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ASSESSMENT OF THE ANTI-INFLAMMATORY ACTIVITY OF *SIDDHARTHAKADI AGAD* BY AN IN VITRO METHOD

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ABSTRACT

Inflammation is a protective physiological response against harmful stimuli such as infection, injury, and toxins; however, chronic inflammation may lead to tissue damage and various pathological conditions. *Siddharthakadi Agad*, a classical polyherbal formulation described in *Charaka Samhita*, is traditionally used in Ayurveda for the management of inflammatory due to its *Shothahara* (anti-inflammatory) properties. The present study was designed to evaluate the anti-inflammatory activity of *Siddharthakadi Agad* using in-vitro methods. The raw ingredients of *Siddharthakadi Agad* were collected from authentic sources, identified, authenticated, and processed to prepare a coarse powder, followed by Bhavana using Bastamutra. Aqueous and Ethanolic extracts of the formulation were prepared using Soxhlet extraction. The anti-inflammatory activity was assessed using the Bovine Serum Albumin (BSA) denaturation assay, with Ibuprofen as the standard drug. The percentage inhibition of protein denaturation was calculated Using Spectrophotometer at 660 nm. Results showed a concentration-dependent increase in anti-inflammatory activity in both extracts. The ethanolic extract demonstrated higher inhibition of protein denaturation (36.9%–86.9%) compared to the aqueous extract (27.8%–75.6%), while Ibuprofen exhibited comparable activity (33.6%–85.28%). The highest activity was observed at 500 µg/ml for all samples. The study concludes that *Siddharthakadi Agad* possesses significant in-vitro anti-inflammatory potential, with the ethanolic extract showing superior efficacy. The observed activity may be attributed to the synergistic effect of its bioactive phytoconstituents such as flavonoids, alkaloids, and phenolic compounds. These findings scientifically validate the traditional Ayurvedic use of *Siddharthakadi Agad* in inflammatory conditions and support its further exploration as a potential herbal anti-inflammatory agent.

INTRODUCTION

Inflammation is a protective response of the body against harmful stimuli such as infection, injury, toxins, and tissue damage. It is characterized by redness, heat, swelling, pain, and loss of function. Acute inflammation is the immediate and early response of living vascularized tissue to injury, characterized by the exudation of plasma proteins and the migration of leukocytes, predominantly neutrophils, to the site of injury. whereas chronic inflammation may lead to tissue damage and various disorders. The inflammatory process involves the release of chemical mediators such as cytokines, prostaglandins, and reactive oxygen species, which contribute to the progression of inflammation. Although modern anti-inflammatory drugs are widely used for treatment, their long-term use may produce adverse effects. Therefore, there is increasing interest in herbal medicines as safer alternatives for the management of inflammatory conditions. In *Ayurveda*, inflammation and swelling are described under the term *Shotha*, which occurs due to the vitiation of *Vata*, *Pitta*, *Kapha*, and *Rakta*. Ayurvedic formulations possessing *Shothahara* (anti-inflammatory) properties are traditionally used in the treatment of inflammatory

disorders.[1] In Charaka Samhita, *Chikitsa Sthana*, Chapter 9 (*Unmada Adhyaya*), *Siddharthakadi Agad* is described as a classical Ayurvedic polyherbal formulation used in the management of various disorders such as bhuta, pichas, alakshmi, jwara etc.[2] The formulation contains multiple herbal ingredients known for their *Shothahara* (anti-inflammatory) properties. Many of its constituents possess anti-inflammatory, antimicrobial, anti-allergic, and immunomodulatory activities, which may help in reducing swelling, pain, itching, and inflammatory reactions. Due to these therapeutic properties. Therefore, the present study aims to evaluate the anti-inflammatory activity of *Siddharthakadi Agad* using in-vitro experimental methods to provide scientific validation for its traditional therapeutic use.

AIM AND OBJECTIVES

To evaluate the Anti-inflammatory activity of *Siddharthakadi Agad.*

MATERIALS AND METHODS

1. COLLECTION OF DRUGS

Raw ingredients of *Siddharthakadi Agad* were collected from different available and authentic sources in 2025.

