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COMPREHENSIVE ANALYSIS OF THE ANTI-INFLAMMATORY POTENTIAL OF *KSHARAGAD* EXTRACTS: AN INVITRO STUDY

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ABSTRACT

Inflammation is a protective response of the body against harmful stimuli such as infection, injury, toxins, and immune reactions. In *Ayurveda*, inflammatory conditions are broadly described under the concept of *Shotha*, which is caused by the imbalance of *Doshas* and other external factors. Classical *Ayurvedic* texts classify *Shotha* into different types based on causative factors and *Dosha* predominance. Modern science explains inflammation as a complex biological process involving immune cells, inflammatory mediators, and vascular changes. The present in-vitro study is undertaken to evaluate the anti-inflammatory activity of *Ksharagad extracts* using the Bovine Serum Albumin (BSA) denaturation assay. The study is designed to assess the potential of *Ksharagad* extracts as an anti-inflammatory formulation and to provide scientific validation for its traditional *Ayurvedic* use in the management of inflammatory conditions.

KEYWORDS: *Ksharagad*, *Shotha*, Anti-inflammatory activity, Bovine Serum Albumin (BSA) denaturation assay.

INTRODUCTION

Inflammation is a fundamental biological process that serves as the body's natural defense against harmful stimuli, such as pathogens, damaged cells, and environmental irritants. It plays an essential role in protecting tissues, eliminating harmful agents, and promoting healing. Acute inflammation is a short-term, protective response that helps repair injured tissues and restore normal physiological function. However, when inflammation becomes prolonged or is not properly regulated, it can contribute to the development of chronic diseases, including cancer, cardiovascular disease, autoimmune disorders, and inflammatory skin conditions.[1–3] Conventional anti-inflammatory therapies, including non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, are effective but are often associated with adverse effects such as gastrointestinal, renal, and cardiovascular complications on long-term use. These limitations have stimulated the search for safer and more effective therapeutic alternatives, particularly from traditional systems of medicine. In *Ayurveda*, inflammatory disorders are broadly described under the concept of ***Shotha***, which arises due to the vitiation of ***Doshas*** in association with etiological factors such as trauma (***Abhighata***), toxins (***Visha***), infections, and other pathogenic influences. Classical *Ayurvedic* literature also emphasizes the therapeutic utility of ***Agad Yogas*** in conditions involving poisoning and various inflammatory process. ***Ksharagad*** is one such *Agad yogas* described in the *Charaka Samhita* (*Visha Chikitsa Adhyaya*) and the

Sushruta Samhita (*Kalpa Sthana, Dundubhisvaniya Adhyaya)*. ***Ksharagad*** described by *Acharya Charak* has been taken for the present study. The formulation consists primarily of ***Palasha Kshara*** along with several ***Vishaghna*** (antitoxic) herbal ingredients, which are traditionally indicated for neutralizing toxins and alleviating inflammatory manifestations. *Acharya Charaka* also mentioned ***Ksharagad*** during the ***Tritiya Vega*** (third stage) of poisoning, when the poison spreads throughout the body and symptoms become more severe. At this stage, ***Ksharagad*** is administered with honey and water to prevent the further spread of poison, to reduce inflammation, and to protect the body from tissue damage through its **Lekhana** (scraping) and **Shothanashaka** (anti-inflammatory) properties. Considering the increasing prevalence of inflammatory disorders and the limitations of existing therapies, scientific evaluation of this classical formulation is essential. Therefore, the present study aimed to investigate the anti-inflammatory activity of *Ksharagad* extracts and validate its traditional therapeutic claims.

AIM AND OBJECTIVES

To evaluate the Anti-inflammatory activity of *Ksharagad* extracts.

MATERIALS AND METHODS

1. Collection and Preparation of *Ksharagad.*

1. ***Palasha***

a. Roots and Seeds of *Palasha* were procured from Herbal Automation, Haridwar in June 2025.
b. Flowers of *Palasha* were collected from Rishikul Campus, UAU, Haridwar in March 2025.
c. Stem, bark and leaves were collected from Rishikul Campus, UAU, Haridwar in April 2025

2. Rhizome of ***Haridra*** *and root of* ***Daruharidra*** were collected from Uttarkashi in March 2025.
3. ***Jatamansi****, and* ***Hingu*** were collected from uttarkashi in June 2025.
4. Inflorescences of **Tulsi (*Surasmanjari*)** was collected from Rishikul Campus, UAU, Haridwar in July 2025.
5. Root of ***Kushtha*** was collected from Dhrali village, Uttarkashi, uttrakhand in June 2025.
6. ***Shweta Sariva, Krishna Sariva*** were procured from Orrisa in Aug 2025.
7. ***Laksha, Harenu, Madhuka, Shunti, Maricha, Pippali, Gairika, Saindhav*** were procured from Herbal Automation, Haridwar in Aug 2025.

