
BASELINE IN-VITRO EVALUATION OF *CINCHONA OFFICINALIS* Q IN THE EGG ALBUMIN DENATURATION ASSAY: A FOUNDATION STUDY WITH A MATCHED ETHANOL CONTROL

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ABSTRACT

Background: Inhibition of heat-induced albumin denaturation is a simple, widely used in-vitro screen for anti-inflammatory potential. *Cinchona officinalis* mother tincture (Q) is used in homoeopathy as China officinalis, yet its behaviour in this assay has not been documented. This basic study provides baseline data and a reproducible, solvent-controlled protocol for further research on mother tinctures.

Objective: To measure the percentage inhibition of albumin denaturation produced by *Cinchona officinalis* Q at 6.25, 12.5, 25, 50 and 100 µL/mL and to compare it with 80% v/v ethanol at the same concentrations.

Methods: Egg albumin, phosphate-buffered saline (PBS, pH 6.4) and test solution were incubated at 37 °C for 20 min and heated at 70 °C for 10 min. Turbidity was read at 660 nm. Q (Dr. Willmar Schwabe; 80% v/v ethanol) and an ethanol control of the same strength were tested in triplicate. Final ethanol content (0.2% to 3.2% v/v) was identical in matched Q and ethanol tubes.

Results: Control absorbance was 0.030. Q produced 0.00% to 0.22% inhibition across the five concentrations, with a small, measurable response from 12.5 µL/mL upwards (maximum 0.22% at 100 µL/mL), whereas the ethanol control produced no inhibition (0.00%) at any concentration.

Conclusion: This foundation study establishes a reproducible protocol with a matched ethanol control and documents the response of Q under defined conditions. Q showed a small but measurable inhibition of albumin denaturation relative to the ethanol control, which showed none, giving an encouraging early signal; the parameters (albumin concentration, concentration range, positive control) can now be optimised to characterise this response in the next phase of research.

KEYWORDS: *Cinchona officinalis*; mother tincture; albumin denaturation; ethanol control; baseline study; in-vitro.

1. INTRODUCTION

Inflammation is a protective tissue response to injury, and protein denaturation is recognised as one of its contributing factors (Sangeetha and Vidhya, 2016; Osman et al., 2016). Agents that prevent heat-induced denaturation of albumin are therefore screened in vitro as possible anti-inflammatory substances. The assay is simple, inexpensive and reproducible, and the turbidity produced is read spectrophotometrically, which makes it well suited to first-line evaluation of new materials.

Cinchona officinalis L. (Rubiaceae) yields the Cinchona alkaloids, including quinine, quinidine, cinchonine and cinchonidine. Cinchonine has been reported to have anti-inflammatory, anti-malarial and other activities (Parveen et al., 2023). In homoeopathy the mother tincture (Q) is prepared from the dried bark and used as China officinalis.

Systematic laboratory work on homoeopathic mother tinctures requires well-characterised baseline data, including the contribution of the ethanol vehicle. The behaviour of Q in the albumin denaturation assay has not been widely reported. This study was therefore designed as a foundation for advanced research: to document the response of Q under defined conditions, with an ethanol control matched in strength and concentration.

2. AIM AND OBJECTIVES

Aim: To assess the effect of *Cinchona officinalis* Q on heat-induced albumin denaturation in vitro.

Primary objective: To measure the percentage inhibition of albumin denaturation produced by *Cinchona officinalis* Q at 6.25, 12.5, 25, 50 and 100 µL/mL and to compare it with 80% v/v ethanol at the same concentrations.

Secondary objective: To establish a reproducible, solvent-controlled protocol that can serve as a baseline for advanced studies on homoeopathic mother tinctures.

3. MATERIALS AND METHODS

3.1 Study design and site

Experimental in-vitro study carried out at INBIOTICS Pvt. Ltd. (Inbiotics R&D).

3.2 Test materials

Table A. Experimental groups.