- ***Haridra, Daruharidra, Vacha and Devdaru***- were collected from Uttarkashi in june 2025.
- ***Shirish***- Shirish bark was collected from the Haridwar campus of Rishikul in March 2025.
- ***Katabhi (shweta Shirish)***- Bark of shweta Shirish was collected from kharikhurd shyampur rishikesh in Aug 2025.
- ***Karanj***-fruit was collected from dehradun in Aug 2025.
- ***Aprajita***- Aprajita seeds was collected from the haridwar campus of Rishikul in july 2025.
- *Shunthi, Pippali, Marich, Aamlaki, Haritaki, Bibhitaki, Priyangu, Hingu, Sarsapa, Manjistha* were procured from Herbal automation, Haridwar. Lastly, about 5 liter of *Basta mutra*/ goat urine was collected from Pathri haridwar.

2. DRUG IDENTIFICATION AND AUTHENTICATION

All collected ingredients of *Siddharthakadi Agad* were identified and authenticated by the experts of the Dravyaguna Department at Rishikul Campus, Haridwar (UAU)with **ref. no – DG/RC/UAU-275 Dated: 20/8/2025**

3. PREPARATION OF CHURN

Apparatus required: Iron mortar pestle, grinder, sieve no. 80, containers, physical balance.

PROCEDURE

- All the ingredients of ***Siddharthakadi Agad*** were taken in dried form in 100 grams of the amount and were coarsely grounded separately with Iron mortar-pestle and then coarse powder were passed through sieve no. 80 separately and then mixed to form the final drug which was then stored in an airtight container for further analysis.
- The final amount came out to be 1791 grams, showing a 9g loss after grinding into the churn.

Bhavana

Apparatus Required: Medium-sized *kharal*, physical balance, spoon, and container.

Procedure: The Bhavana process was carried out in the Gurukul kangri universitiy. *Basta mutra* was collected in sterile bottles during the morning hours. For the Bhavana process, 300g of the drug was taken. Each Bhavana required approximately 2–3 days to complete, and about 5–6 hours of trituration was performed daily. A total of Three Bhavanas were administered to *Siddharthakadi Agad*. After completion of the Bhavana process, the weight of the drug increased to 310 g. The final product was stored in an airtight container for further use.

4. ANALYTICAL STUDY

EXTRACTION

SOXHLET EXTRACTION OF THE DRUG-The Purpose of the extraction of crude drugs is to obtain active therapeutic constituents, remove undesirable/inert substances, and concentrate the medicinally useful portion.

- **Method**- Distilled water and Ethanol were used as solvents; hence, two extracts of the drug were obtained. Aqueous extract and Ethanolic extract.
- **Procedure of Aqueous and Ethanolic Extraction**

ALCOHOLIC EXTRACT

100 g of the drug was placed into the Soxhlet chamber, which was then filled with 200 mL of ethanol. An additional 250 mL of ethanol was poured into the round-bottom flask along with a few glass beads, and the complete apparatus was assembled on a heating mantle, and the temperature was kept below the boiling point of ethanol, at 60°C, and the experiment was run for 5-6 days in a row. The contents of the round-bottom flask were collected and poured into a petri dish, where they were allowed to evaporate in a water bath for 1 to 2 days before being dried at room temperature and stored in a container.

AQUEOUS EXTRACT

The Soxhlet chamber was filled with 100g of drug, 200ml of distilled water, and 250ml of distilled water in a round-bottom flask. The entire apparatus was then connected to a heating mantle, and the temperature was kept below the water's boiling point—80°C—for five days in a row. The contents of the round-bottom flask were collected and poured into a petri dish, where they were allowed to evaporate in a water bath for 1 to 2 days before being dried at room temperature, and stored in a container.

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY USING BOVINE SERUM ALBUMIN ASSAY

MATERIALS REQUIRED

- Bovine Serum Albumin (BSA)
- Test sample (plant extract / drug solution)
- Standard anti-inflammatory drug (e.g., Ibuprofen)
- Phosphate Buffer Saline (PBS), pH 6.4 or 7.4
- Distilled water
- Hydrochloric acid (HCl) or Sodium hydroxide (NaOH) (for pH adjustment)

APPARATUS / EQUIPMENT

- Test tubes
- Micropipettes and tips
- Water bath (maintained at ~37°C and 60–70°C)
- Incubator (optional)
- UV–Visible spectrophotometer
- pH meter
- Measuring cylinders

Principle

Protein denaturation is one of the major causes of inflammation. During inflammatory conditions, proteins lose their native structure and function. Substances capable of preventing protein denaturation may therefore exhibit anti-inflammatory activity. This assay evaluates the ability of the test drug formulation to stabilize egg albumin and inhibit heat-induced protein denaturation.