All the ingredients except *Hingu* had been thoroughly washed under running water and dried.

Drug Identification and Authentication

All collected ingredients of *Kshar Agad* were identified and authenticated by the experts of the Dravyaguna Department at Rishikul Campus, Haridwar (UAU) with. Ref. no – DG/RC/UAU-276.

Preparation of *Ksharagad*

The preparation of *Ksharagad* was divided into two main steps: **(1) Preparation of *Kshara*** and **(2) Preparation of *Ksharagad Churna***.

1. Preparation of *Kshara*[4]-Following *Sushruta Saṁhita* (*Sutrasthana* 11th *Adhyaya* – "*Kṣara Karma Vidhi Adhyaya*"). *Palasha Kshara* was prepared in three phases.

a. Ash Preparation: Dried *Palasha Panchanga* (≈9 kg) was cleaned, coarsely powdered, and incinerated to obtain whitish-grey ash.

b. *Kshara Jala*: 910 g of ash was mixed with six times its weight in water, allowed to settle for 24 hours, and filtered 21 times through muslin cloth to obtain clear *Kshara Jala.*

c. *Kshara* Formation: The filtrate was heated to evaporate water, yielding 90 g of *Kshara*.

2. Preparation of *Ksharagad Churna*

All ingredients, except *Palash panchang* were individually coarsely powdered, sieved (No. 80), and mixed with *Palasha Kshara*. The final *churna* weighed 1.583 g, with a minimal loss of 17 g during processing, and was stored in an airtight container for further analysis.

Aqueous Extraction

For the aqueous extraction, 100 g of the powdered drug was placed in the Soxhlet chamber (thimble). About 200 ml of distilled water was added into the chamber, while 250 ml of distilled water was poured into a 500 ml round-bottom flask. The complete Soxhlet apparatus was assembled and connected to a heating mantle. The extraction process was carried out continuously for five days, maintaining the temperature below the boiling point of water at approximately 80°C.

After completion of extraction, the contents collected in the round-bottom flask were transferred into a Petri dish and concentrated by evaporation on a water bath for 1–2 days. The obtained extract was then dried at room temperature and stored for further research work.

Ethanolic Extraction

For the ethanolic extraction, 100 g of the powdered drug was placed into the Soxhlet chamber. Approximately 100 ml of ethanol was added into the chamber, while 250 ml of ethanol along with a few glass beads was added to the round-bottom flask. The Soxhlet apparatus was assembled properly and connected to a heating mantle. The extraction was carried out continuously for five days at a temperature maintained below the boiling point of ethanol, around 60°C.

After completion of the extraction process, the contents of the round-bottom flask were collected in a Petri dish and concentrated by evaporation using a water bath for 1–2 days. The dried extract obtained at room temperature was then preserved for further research work.

Table showing yield of Kshar *Agad* extracts

S.No.	Extraction Method	Solvent	Weight of Dry Drug (g)	Weight of Extract (g)	Percentage Yield (%)
1	Heat extraction	Aqueous	100	29.2	29.2%
2	Heat extraction	Ethanol	100	26.8	26.8%

Evaluation Of Anti-Inflammatory Activity Using Bovine Serum Albumin Denaturation Assay

MATERIAL REQUIRED

S. No.	Material / Reagent	Specification / Purpose
1	Bovine Serum Albumin	Stable and reproducible protein source
2	Phosphate-buffered saline (PBS)	pH 6.4; reaction medium and blank
3	Test sample / extract	Different concentrations for evaluation
4	Ibuprofen	Standard anti-inflammatory
6	Test tubes / Microcentrifuge tubes	Reaction mixture preparation
9	Water bath	Maintained at 37 °C and 70 °C
10	Incubator	For incubation at 37 °C
11	UV–Visible spectrophotometer	Measurement of absorbance at 660 nm

The protein denaturation assay was performed according to the method described by Gambhire *et al.*[5], with minor modifications as reported by Dharmadeva *et al.*[6]

The reaction mixture (5 ml) was made up of 2 ml of extract, 2.80 ml of phosphate buffered saline (PBS, pH 6.4), and 0.2 ml of 1% bovine albumin. After mixing the mixture, it was incubated for 15 minutes at 37 °C in a water bath before being heated for 5 minutes at 70 °C. After cooling, a UV/VIS spectrometer was used to measure the turbidity at 660 nm. The control was phosphate buffer solution and ibuprofen as a reference drug. Using the following formula, the percentage inhibition of protein denaturation was determined

