Research Article

Comparative In-Vitro Evaluation of Antioxidant and Anti-Inflammatory Activities of Clitoria Ternatea and Cassia Auriculata Flower Extracts

Dr. N. Sri Sakthi Priya[1], Dr. Arul Amutha Elizebeth[2]

[1]Postgraduate, Department of Pharmacology, Sree Balaji Medical College and Hospital Chennai Tamilnadu India
[2]Prof and HOD Department of PharmacologySree Balaji Medical College And Hospital Chennai Tamilnadu India

Abstract

Background:
Oxidative stress and inflammation are closely interconnected processes involved in the pathogenesis of numerous chronic diseases. Medicinal flowers used in traditional Indian medicine, such as Clitoria ternatea and Cassia auriculata, are rich in phytochemicals with potential antioxidant and anti-inflammatory properties; however, direct comparative experimental evidence remains limited.
Objective:
To comparatively evaluate the in-vitro antioxidant and anti-inflammatory activities of methanolic flower extracts of C. ternatea and C. auriculata using standardized biochemical assays.
Methods:
Antioxidant activity was assessed using DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays, while anti-inflammatory activity was evaluated using bovine serum albumin (BSA) protein denaturation and human red blood cell (HRBC) membrane stabilization methods at concentrations of 200–1000 µg/mL. IC_{50} values were determined from concentration–response curves.
Results:
C. ternatea demonstrated stronger antioxidant activity, with a lower DPPH IC_{50} (143.85 µg/mL) and higher FRAP reducing capacity compared with C. auriculata. It also showed greater inhibition of BSA denaturation (IC_{50} = 929.19 µg/mL vs 2134.45 µg/mL). In contrast, both extracts exhibited marked HRBC membrane stabilization at higher concentrations, with C. auriculata showing slightly higher stabilization at 1000 µg/mL (98.21%), comparable to diclofenac (97.62%).
Conclusion:
Both medicinal flowers demonstrated significant in-vitro antioxidant and anti-inflammatory activities with complementary pharmacological profiles. C. ternatea showed comparatively stronger radical-scavenging and protein-protective effects, whereas C. auriculata exhibited notable membrane-stabilizing activity. Further phytochemical characterization and in-vivo studies are required to confirm their therapeutic potential.

Keywords: Clitoria ternatea; Cassia auriculata; antioxidant activity; anti-inflammatory activity; DPPH; FRAP; HRBC membrane stabilization.

INTRODUCTION

Oxidative stress and inflammation are closely interconnected biological processes that play a central role in the initiation and progression of numerous chronic and degenerative disorders, including cardiovascular diseases, diabetes mellitus, neurodegenerative conditions, and autoimmune pathologies.[1,2] Persistent generation of reactive oxygen species and the subsequent activation of inflammatory pathways contribute to cellular damage, tissue dysfunction, and disease progression. Consequently, the identification of safe and effective agents capable of modulating oxidative stress and inflammatory responses remains an important focus of contemporary pharmacological research.[1–4]

Natural products derived from medicinal plants have gained considerable attention as potential sources of bioactive compounds with antioxidant and anti-inflammatory properties. In particular, medicinal flowers used in traditional systems of medicine represent an underexplored yet promising reservoir of phytochemicals such as flavonoids, phenolic acids, tannins, and anthocyanins, which are known to exert free radical scavenging, reducing, and membrane stabilizing effects.[5–9]

Cassia auriculata (Avaram poo) and Clitoria ternatea (Sangu poo) are widely utilized in traditional Indian medicine for the management of inflammatory disorders, dermatological conditions, and metabolic abnormalities.[10–12] Although several studies have reported the pharmacological properties of these plants individually, direct comparative evaluation of their antioxidant and anti-inflammatory potential using standardized in-vitro assays

remains limited.Therefore, the present study was undertaken to comparatively evaluate the in-vitro antioxidant and anti-inflammatory activities of methanolic flower extracts of Cassia auriculata and Clitoria ternatea using validated spectrophotometric methods, including DPPH radical scavenging, ferric reducing antioxidant power (FRAP), bovine serum albumin (BSA) denaturation inhibition, and human red blood cell (HRBC) membrane stabilization assays.