Group	Composition
Control	Egg albumin + PBS (pH 6.4), without sample or solvent
Test	<i>Cinchona officinalis</i> Q, Dr. Willmar Schwabe; solvent ethanol 80% v/v
Comparator	Ethanol 80% v/v, identical to the solvent of Q, at the same $\mu\text{L/mL}$ series

The ethanol comparator was prepared at the same strength (80% v/v) and the same concentrations (6.25–100 $\mu\text{L/mL}$) as Q, so that every Q tube and its matching ethanol tube contained an equal ethanol load.

3.3 Procedure

The assay followed Mizushima and Kobayashi (1968) and Abbas et al. (2021) with slight modifications.

1. Two millilitres of test or ethanol solution (diluted in distilled water) were mixed with 0.2 mL of freshly prepared egg albumin solution.
2. The volume was made up to 5 mL with PBS (pH 6.4). All determinations were in triplicate.
3. Mixtures were incubated at 37 °C for 20 min, then heated at 70 °C for 10 min to induce denaturation.
4. Tubes were cooled to room temperature.
5. Absorbance (turbidity) was read at 660 nm using a UV-Visible spectrophotometer (Model: UV-VIS Spectrophotometer 117 TS).

3.4 Final ethanol content

Test and comparator solutions (2 mL), diluted in distilled water, were made up to a final reaction volume of 5 mL with PBS. The final concentration of Q or ethanol was therefore 2.5–40 $\mu\text{L/mL}$ (0.25–4% v/v), and, at 80% v/v, the final ethanol content was 0.2–3.2% v/v (Table B).

Table B. Final ethanol content per reaction tube.

Aliquot (μL/mL)	conc.	Volume in tube (μL)	Final Q or ethanol in 5 mL (μL/mL)	Final ethanol (% v/v)
6.25		12.5	2.5	0.2
12.5		25	5	0.4
25		50	10	0.8
50		100	20	1.6
100		200	40	3.2

3.5 Calculation and analysis

Percentage inhibition = [(Absorbance of control – Absorbance of sample) / Absorbance of control] × 100

Results are expressed as mean ± SD of triplicates.

4. RESULTS

The absorbance of the control was 0.030. The results are given in Tables 1 and 2, Graph 1 and Fig. 1.

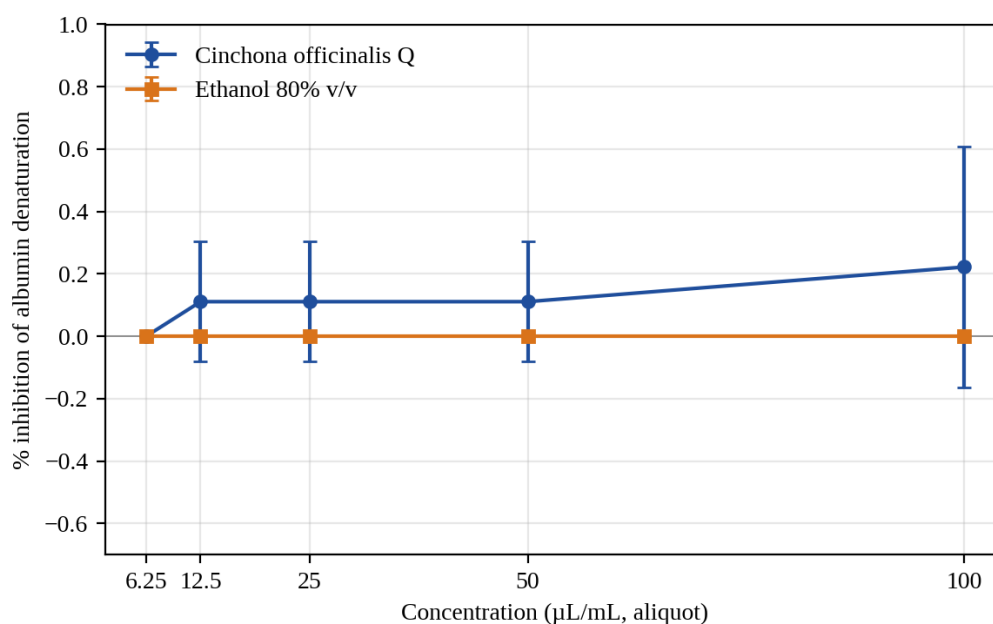
Table 1. Inhibition of albumin denaturation by *Cinchona officinalis* Q (control absorbance = 0.030.)