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

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

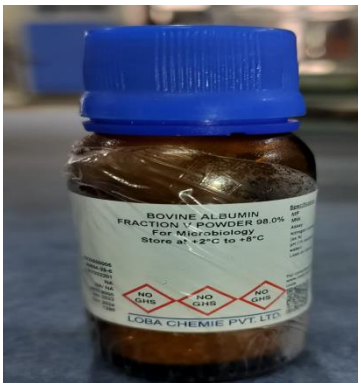


Fig 1:a Bovine Serum Albumin.

Preparation of PBS; Disodium hydrogen phosphate-1.79gms, Potassium dihydrogen phosphate-1.36gms and Sodium chloride – 7gms. These salts were mixed with 1 L of distilled water to make phosphate buffer saline.

Fig1.b: Reaction mixture was made up of 2 ml of extract, 2.80 ml of phosphate buffered saline (PBS, pH 6.4), and 0.2 ml of 1% bovine albumin.

Fig.1. d After mixing the mixture at varying concentrations (100,200,300,400,500µgm/ml), it was incubated for 15 minutes at 37 C. After cooling, a UVspectrometer was used to measure the turbidity at 660 nm. Ibuprofen was used as a Standard drug.

Fig 1.e: Spectrophotometer.

IN-VITRO ANTI-INFLAMMATORY STUDY OF *KSHARAGAD*

Table 1: Showing Anti-inflammatory activity of Ibuprofen (standard drug) at different concentrations.

S.No.	Concentration of IBUPROFEN (µg/ml)	Optical Density (660 nm)	% Inhibition
1	100	0.664	33.6
2	200	0.561	43.9
3	300	0.399	60.69
4	400	0.302	69.80
5	500	0.147	85.25

Table 2: Showing Anti-inflammatory activity of *Kshar Agad* extracts at different Concentrations.

S. No.	Concentration of Sample (µg/ml)	Aqueous Extract O.D.	Aqueous Extract % Inhibition	Ethanolic Extract O.D.	Ethanolic Extract % Inhibition
1	100	0.754	24.6	0.679	32.1
2	200	0.636	36.4	0.572	42.8
3	300	0.482	51.8	0.407	59.3
4	400	0.391	63.7	0.318	68.2
5	500	0.276	72.4	0.169	83.1

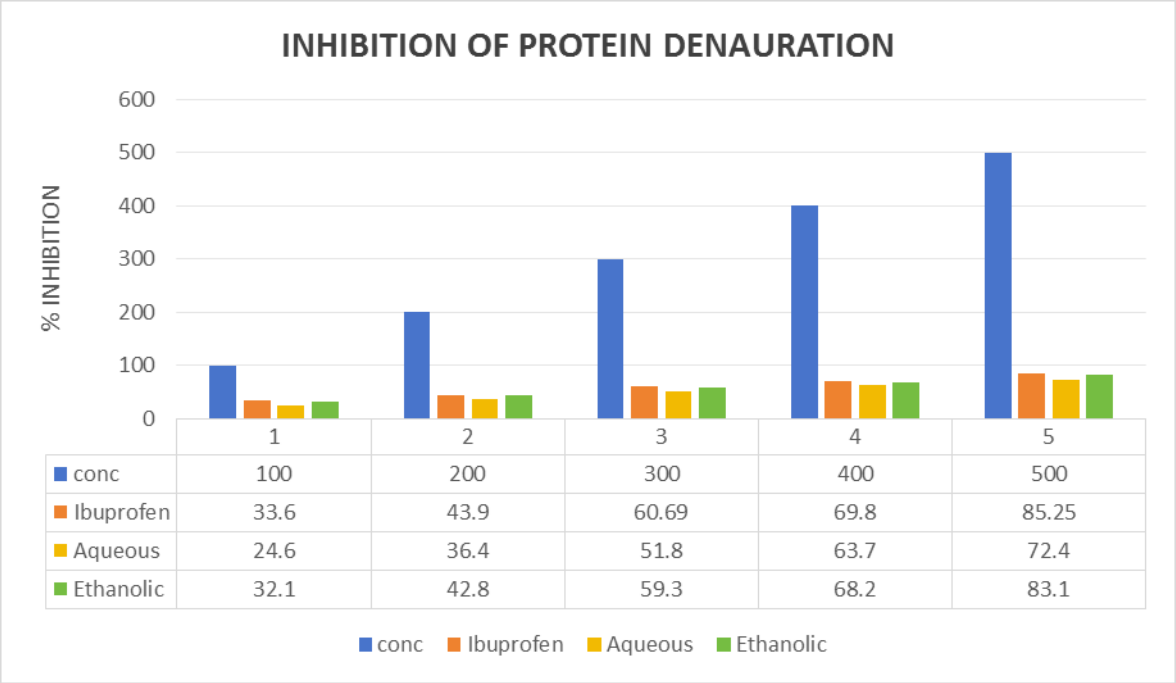
The anti-inflammatory activity of Ibuprofen and *Kshar Agad* extracts was evaluated at different concentrations using Bovine Serum Albumin assay. The activity was assessed by measuring the optical density (OD) at 660 nm and calculating the percentage inhibition of protein denaturation. A decrease in optical density along with an increase in percentage inhibition indicates stronger anti-inflammatory activity.