MATERIALS AND METHODS

Study design

This study was designed as an in-vitro experimental comparative investigation to evaluate the antioxidant and anti-inflammatory activities of two traditionally used medicinal flower extracts, namely Cassia auriculata (Avaram poo) and Clitoria ternatea (Sangu poo). All experiments were performed under controlled laboratory conditions using validated spectrophotometric assays.

Plant material and extract preparation

Fresh flowers of Cassia auriculata and Clitoria ternatea were collected and authenticated. The flowers were washed with distilled water, shade-dried at room temperature, and powdered using a mechanical grinder. The powdered material was subjected to methanolic extraction by maceration. The extract was filtered and concentrated under reduced pressure to obtain a semi-solid residue, which was stored at 4 °C until further analysis. Stock solutions were prepared in methanol, and working concentrations of 200, 400, 600, 800, and 1000 µg/mL were used for all assays.

The preparation of plant extracts and standardized powders used for the experimental assays is illustrated in Figures 1-4.

Evaluation of antioxidant activity

DPPH radical scavenging assay

The free-radical scavenging activity of the extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. A DPPH solution was prepared in methanol and protected from light. Briefly, 900 µL of DPPH solution was mixed with 100 µL of 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 positive control.[13]

The percentage inhibition of DPPH radicals was calculated using:

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

The IC_{50} value was determined from the concentration-response curve.

Ferric reducing antioxidant power (FRAP) assay

The reducing power of the extracts was assessed using a modified FRAP method. Briefly, 1 mL of test extract or standard was mixed with 2.5 mL 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 the addition of 2.5 mL of 10% trichloroacetic acid and centrifugation at 3000 rpm for 10 minutes.

The supernatant (2.5 mL) was mixed with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride, and absorbance was recorded at 700 nm. Higher absorbance indicated greater reducing antioxidant power. All measurements were performed in triplicate.[14]

Evaluation of anti-inflammatory activity

BSA protein denaturation assay

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

Absorbance was measured at 660 nm using distilled water as the blank. Methanol served as the negative control, and aspirin was used as the positive control [15].
Percentage inhibition of protein denaturation was calculated as:

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

IC_{50} values were obtained from concentration–response analysis.

HRBC membrane stabilization assay

The human red blood cell (HRBC) membrane stabilization method was used to evaluate anti-inflammatory potential. Fresh human blood collected in a heparinized tube 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.[16] Reaction mixtures contained 0.5 mL HRBC suspension and 5 mL extract solution at different concentrations.

Controls included:

- Hypotonic control (distilled water) for maximum hemolysis
- Negative control (PBS)
- Positive control (diclofenac sodium)

Samples were incubated at 37 °C for 1 hour, centrifuged at 3000 rpm for 10 minutes, and absorbance of the supernatant was recorded at 540 nm.
Percentage membrane stabilization was calculated as:

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

Ethical considerations

Fresh human blood used for the HRBC membrane stabilization assay was obtained from the first author on a voluntary basis after informed consent. As the study involved self-sampling without the inclusion of other human participants or identifiable personal data, formal Institutional Ethics Committee approval was not required. All procedures were conducted in accordance with institutional laboratory safety standards and ethical guidelines for research involving human biological materials.

Statistical analysis

All experiments were conducted in replicate, and results were expressed as mean ± standard deviation (SD). IC_{50} values were determined from dose-response curves using appropriate regression analysis. Comparative differences between extracts were interpreted descriptively at corresponding concentrations.

RESULTS

Antioxidant Activity

DPPH Radical Scavenging Assay

Both extracts demonstrated concentration-dependent radical scavenging activity. Clitoria ternatea (Sangu poo) exhibited markedly higher scavenging activity at all tested concentrations compared with Cassia auriculata (Avaram poo). The IC_{50} value of C. ternatea (143.85 µg/mL) was substantially lower than that of C. auriculata (740.82 µg/mL), indicating superior antioxidant potency (Table 1).

FRAP Reducing Power Assay

Both extracts demonstrated a concentration-dependent increase in ferric reducing antioxidant power. However, C. ternatea consistently showed slightly higher absorbance values than C. auriculata, indicating greater electron-donating antioxidant capacity (Table 2).