Conc. (μL/mL, aliquot)	Abs 1	Abs 2	Abs 3	Mean absorbance	% Inhibition	Final ethanol (% v/v)
6.25	0.0300	0.0300	0.0300	0.0300	0.00	0.2
12.5	0.0300	0.0300	0.0299	0.0300	0.11	0.4
25	0.0300	0.0300	0.0299	0.0300	0.11	0.8
50	0.0300	0.0300	0.0299	0.0300	0.11	1.6
100	0.0300	0.0298	0.0300	0.0299	0.22	3.2

Table 2. Inhibition of albumin denaturation by ethanol 80% v/v (same concentrations as Q.)

Conc. (μL/mL, aliquot)	Abs 1	Abs 2	Abs 3	Mean absorbance	% Inhibition	Final ethanol (% v/v)
6.25	0.0300	0.0300	0.0300	0.0300	0.00	0.2
12.5	0.0300	0.0300	0.0300	0.0300	0.00	0.4
25	0.0300	0.0300	0.0300	0.0300	0.00	0.8
50	0.0300	0.0300	0.0300	0.0300	0.00	1.6
100	0.0300	0.0300	0.0300	0.0300	0.00	3.2

Q produced inhibition of up to 0.22%, appearing from 12.5 $\mu\text{L}/\text{mL}$ and highest at 100 $\mu\text{L}/\text{mL}$. Ethanol (80% v/v) showed no inhibition (0.00%) at any concentration (Table 2), so the response seen with Q stands above the solvent control (Graph 1). [Still to do: add photographs of the 6.25 and 12.5 $\mu\text{L}/\text{mL}$ tubes to Fig. 1.]



Graph 1. Percentage inhibition of albumin denaturation by Q and by ethanol 80% v/v at the same concentrations (mean $\pm$ SD, n = 3).

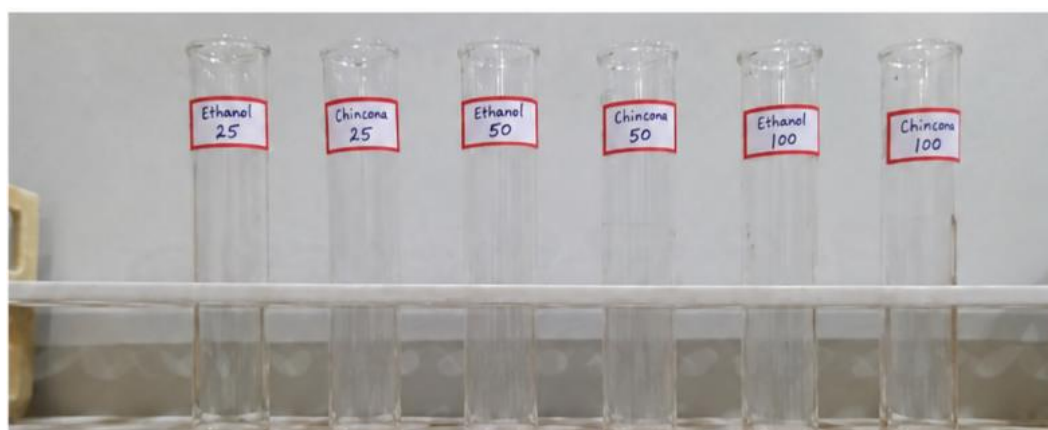


Fig. 1. Reaction tubes after heating and cooling: ethanol (80% v/v) and *Cinchona officinalis* Q at 25, 50 and 100 $\mu\text{L}/\text{mL}$ (aliquot concentration), as labelled on the tubes.