The standard drug, Ibuprofen, showed a concentration-dependent anti-inflammatory effect. As the concentration increased from 100 to 500 µg/ml, the optical density decreased from 0.664 to 0.147, while the percentage inhibition increased from 33.6% to 85.25%. The maximum inhibition was observed at 500 µg/ml, demonstrating the strong anti-inflammatory potential of ibuprofen.

Similarly, both aqueous and ethanolic extracts of *Kshar Agad* exhibited significant anti-inflammatory activity in a dose-dependent manner. In the aqueous extract, the optical density decreased from 0.754 to 0.276 with

increasing concentration, whereas the percentage inhibition increased from 24.6% to 72.4%. The ethanolic extract showed comparatively better activity, with OD values decreasing from 0.679 to 0.169 and percentage inhibition increasing from 32.1% to 83.1% as the concentration increased from 100 to 500 µg/ml.

On comparison, the ethanolic extract demonstrated higher anti-inflammatory activity than the aqueous extract at all tested concentrations and showed activity nearly comparable to the standard drug ibuprofen. At 500 µg/ml, the ethanolic extract produced 83.1% inhibition, which was close to the 85.25% inhibition shown by ibuprofen, whereas the aqueous extract exhibited 72.4% inhibition. These findings suggest that the ethanolic extract of *Kshar Agad* contains a higher amount of bioactive phytoconstituents responsible for anti-inflammatory activity. Overall, the results indicate that *Kshar Agad* possesses significant anti-inflammatory potential, particularly in its ethanolic extract form.



	1	2	3	4	5
conc	100	200	300	400	500
Ibuprofen	33.6	43.9	60.69	69.8	85.25
Aqueous	24.6	36.4	51.8	63.7	72.4
Ethanolic	32.1	42.8	59.3	68.2	83.1

Fig.2: Comparative Evaluation of Protein Denaturation Inhibition by Standard Ibuprofen and *Kshar Agad* extracts at different concentrations.

DISCUSSION

The present study demonstrated significant anti-inflammatory activity of *Ksharagad* extracts in a concentration-dependent manner, particularly in the ethanolic extract, which showed activity comparable to the standard drug Ibuprofen.[7,8] The observed anti-inflammatory effect may be attributed to the collective pharmacodynamic properties of the 17 ingredients present in *Ksharagad*, namely *Palasha, Haridra, Daruharidra, Sunthi, Maricha, Pippali, Kushtha, Laksha, Gairika, Saindhav, Harenu, Surasmanjari, Hingu, Krishna Sariva, Shweta Sariva, Jatamansi, and Madhuka,* all of which are reported to possess significant *Shothahara, Krimighna, Vishaghna,* and *Raktaprasadana* activities.[9,10] *Acharya Charaka* has described the utility of this formulation in conditions such as *Twak Dosha, Krimi, Sotha, Gulma, Arsha, Bhagandra, Pandu, Kasa,* and other disorders associated with inflammation, toxins, and derangement of *Doshas*, indicating its broad therapeutic potential.[11]

From the *Ayurvedic* perspective, the anti-inflammatory activity of ***Ksharagad*** can be correlated with its predominant ***Rasa Panchaka*** properties. Most of the drugs in the formulation possess ***Katu, Tikta, Kashaya,*** and ***Madhura Rasa***.[12,13] ***Katu Rasa*** exhibits ***Krimighna, Vishaghna, Shothahara, Deepana, Pachana, Lekhana,*** and ***Kleda Shoshana*** properties, thereby helping in the alleviation of ***Kapha Dosha*** and reduction of inflammatory exudates.[14] ***Tikta Rasa*** is known for its ***Krimighna, Kandughna, Raktaprasadana, Vishaghna,*** and ***Dahaprashamana*** actions, which contribute to detoxification, reduction of burning sensation, and purification of blood, thereby assisting in the management of inflammatory conditions.[14] ***Kashaya Rasa*** possesses ***Sanshamana, Lekhana,*** and ***Kleda Shoshana*** properties and acts on **Kapha,** ***Pitta,*** and ***Rakta Dushti***, further supporting its anti-inflammatory role.[12] ***Madhura Rasa*** provides ***Vishaghna, Twachya,*** and ***Pitta-Vata Shamaka*** effects, promoting tissue healing and soothing inflamed tissues.[13]

