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Research Article

Champak Agad

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Comprehensive analysis of the Anti-Inflammatory Activity of Champak Agad: An In-Vitro Study

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Introduction: Prolonged inflammation can cause several health problems like cardiovascular diseases, arthritis, Crohn's disease, ulcerative colitis, and even cancer. Herbal anti-inflammatory formulations can be used to reduce the symptoms of inflammation as they have fewer side effects compared to synthetic drugs. One such herbal formulation is Champak Agad. This study was implemented to evaluate the possibility of Champak Agad's anti-inflammatory properties, which may be important for treating inflammatory diseases.

Materials and Methods: Champak Agad was prepared according to the classical reference. The anti-inflammatory activity of Champak Agad was examined using an egg albumin protein denaturation assay in which the aqueous and alcoholic extract of the drug was used and diclofenac was used a standard drug.

Result: In this study, the drug extracts showed varying degrees of inhibition according to different drug concentrations. This investigation demonstrated that the aqueous extract exhibited higher anti-inflammatory activity than the alcoholic extract. The aqueous extract showed an inhibition rate of 85% with a 200mg/ml drug dose and the inhibition rate observed with the alcoholic extract was 82% with a 200mg/ml drug dose.

Discussion: By inhibiting protein denaturation in a concentration-dependent manner, the current study shows that Champak Agad extracts have strong anti-inflammatory properties.

Keywords: Inflammation, Champak Agad, anti-inflammatory, protein denaturation assay

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Ankita Nautiyal, Post Graduate Scholar, PG Department of Agad Tantra Evum Vidhi Vaidyak, Uttarakhand Ayurved University, Rishikul Campus, Haridwar, Uttarakhand, India. Email: nautiyal.ankita94@gmail.com	Nautiyal A, Tiwari RC, Mittal B, Belwal NS, Comprehensive analysis of the Anti-Inflammatory Activity of Champak Agad: An In-Vitro Study. J Ayu Int Med Sci. 2025;10(3):91-98. Available From https://jaims.in/jaims/article/view/4071/	

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Introduction

Inflammation is a natural biological response to injury or infection, playing a critical role in the body's healing process. However, chronic inflammation can lead to a host of diseases, including arthritis, cardiovascular disorders, and even cancer. Traditionally, synthetic anti-inflammatory drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, have been the cornerstone of inflammation management. While these drugs are effective, their long-term use is often associated with significant side effects, including gastrointestinal bleeding, cardiovascular risks, and renal impairment. These limitations have spurred a growing interest in natural alternatives, particularly plant-based anti-inflammatory agents, which offer a safer and potentially more sustainable approach to managing inflammation. Plants have been used for centuries in traditional medicine systems across the world to treat inflammatory conditions. Numerous studies have identified a wide array of bioactive compounds in plants, such as flavonoids, alkaloids, terpenoids, and polyphenols, which exhibit potent anti-inflammatory properties. These natural compounds work through multiple mechanisms, including the inhibition of pro-inflammatory enzymes, scavenging of free radicals, and modulation of immune responses. Unlike synthetic drugs, which often target a single pathway, plant-based anti-inflammatory agents tend to exert their effects through a multi-targeted approach, potentially leading to fewer side effects and a broader therapeutic impact. One such *Agad* is *Champak Agad. Acharya Vagbhat* has mentioned *Champak Agad* in *Keetlootadivishpratishedham* chapter of *Uttarsthan*. This *Agad* is indicated to be useful in all *Loota* and *Keeta Visha* and can treat symptoms of insect bites.**[1]** Most of symptoms of Insect bites are manifested on skin like Pain, inflammation, burning sensation, redness, and secondary infections due to breaking of skin barrier. The benefits of natural anti-inflammatory plants extend beyond their efficacy. They are often more accessible, cost-effective, and culturally acceptable, particularly in regions where traditional medicine is deeply rooted. Moreover, growing body of scientific evidence supporting their therapeutic potential has led to increased integration of plant-based remedies into modern healthcare practices.

As demand for safer & more holistic treatment options continues to rise, natural anti-inflammatory plants represent promising avenue for addressing global burden of inflammatory diseases. This article explores potential of these natural remedies, comparing their benefits & mechanisms of action to those of synthetic anti-inflammatory drugs, & high. their role in future of inflammation management.

Aim and Objectives

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

Materials and Methods

1. Collection of Drugs

The raw material of *Champak Agad* was collected from different available & authentic sources in 2023.

