with 66.15 % inhibition for Jasmine, confirming the superior radical-quenching capacity of Clitoria ternatea extract.

Table 1. DPPH Radical Scavenging Activity of Sangu Poo and Jasmine

Concentration (µg/mL)	Mean Absorbance (Sangu)	% Inhibition (Sangu)	Mean Absorbance (Jasmine)	% Inhibition (Jasmine)
200	0.394	67.63	0.871	28.43
400	0.382	68.61	0.795	34.68
600	0.340	72.06	0.635	47.82
800	0.331	72.80	0.510	58.09
1000	0.325	73.29	0.412	66.15

IC_{50}:

Sangu poo = 143.85 µg/mL
Jasmine = 660.34 µg/mL

FRAP Reducing Power Assay

Both extracts exhibited a progressive increase in absorbance with rising concentration, indicating dose-dependent reducing antioxidant capacity.Across all tested concentrations, Sangu poo showed slightly higher absorbance values than Jasmine, suggesting greater electron-donating ability.However, both extracts demonstrated lower reducing power than the ascorbic acid reference standard.

Table 2. FRAP Reducing Power of Sangu Poo and Jasmine

Concentration (µg/mL)	Sangu (Absorbance)	Jasmine (Absorbance)	Ascorbic Acid (Absorbance)
200	0.125	0.104	0.248
400	0.136	0.114	0.462
600	0.142	0.120	0.681
800	0.157	0.137	0.875
1000	0.199	0.143	1.066

Anti-Inflammatory Activity

BSA Protein Denaturation Assay

Both extracts inhibited heat-induced protein denaturation in a concentration-dependent manner.Jasmine demonstrated slightly stronger anti-denaturation activity, with an IC_{50} of 842.64 µg/mL, compared with 929.19 µg/mL for Sangu poo. At 1000 µg/mL, Jasmine produced approximately 60.60 % inhibition, whereas Sangu poo achieved 55.90 % inhibition, indicating marginally greater protein-stabilizing anti-inflammatory potential for Jasmine.

Table 3. Inhibition of BSA Protein Denaturation

Concentration (µg/mL)	Mean Absorbance (Sangu)	% Denaturation Inhibition (Sangu)	Mean Absorbance (Jasmine)	% Denaturation Inhibition (Jasmine)
200	0.009	0.67	0.015	1.12
400	0.194	14.48	0.247	18.43
600	0.382	28.51	0.463	34.55
800	0.527	39.33	0.621	46.34
1000	0.749	55.90	0.812	60.60

IC_{50}:

Sangu poo = 929.19 µg/mL

Jasmine = 842.64 µg/mL

HRBC Membrane Stabilization Assay

Both extracts demonstrated high membrane-stabilizing activity at increasing concentrations, reflecting anti-inflammatory potential through protection of erythrocyte membrane integrity. Sangu poo showed greater stabilization, reaching 96.96 % at 1000 µg/mL, whereas Jasmine achieved approximately 90.94 % stabilization at the same concentration.The membrane-protective activity of Sangu poo approached that of the diclofenac standard, indicating strong anti-inflammatory efficacy in this model.

Table 4. HRBC Membrane Stabilization Activity

Concentration (µg/mL)	% Stabilization (Sangu)	% Stabilization (Jasmine)	% Stabilization (Diclofenac)
200	91.84	86.89	89.09
400	93.44	87.72	91.90
600	94.70	88.62	94.04
800	95.47	89.45	95.89
1000	96.96	90.94	97.62

Table 5. Comparative IC_{50} Values of Sangu Poo and Jasmine

Assay	Sangu Poo IC_{50} (µg/mL)	Jasmine IC_{50} (µg/mL)
DPPH Radical Scavenging	143.85	660.34
BSA Denaturation	929.19	842.64

Discussion

Comparative evaluation of the biological activities of Clitoria ternatea (Sangu poo) and Jasminum species (Jasmine) demonstrates clear variation in antioxidant and anti-inflammatory response patterns, indicating the involvement of distinct phytochemical-mediated mechanisms. Quantitative findings reveal that Sangu poo possesses markedly stronger free-radical scavenging activity, reflected by a substantially lower DPPH IC_{50} of 143.85 µg/mL compared with 660.34 µg/mL for Jasmine. At the highest tested concentration (1000 µg/mL), Sangu poo achieved approximately 73.29 % inhibition, exceeding the 66.15 % inhibition observed with Jasmine. A similar trend was evident in the FRAP assay, where Sangu poo demonstrated higher reducing power (absorbance 0.199) relative to Jasmine (0.143) at 1000 µg/mL, confirming superior electron-donating antioxidant capacity.