5. DISCUSSION

This foundation study documented the response of *Cinchona officinalis* Q in the egg albumin denaturation assay at five concentrations, with triplicate readings and a clearly defined

solvent load. Q showed a small inhibition of denaturation, appearing from 12.5 $\mu\text{L/mL}$ and reaching a maximum of 0.22% at 100 $\mu\text{L/mL}$, whereas the ethanol control showed none at any concentration. Although the response is modest and the absorbance differences are small (0.0001, at the limit of instrument resolution), the difference from the control is an encouraging early indication of a protective effect on albumin, and the data provide a reliable baseline against which later work can be compared.

A principal strength of the design is the ethanol comparator, matched to Q in strength (80% v/v), concentration and final ethanol content (0.2–3.2% v/v) at every point. This allows any solvent effect to be separated from the effect of the tincture, which is essential for interpreting mother-tincture studies, where the vehicle is itself a variable. The ethanol comparator showed 0.00% inhibition at every concentration, up to a final ethanol content of 3.2% v/v, indicating that, under these conditions, the solvent did not contribute to the readings and the small response seen with Q arises from the tincture rather than the vehicle.

The low control absorbance (0.030) shows that little denaturation occurred in the absence of sample. This is a useful methodological finding: it indicates that the albumin concentration and heating conditions can be optimised to give a wider dynamic range, which would improve sensitivity in subsequent experiments.

Earlier reports of anti-inflammatory activity concern cinchonine and Cinchona bark extracts in cell-based systems (Parveen et al., 2023; Schink et al., 2018). These used different preparations, species and endpoints and are not directly comparable with the present assay, which is a first-line screen. The present results should be read as the starting point for a stepwise programme of investigation, not as a final statement on the tincture.

6. STRENGTHS AND LIMITATIONS

Strengths

- Ethanol control matched in strength, concentration and final solvent content.
- Concentrations expressed in $\mu\text{L/mL}$ with final ethanol content stated, allowing exact replication.
- Triplicate determinations at five concentrations with a defined, inexpensive method.

Limitations

- No positive control (e.g. diclofenac sodium) and no sample colour blank.
- Concentration of the liquid mother tincture not standardised by marker or dry-residue content.

- Final ethanol content was calculated from the aliquot volumes in a 5 mL final volume (Table B) rather than measured directly.
- A single, non-specific assay on the crude mother tincture.

7. FUTURE SCOPE

The findings define a clear sequence for advanced research:

1. Optimise the albumin concentration and heating conditions to widen the dynamic range of the assay.
2. Include a diclofenac sodium positive control and a sample colour blank.
3. Standardise the tincture (for example by total alkaloid content expressed as quinine) and test a wider concentration range.
4. Extend to complementary models: membrane stabilisation, proteinase inhibition and cell-based inflammatory assays.
5. Apply the validated protocol to other mother tinctures, and evaluate dynamised preparations separately, in their own right, using the same solvent-controlled design.

8. CONCLUSION

This basic study establishes a reproducible, solvent-controlled protocol for evaluating *Cinchona officinalis* Q in the egg albumin denaturation assay and provides baseline data at 6.25–100 $\mu\text{L/mL}$. Under the conditions tested, Q showed a small but measurable inhibition of albumin denaturation (maximum 0.22% at 100 $\mu\text{L/mL}$) against 0.00% for the matched ethanol control, an encouraging preliminary indication of activity that is free of solvent confounding. The study identifies the parameters to refine and the assays to add, and so provides a sound foundation for advanced research on homoeopathic mother tinctures.

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