- *Haridra* was collected from the Rishikul campus, Haridwar in January 2023
- *Daruharidra* was collected from the Dharali regions of Uttarakashi in June 2023.
- *Pattrang* was acquired from Coimbatore, Tamil Nadu in July 2023.
- *Nagkesar* was acquired from Odisha in July 2023.
- *Tagar mool* and *Manjistha* were bought from Herbal Automation, Haridwar in July 2023.

2. Drug Identification and Authentication

All collected ingredients of *Champak Agad* were identified and authenticated by the experts of the Dravyaguna Department at Rishikul Campus, Haridwar (UAU).

3. Preparation of *Churna*

Apparatus required

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

Procedure

All ingredients of *Champak Agad* were taken in dried form in 100 grams of amount each and were coarsely ground separately with Iron mortar-pestle and then they were made into fine powder form by grinding and passed through sieve no. 80 separately and then mixed to form final drug which was then stored in an airtight container for further analysis.

4. Analytical Study

Extraction Process

- Extraction involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures.
- It is the method of removing active constituents from a solid or liquid using a liquid solvent.
- The purpose of this extraction procedure for crude drugs is to attain the therapeutically desired portion.
- There are various types of extraction procedures like maceration, infusion, percolation, etc.
- For the present study Soxhlet Extraction method was chosen.

5. Soxhlet Extraction Method

For the aim of extraction, distilled water and ethanol were used as solvents hence, two extracts of the drug were obtained.

- Aqueous extract
- Alcohol extract - ethanolic

Materials Required

- Round bottom flask (500 ml)
- Screw cap tube
- Precision electronic scales
- Tarred Petri dish
- Solvents: 95% ethanol and distilled water
- Soxhlet chamber/Thimble (500 ml)
- Heating mental
- Condenser along with 2 tubes
- Cotton
- Water bath
- Glass beads
- Filter paper

For Ethanolic Extraction

100g of drug was taken and put in Soxhlet chamber, which was then filled with 100ml of ethanol. Another 250ml of ethanol was added to round-bottom flask along with some glass beads, and entire setup was connected to a heating mantle.

The temperature was kept below the boiling point of ethanol, at 60°C, and the experiment was run for 5 days in a row. The contents of the round-bottom flask were collected and placed in 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 30 ml screw cap bottle.

Then preserved in the refrigerator for further research work.

Figure 1.a: Champak Agad

Figure 1.b: Soxhlet heat extraction

Figure 1.c: Water Bath

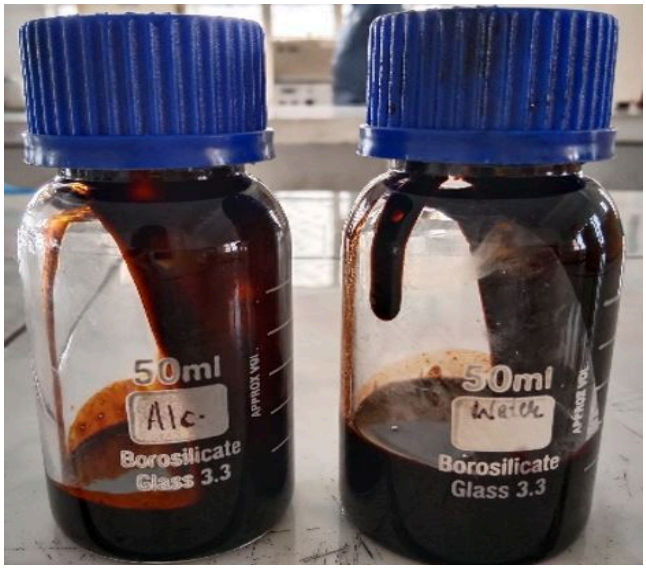

Figure 1.d: Final prepared Ethanolic extract and Aqueous extract

For Aqueous Extraction

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 then collected in a petri dish, allowed to evaporate in a water bath for one to two days, and then dried at room temperature.

The final extract was preserved in the refrigerator in a 30 ml screwcap bottle.

Evaluation Of Anti-Inflammatory Activity Using Egg Albumin Protein Denaturation Assay

Materials Required

- Sample extracts
- Diclofenac sodium
- Fresh egg
- Phosphate buffer saline
- Glass test tubes
- Pipette
- Water bath
- Spectrophotometer

Protein denaturation is considered to be one of the reasons for inflammation hence, this assay was done to investigate the potential of the drug formulation to stabilize the albumin and prevent it from denaturation.