These observations are consistent with earlier phytochemical and pharmacological reports describing Clitoria ternatea flowers as rich sources of anthocyanins, flavonoids, and phenolic compounds, which contribute to efficient radical neutralization and redox regulation [9]. Previous in-vitro antioxidant investigations of C. ternatea extracts have likewise reported moderate-to-strong DPPH scavenging and ferric-reducing potential, supporting its relevance as a natural antioxidant candidate [14,15]. In contrast, antioxidant activity reported for Jasminum species is generally moderate, often attributed to phenolic acids, flavonoid glycosides, and volatile constituents, which may explain the comparatively higher IC_{50} observed [16].

With respect to anti-inflammatory activity, divergent mechanistic behaviour becomes evident. Jasmine demonstrated slightly stronger inhibition of heat-induced protein denaturation, with an IC_{50} of 842.64 µg/mL compared with 929.19 µg/mL for Sangu poo, achieving 60.60 % inhibition at 1000 µg/mL versus 55.90 % for Sangu poo. Similar protein-stabilizing and anti-denaturation properties of Jasminum extracts have been documented in earlier experimental models, where phenolic and flavonoid constituents were proposed to protect protein conformation under thermal or inflammatory stress conditions [7].

Conversely, Sangu poo exhibited greater HRBC membrane-stabilizing activity, reaching approximately 96.96 % stabilization at 1000 µg/mL, compared with 90.94 % for Jasmine and approaching the activity of the diclofenac reference standard (~97.62 %). Membrane stabilization in this model is considered analogous to lysosomal membrane protection during inflammation, suggesting that C. ternatea may exert anti-inflammatory effects through cellular membrane preservation in addition to antioxidant action. Comparable membrane-protective effects of Clitoria ternatea extracts have been described previously, further supporting this interpretation[14,17].

Strengths

The work utilised multiple complementary in-vitro assays, including DPPH radical scavenging, FRAP reducing power, BSA protein denaturation inhibition, and HRBC membrane stabilization, allowing comprehensive evaluation of antioxidant and anti-inflammatory activity. Quantitative dose–response analysis with IC_{50} determination enabled reliable comparison between Clitoria ternatea and Jasminum extracts, while the use of standard reference compounds such as ascorbic acid, aspirin, and diclofenac supported methodological validity. The findings also provide direct comparative pharmacological evidence for two traditionally used medicinal flowers and may assist in prioritising plant candidates for further phytochemical and therapeutic investigation.

Limitations and Future direction

The experimental design was confined to in-vitro biochemical and membrane-based models, which may not adequately represent the complex physiological and pharmacokinetic conditions of living systems, thereby limiting direct clinical extrapolation of the observed antioxidant and anti-inflammatory effects. The use of crude methanolic extracts without isolation or quantification of specific bioactive constituents further restricts precise correlation between phytochemical composition and pharmacological activity. In addition, statistical evaluation

was largely descriptive, and detailed inferential analysis or mechanistic pathway investigation was not performed. Assessment of toxicity, bioavailability, and in-vivo efficacy also remained beyond the study scope. Consequently, future research incorporating chromatographic phytochemical characterization, molecular mechanism studies, animal experimentation, and well-designed clinical evaluation will be necessary to establish therapeutic relevance and translational potential.

Conclusion

Clitoria ternatea demonstrates superior antioxidant potential and membrane-stabilizing anti-inflammatory activity, whereas Jasminum species exhibit comparatively stronger inhibition of protein denaturation, indicating the involvement of distinct yet complementary anti-inflammatory mechanisms between the two medicinal flowers. The differential activity observed across radical scavenging, reducing power, protein stabilization, and membrane protection models highlights the importance of multi-assay phytopharmacological evaluation for accurate characterization of traditional medicinal plants. Collectively, these findings reinforce the therapeutic relevance of medicinal flowers as promising natural sources of bioactive compounds with antioxidant and anti-inflammatory potential. Further studies involving phytochemical profiling, isolation of active constituents, mechanistic pathway analysis, toxicity assessment, and in-vivo validation will be essential to substantiate their clinical applicability and support future development of plant-derived anti-inflammatory agents.

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