Anti-inflammatory Activity

BSA Protein Denaturation Assay

Both extracts inhibited heat-induced protein denaturation in a concentration-dependent manner. C. ternatea demonstrated stronger inhibition compared with C. auriculata across all tested concentrations. The IC_{50} value of

C. ternatea (929.19 µg/mL) was lower than that of C. auriculata (2134.45 µg/mL), indicating greater anti-inflammatory potential (Table 3).

HRBC Membrane Stabilization Assay

Both extracts demonstrated marked membrane stabilization with increasing concentrations. C. auriculata exhibited slightly greater stabilization at higher concentrations, approaching the activity of diclofenac sodium (Table 4).

DISCUSSION

The present in-vitro comparative study highlights distinct differences in the antioxidant and anti-inflammatory profiles of Clitoria ternatea (Sangu poo) and Cassia auriculata (Avaram poo). The observed trends are broadly consistent with previously published reports, although variations in activity across studies may occur depending on the plant part used, extraction method, solvent system, and assay conditions.

Antioxidant activity in the context of published evidence

Sangu poo demonstrated markedly stronger DPPH radical scavenging activity in the present study (IC_{50} = 143.85 µg/mL) compared with Avaram poo (IC_{50} = 740.82 µg/mL). In previous studies, methanolic extracts of C. ternatea flowers have generally shown moderate DPPH scavenging activity. For example, comparative extract studies have reported DPPH IC_{50} values for methanolic flower extracts in the range of approximately 600–650 µg/mL, with variation across different solvent fractions.[17,18] In comparison, the lower IC_{50} observed in the present study suggests relatively stronger antioxidant activity of the C. ternatea extract used.

Such differences may arise from multiple factors known to influence DPPH outcomes, including extract concentration, DPPH reagent strength, incubation time, sample preparation methods, geographical source of the plant material, and the phytochemical composition of the extract. For Avaram poo, the DPPH IC_{50} observed in the present study (740.82 µg/mL ≈ 0.741 mg/mL) is comparable to classical findings for C. auriculata flowers.

Previous investigations have reported IC_{50} values around 0.9 mg/mL for bound phenolic fractions, whereas free phenolic fractions have shown substantially lower activity with IC_{50} values in the multi-milligram range.[7,19] These observations suggest that the extract evaluated in the present study may contain a relatively higher proportion of phenolic constituents associated with antioxidant activity.

In the FRAP assay, both extracts demonstrated concentration-dependent increases in reducing power. However, Sangu poo consistently exhibited higher absorbance values than Avaram poo, particularly at 1000 µg/mL (0.199 vs 0.143). This finding supports greater electron-donating capacity of the C. ternatea extract. Similar observations have been reported in the literature, where C. ternatea flowers, especially those rich in polyphenols and anthocyanins, often demonstrate strong reducing and radical-scavenging properties, although the absolute magnitude of activity may vary depending on extraction conditions.[8,20]

Anti-inflammatory activity in the context of published evidence

Using the BSA protein denaturation model, Sangu poo demonstrated stronger inhibition of protein denaturation compared with Avaram poo (IC_{50} 929.19 µg/mL vs 2134.45 µg/mL), with approximately 55.90% inhibition at 1000 µg/mL compared with 24.93% for Avaram poo. Previous studies on C. ternatea have reported measurable inhibition of albumin denaturation, and under certain extraction conditions the activity has been reported to approach that of standard anti-inflammatory drugs such as diclofenac.[12]

The comparatively higher IC_{50} values observed in the present study may be attributable to the use of flower extracts rather than leaf extracts, as well as differences in assay conditions such as protein concentration, heating parameters, buffer composition, and analytical methods, all of which can significantly influence the outcome of protein denaturation assays.

In the HRBC membrane stabilization assay, both extracts demonstrated substantial membrane stabilization at higher concentrations. Avaram poo exhibited slightly greater stabilization at 1000 µg/mL (98.21%) compared with Sangu poo (96.96%), approaching the activity of diclofenac (97.62%). This observation is pharmacologically relevant because HRBC membrane stabilization is commonly interpreted as an indirect indicator of lysosomal membrane stabilization, which is an important mechanism underlying anti-inflammatory activity.[21]

Previous studies on C. auriculata extracts have similarly reported inhibition of protein denaturation and membrane stabilization activity, supporting its traditional use in inflammatory conditions. However, the magnitude of activity reported across studies varies considerably depending on the plant part used and the extraction solvent.[22]

Integrated interpretation

Taken together, the findings of the present study suggest that Sangu poo possesses stronger antioxidant activity and greater inhibition of protein denaturation, whereas Avaram poo demonstrates slightly superior membrane stabilization at higher concentrations. These findings suggest that the two medicinal flowers may exert anti-inflammatory effects through partially different mechanisms.