Method

The Bovine serum albumin assay was carried out according to the method described by **Gambhire et al.**, with minor modifications as reported by **Dharmadeva et al.**[3,4]

Procedure

The reaction mixture (5 mL) consists of 2 mL of the extract, 2.8 mL of phosphate-buffered saline (PBS, pH 6.4), and 0.2 mL of 1% bovine serum albumin. The mixtures was thoroughly mixed and incubated in a water bath at 37 °C for 15 min, followed by heating at 70 °C for 5 min. After cooling to room temperature, turbidity was measured at 660 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800)., and Ibuprofen was used as the standard drug. The percentage inhibition of protein denaturation was calculated using the following formula.

Calculation

$$\%\ inhibition of denaturation = 100 \times (1 - A2/A1)$$

Where **A1 = absorption of the control sample**
A2 = absorption of the test sample.

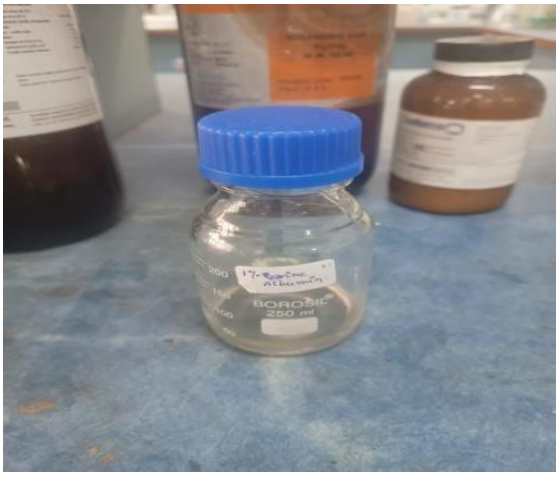


Figure1.a: BSA.

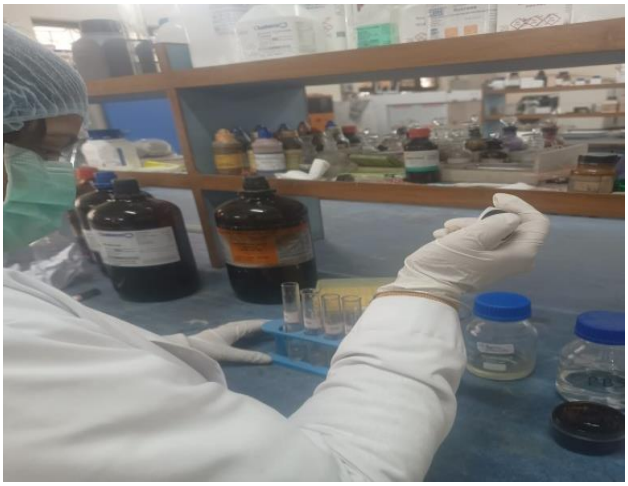


Figure1.b: Mixing Aq and EtOH extract with BSA at different concentrations.

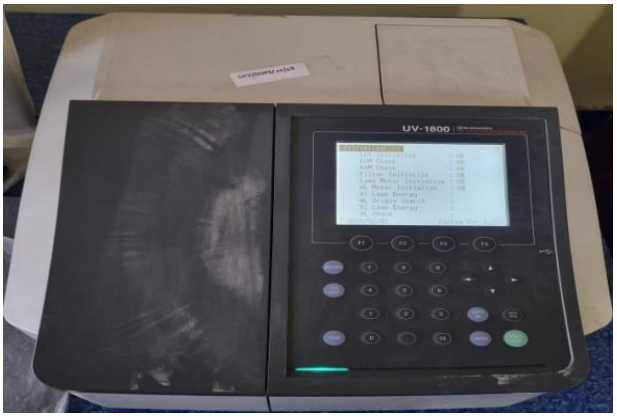


Figure 1.c: Prepared reaction mixtures.