The ***Guna*** of the formulation also plays a significant role in its pharmacological action. The majority of the ingredients possess ***Laghu, Ruksha, Snigdha,*** and ***Tikshna Guna***.[13,14] ***Laghu*** *and* ***Ruksha Guna*** aid in ***Kapha Shamana*** and removal of accumulated toxins and excessive secretions, whereas ***Tikshna Guna*** facilitates

rapid penetration into microchannels and enhances therapeutic action.[14] ***Snigdha*** **Guna** supports tissue nourishment, healing, and restoration of skin integrity. *Acharya Sushruta* has described the ***Lekhana*** and ***Ropana*** properties of ***Laghu Guna***, which may help in reducing inflammatory swelling and promoting tissue repair.[15]

Most of the ingredients of ***Ksharagad*** possess ***Ushna Virya***, while a few exhibit ***Sheeta Virya***.[13,14] ***Ushna Virya*** contributes to ***Vata-Kapha Shamana, Deepana, Pachana,*** and rapid metabolic action at the cellular level, thereby reducing ***Ama*** and inflammatory processes.[14] ***Sheeta Virya*** helps in ***Pitta Shamana*** and ***Rakta Prasadana***, reducing burning sensation and inflammation.[13] The presence of both ***Ushna*** and ***Sheeta Virya*** provides a balanced action against inflammatory pathology involving all three ***Doshas***.

Furthermore, the predominance of ***Katu*** and ***Madhura Vipaka*** in ***Ksharagad*** contributes to its ***Tridoshahara*** effect.[12,14] ***Katu Vipaka*** imparts ***Kaphahara*** and ***Lekhana*** action, helping in reducing edema and inflammatory accumulations.[14] ***Madhura Vipaka*** provides ***Vata-Pitta Shamana*** and promotes healing and rejuvenation of tissues.[12]

In addition to these *Ayurvedic* attributes, the anti-inflammatory activity of *Ksharagad* is further supported by the presence of several bioactive phytoconstituents in its ingredients. Herbs such as ***Haridra, Hingu, Shunthi, Maricha, Kushta, Harenu, Shweta Sariva, Krishna Sariva, Madhuka, Laksha,*** and ***Surasamanjiri*** possess well-documented anti-inflammatory activity due to constituents including **curcuminoids, ferulic acid, gingerols, sesquiterpenes, flavonoids, coumarins, terpenoids,** and **glycyrrhizin**. Moreover, potent antioxidant compounds such as **curcuminoids** (Haridra), **flavonoids** (*Palasha, Daruharidra, Jatamansi, Pippali, Maricha, Kushta*, and *Krishna Sariva*), **gingerols** (*Shunthi*), **ferulic acid** (*Hingu)*, and **glycyrrhizin** (*Madhuka*) help neutralize reactive oxygen species and reduce oxidative stress. Thus, the significant anti-inflammatory activity observed in the present study may be attributed to the combined effects of the ***Rasa, Guna, Virya,*** and ***Vipaka*** properties of the constituent drugs along with their rich phytochemical composition. The higher activity shown by the ethanolic extract suggests that ethanol may have extracted greater amounts of bioactive phytoconstituents responsible for protein denaturation inhibition, antioxidant activity, and anti-inflammatory effects.[16,17]

CONCLUSION

The present in-vitro study demonstrated that *Ksharagad* extracts possesses significant anti-inflammatory activity, as evidenced by its ability to inhibit protein denaturation in the Bovine Serum Albumin assay. Both aqueous and ethanolic extracts exhibited concentration-dependent anti-inflammatory effects; however, the ethanolic extract showed comparatively higher activity and produced inhibition values nearly comparable to the standard drug ibuprofen at higher concentrations. These findings indicate that the ethanolic extract may contain a greater concentration of bioactive phytoconstituents responsible for the observed pharmacological action.

Overall, the study provides scientific support for the traditional *Ayurvedic* use of *Ksharagad* in the management of inflammatory disorders.

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