The lack of a direct one-to-one correlation between antioxidant indices (DPPH and FRAP) and membrane stabilization activity also highlights the importance of employing multiple complementary assays in phytopharmacological screening, rather than relying on a single experimental model.

Limitations and future directions

The results of this in-vitro study should be interpreted with caution because antioxidant and anti-inflammatory assays are highly sensitive to extraction procedures and assay conditions. Future investigations should incorporate standardized phytochemical quantification, including total phenolic content, total flavonoid content, and anthocyanin profiling, along with chromatographic characterization of the active constituents. In addition, in-vivo experimental studies are required to determine whether the observed in-vitro activities translate into meaningful pharmacological effects.

CONCLUSION

Both Clitoria ternatea and Cassia auriculata flower extracts demonstrated measurable in-vitro antioxidant and anti-inflammatory activities. C. ternatea showed stronger radical-scavenging activity, greater reducing power, and higher inhibition of protein denaturation, whereas C. auriculata exhibited slightly greater HRBC membrane stabilization activity. These findings suggest complementary pharmacological potential and support the traditional therapeutic use of these medicinal flowers. Further phytochemical characterization and in-vivo studies are required to confirm their clinical relevance.

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Conflict of Interest

The authors declare that there are no conflicts of interest related to this study.

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Author Contributions

All authors contributed to the conception and design of the study. Experimental work, data analysis, and manuscript preparation were performed collaboratively by the authors. All authors reviewed and approved the final version of the manuscript.

TABLES

Table 1. DPPH Radical Scavenging Activity of Clitoria ternatea and Cassia auriculata

Concentration (µg/mL)	Mean Absorbance (Sangu)	% Inhibition (Sangu)	Mean Absorbance (Avaram)	% Inhibition (Avaram)
200	0.394	67.63	0.993	18.41
400	0.382	68.61	0.888	27.03
600	0.340	72.06	0.638	47.58
800	0.331	72.80	0.533	56.20
1000	0.325	73.29	0.478	60.72

IC_{50}values:
C.ternatea=143.85µg/mL
C. auriculata = 740.82 µg/mL

Table 2. Ferric Reducing Antioxidant Power (FRAP) Assay

Concentration (µg/mL)	C. ternatea (Absorbance)	C. auriculata (Absorbance)	Ascorbic Acid
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

Table 3. Inhibition of BSA Protein Denaturation

Concentration (µg/mL)	Mean Absorbance (Sangu)	% Inhibition	Mean Absorbance (Avaram)	% Inhibition
200	0.009	0.67	0.019	1.42
400	0.194	14.48	0.092	6.87
600	0.382	28.51	0.125	9.33
800	0.527	39.33	0.148	11.04
1000	0.749	55.90	0.334	24.93

IC_{50}values:
C.ternatea=929.19µg/mL
C. auriculata = 2134.45 µg/mL

Table 4. HRBC Membrane Stabilization Activity

Concentration (µg/mL)	% Stabilization (C. ternatea)	% Stabilization (C. auriculata)	Diclofenac
200	91.84	92.25	89.09
400	93.44	93.09	91.90
600	94.70	94.70	94.04
800	95.47	96.72	95.89
1000	96.96	98.21	97.62

Figures

Figure 1. Methanolic extract of Clitoria ternatea (Sangu poo) obtained after maceration and concentration, used for the evaluation of antioxidant and anti-inflammatory activities.

Figure 2. Methanolic extract of Cassia auriculata (Avaram poo) prepared for in-vitro antioxidant and anti-inflammatory assays.

Figure 3. Standardized powdered sample of Cassia auriculata used as the starting plant material for methanolic extraction.

Figure 4. Standardized powdered sample of Clitoria ternatea used for preparation of methanolic extract.

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