Figure 1.d: Spectrophotometer.

OBSERVATIONS AND RESULTS

Table 1: Anti-inflammatory activity of *Siddharthakadi* Agad extracts at different concentrations.

FINDINGS OF BSA FOR AQUEOUS AND ETHANOLIC EXTRACT

S.N.	Conc. Of sample /standard in(µg/ml)	Ethanolic extract		Aqueous extract		Ibuprofen	
		O.D. (660nm)	%Inhibition	O.D. (660nm)	%Inhibition	O.D. (660nm)	%Inhibition
1	100	0.631	36.9	0.722	27.8	0.664	33.6
2	200	0.521	47.9	0.604	39.6	0.561	43.9
3	300	0.362	63.8	0.454	54.6	0.393	60.69
4	400	0.274	72.6	0.363	63.7	0.302	69.80
5	500	0.131	86.9	0.244	75.6	0.147	85.28

The present study demonstrated a significant concentration-dependent increase in the inhibition of protein denaturation by both ethanolic and aqueous extracts. Among the tested concentrations, the ethanolic extract consistently exhibited greater inhibitory activity compared to the aqueous extract. The highest inhibition was observed at a concentration of 500 µg/ml for both extracts. However, the ethanolic extract showed comparatively higher inhibitory activity than the aqueous extract at this maximum concentration. These findings indicate that both extracts possess notable anti-inflammatory potential, with the ethanolic extract exhibiting superior efficacy.

At **100 µg/ml** concentration, the maximum inhibition was observed with the **ethanolic extract (36.9%)**, followed by the **aqueous extract (27.8%)** and **ibuprofen (33.6%)**.

At **200 µg/ml** concentration, the maximum inhibition was observed with the **ethanolic extract (47.9%)**, followed by the **aqueous extract (39.6%)** and **ibuprofen (43.9%)**.

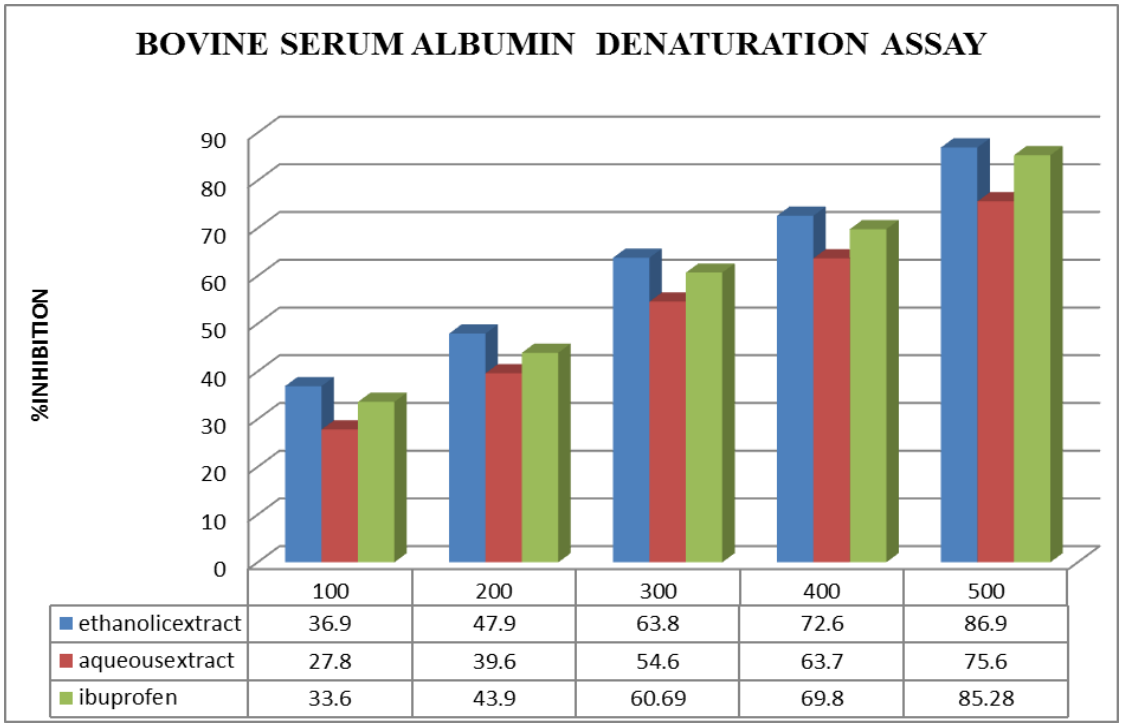
At **300 µg/ml** concentration, the maximum inhibition was observed with the **ethanolic extract (63.8%)**, followed by the **aqueous extract (54.6%)** and **ibuprofen (60.69%)**.