- Protein denaturation study was assayed according to the method of Gambhire *et al*.**[2]**, with some modifications as described in Dharmadeva, *et al.***[3]**
- The reaction mixture having a total volume of 5ml consisted of 0.2 ml of fresh egg albumin, 2.8 ml of buffered phosphate saline (PBS, pH 6.4), and 2.0 ml of different concentrations of sample extracts, i.e. 25mg/ml, 50mg/ml, 100mg/ml,150mg/ml and 200mg/ml.
- The positive control consisted of 0.2 ml of fresh egg albumin, 2.8 ml of PBS (pH 6.4), and 2.0 ml of diclofenac sodium at different concentrations i.e. 5mg/ml, 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml.

- Negative control samples contained the same amount of egg albumin and PBS with 2.0 ml of distilled water.
- The mixture was incubated at 37± 2°C for 15 min and then heated at 70°C for 5 min to induce denaturation.
- After cooling, the absorbance was recorded at 660 nm using the vehicle as a blank. The percentage inhibition of protein denaturation was calculated using the formula

(%) Percentage inhibition = $A_c - A_1/A_c \times 100$

Where A_1 is the absorbance of the test sample and A is the absorbance of the control

Figure 2.a: Fresh egg albumin

Figure 2.b: Prepared reaction mixtures

Figure 2.c: Incubation of reaction mixtures in water bath

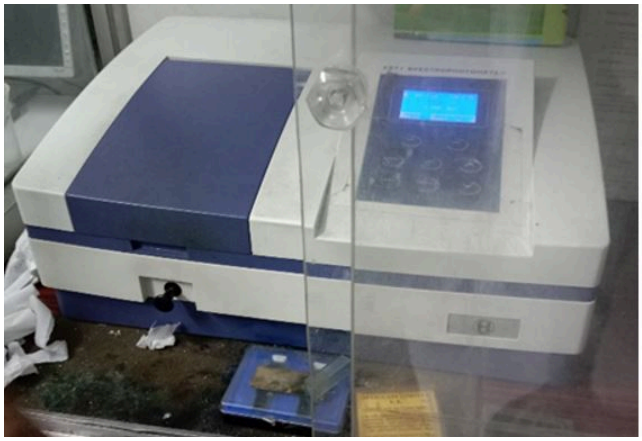

Figure 2.d: Spectrophotometer

Observations and Results

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

SN	Conc. of Diclofenac (mg/ml)	Optical density (660nm)	% inhibition
1.	12.5	0.54	28
2.	25	0.42	44
3.	50	0.18	76
4.	75	0.12	84
5.	100	0.05	93.33

Table 2: Anti-inflammatory activity of *Champak Agad* extracts at different concentrations

SN	Conc. of sample (mg/ml)	Ethanolic extract		Aqueous extract	
		O.D.	% Inhibition	O.D.	% Inhibition
1.	25	0.450	22.41	0.302	25
2.	50	0.355	38.79	0.235	42.5
3.	100	0.268	53.80	0.132	67.5
4.	150	0.194	66.55	0.090	77.5
5.	200	0.102	82.50	0.06	85

The present study demonstrated concentration-dependent inhibition of egg albumin protein denaturation by extracts of *Champak Agad* at concentrations of 25 mg/ml, 50 mg/ml, 100 mg/ml, 150 mg/ml, and 200 mg/ml. Diclofenac sodium, used as a standard drug at a range between 25mg/ml and 100 mg/ml, also showed concentration-dependent inhibition of protein denaturation, being used as a reference.

The maximum inhibition of 25% at the concentration of 25 mg/ml was observed with aqueous extract, while the ethanol extract achieved 22.41%. The inhibition increased further to 42.5% and 38.79% at a concentration of 50 mg/ml in water and ethanol extract respectively.

In this case, a more pronounced effect was observed compared with lower concentrations. The inhibition rate with aqueous extract at a concentration of 100mg/ml reached 67.5%, while for the ethanol extract it gave 53.8%. At 150mg/ml, the inhibition reached 77.5% with the aqueous extract and 66.55% with the ethanolic extract.

At the highest concentration of 200 mg/ml, the aqueous extract showed the highest inhibition (85%), while the ethanolic extract followed closely with 82.5%.

Champak Agad extracts exhibited a clear concentration-dependent increase of inhibition rates, with the greatest activity found at 200 mg/ml. Importantly, the aqueous extract demonstrated better efficacy than the ethanol extract.