At **400 µg/ml** concentration, the maximum inhibition was observed with the **ethanolic extract (72.6%)**, followed by the **aqueous extract (63.7%)** and **ibuprofen (69.80%)**.

At **500 µg/ml** concentration, the maximum inhibition was observed with the **ethanolic extract (86.9%)**, followed by the **aqueous extract (75.6%)** and **ibuprofen (85.28%)**.

These findings indicate that both extracts possess considerable anti-inflammatory potential, with the ethanolic extract showing comparatively superior efficacy throughout the study.

GRAPHICAL REPRESENTATION OF INHIBITION OF BOVINE SERUM ALBUMIN ASSAY



	100	200	300	400	500
ethanolicextract	36.9	47.9	63.8	72.6	86.9
aqueousextract	27.8	39.6	54.6	63.7	75.6
ibuprofen	33.6	43.9	60.69	69.8	85.28

DISCUSSION

The ingredients of *Siddharthakadi Agad*, including *Brassica campestris*, *Curcuma longa*, *Berberis aristata*, *Triphala*, and other medicinal plants, are predominantly characterized by *Tikta* (bitter) and *Katu* (pungent) *rasa*, *Laghu* and *Ruksha guna*, *Ushna virya*, and *Katu vipaka*. These properties are associated with *Deepana-Pachana*, *Krimighna* (antimicrobial), *Vishaghna* (detoxifying), and *Shothahara* (anti-inflammatory actions), which collectively support their traditional therapeutic use.

Most of the ingredients exhibit well-established anti-inflammatory, antimicrobial, and antioxidant activities. Plants such as *Brassica campestris*, *Acorus calamus*, *Ferula narthex*, *Pongamia pinnata*, *Cedrus deodara*, and *Rubia cordifolia* show anti-inflammatory effects through modulation of inflammatory mediators and pathways like NF-κB and COX. Similarly, *Terminalia chebula*, *Terminalia bellirica*, and *Emblica officinalis* demonstrate strong anti-inflammatory and antioxidant activity due to their rich phenolic content. Spices such as *Piper longum*, *Piper nigrum*, and *Zingiber officinale* further enhance these effects through bioactive compounds like piperine and gingerols.[5-8]

Additionally, *Curcuma longa* and *Berberis aristata* exhibit potent anti-inflammatory and antioxidant effects, while several other ingredients contribute significant antimicrobial activity against a wide range of pathogens. *Bastamutra* also supports detoxifying and antimicrobial actions as per traditional and modern evidence.[9] In conclusion, the formulation demonstrates synergistic anti-inflammatory, antimicrobial, and antioxidant activities, validating its traditional Ayurvedic use in the management of inflammatory, infectious, and oxidative stress-related conditions.[10,11]

CONCLUSION

The present study on *Siddharthakadi Agad* demonstrated significant concentration-dependent anti-inflammatory activity in both aqueous and ethanolic extracts using the bovine serum albumin denaturation assay. Among the two extracts, the ethanolic extract exhibited comparatively higher inhibition of protein denaturation at all tested concentrations, indicating superior anti-inflammatory potential.

The formulation comprises multiple medicinal plants predominantly characterized by Tikta (bitter) and Katu (pungent) rasa, Laghu and Ruksha guna, Ushna virya, and Katu vipaka, which are associated with *Deepana-Pachana*, *Krimighna*, *Vishaghna*, *and Shothahara* actions. The presence of bioactive phytoconstituents such as flavonoids, phenolics, alkaloids, and terpenoids in the individual ingredients contributes to anti-inflammatory activities.

Overall, the findings validate the traditional Ayurvedic use of *Siddharthakadi Agad* and suggest that the formulation possesses significant therapeutic potential in managing inflammatory conditions, supported by both classical principles and modern scientific evidence.

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