The anti-inflammatory effect of both extracts is significant, but both are slightly less potent than diclofenac. Therefore, these observations suggested that while diclofenac is a stronger inhibitor, the aqueous and ethanol extracts of *Champak Agad* are promising natural alternatives with substantial anti-inflammatory properties.

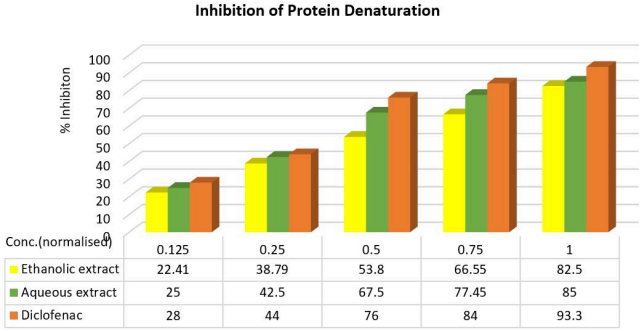

Figure 3. Comparison of Champak Agad (25–200 mg) and Diclofenac (12.5–100 mg) using normalized concentrations (0–1) to enable direct visualization of their relative effects despite different dosage ranges.

Discussion

The anti-inflammatory actions shown by *Champak Agad* can be attributed to the synergetic effects of its ingredients, supported by both Ayurvedic principles and modern scientific studies: *Haridra, Daruharidra, Tagar, Manjishtha, Nagkesar,* and *Pattang*. These agents are recognized to have *Shothagna* (anti-inflammatory), *Jantughna* (antimicrobial), and *Rasayana* (rejuvenating) properties, which make them fit for inflammatory conditions.

Modern studies offer a better understanding of the mechanisms of action of these ingredients. Curcumin, the active compound in *Haridra*, is one of the examples of such an ingredient that was found to substantially reduce pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, and MCP-1, with downregulation of COX-2 and 5-LOX enzymes which is critical in the synthesis of inflammatory mediators like prostaglandins and leukotrienes.**[4,5]**

Similarly, berberine acts through its signaling pathways to modulate inflammation in many systems by upregulating COX-2 after pro-inflammatory cytokines, with certain pathways eliciting their behaviour.**[6]** *Manjishtha* (Rubia cordifolia) causes significant inhibition of the production of pro-inflammatory cytokines, with the consequent amelioration of knee swelling/thickening inflammation during models of rheumatoid arthritis. **[7]** *Tagar* extracts have been shown to produce a dose-dependent reduction in carrageenan-induced paw edema; additionally, *Tagar* supports acute inflammatory response.**[8]** *Nagkesar* (Mesua ferrea) modulates NF-κB signalling to exhibit anti-inflammatory properties and reduce the levels of key cytokines such as IL-1 and TNF-α.**[9]** *Pattang* (Caesalpinia sappan) suppresses the expression of IL-1β and TNF-α, inhibiting NO production via iNOS downregulation, and reduces COX-2 activity through NF-κB signal pathway inhibition.**[10]**

The *Tikta Rasa* and *Ushna Virya* characteristics of these ingredients enhance digestion, metabolism, and thus nutrient absorption, boosting modulated immunity. Proper digestion aids in the availability of antioxidants and nutrients that relieve oxidative stress and inflammation.**[11]** Modern immune research backs metabolic reprogramming that allows immune cells to redirect themselves to an anti-inflammatory state**[12]** and hence supports the anti-inflammatory actions of *Champak Agad.* The concentration-dependent inhibition of protein denaturation by *Champak Agad* is consistent with the combined effective action of its constituents. These results bridge the world of ancient *Ayurvedic* wisdom with contemporary evidence as they vouch for the therapeutic scope of *Champak Agad* as a natural anti-inflammatory drug.

Conclusion

The present study demonstrates that *Champak Agad* extracts exhibit significant anti-inflammatory activity through concentration-dependent inhibition of protein denaturation. The ethanolic and aqueous extracts were found to inhibit denaturation with increasing rates when higher concentrations were used, where the aqueous extract always outperformed the ethanolic extract consistently. The maximum inhibition of the aqueous extract was achieved at the highest concentration tested of 200 mg/ml, confirming its superior effectiveness.

Hence these findings prove that *Champak Agad* extracts, particularly the aqueous one, are highly potential natural anti-inflammatory agents.

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