



**IN-VITRO EVALUATION OF ANTI-PSORIATIC ACTIVITY AND PHYTOCHEMICAL SCREENING OF ARGEMONE MEXICANA LEAF EXTRACT**

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**ABSTRACT**

Psoriasis is a chronic inflammatory skin disorder associated with oxidative stress and immune dysregulation. Medicinal plants rich in bioactive phytoconstituents are increasingly being explored as safer alternative therapeutic agents. The present study was designed to evaluate the phytochemical profile and in-vitro anti-psoriatic potential of *Argemone mexicana* leaf extract. Preliminary phytochemical screening revealed the presence of major secondary metabolites such as alkaloids, flavonoids, tannins, glycosides, and terpenoids. The antioxidant activity of the extract was assessed using DPPH radical scavenging assay, nitric oxide scavenging assay, and Ferric Reducing Antioxidant Power (FRAP) assay, while anti-inflammatory potential was evaluated by protein denaturation method. The results demonstrated significant antioxidant and anti-inflammatory activities in a concentration-dependent manner, indicating the presence of potent bioactive compounds. The findings suggest that *Argemone mexicana* leaf extract possesses promising in-vitro anti-psoriatic potential and may serve as a potential source for developing natural therapeutic agents against the oxidative stress and inflammatory skin disorders.

**KEYWORDS:** *Argemone mexicana*, psoriasis, DPPH assay, nitric oxide scavenging, FRAP assay, protein denaturation, anti-inflammatory activity.

**INTRODUCTION**

Psoriasis is a long-term, non-infectious autoimmune skin condition that affects about 2–3% of people worldwide. It is characterized by an abnormally fast skin cell turnover, where keratinocytes mature in roughly 3–5 days instead of the normal 28–30 days. This rapid process leads to the buildup of immature skin cells, forming thick, red, inflamed, and scaly plaques on the skin.

Conventional treatments can sometimes be associated with potential long-term side effects, which have encouraged interest in safer, plant-derived therapeutic options. One such candidate is *Argemone mexicana*, which contains biologically active compounds such as berberine and sanguinarine. These compounds are

thought to act on key pathways involved in psoriasis by reducing pro-inflammatory cytokines, inhibiting activation of the NF-κB signaling pathway, and scavenging reactive oxygen species (ROS), thereby helping to reduce oxidative stress and inflammation.

**MATERIAL AND METHODS**

**1. Plant Collection and Sample Preparation**

Collection and authentication: Fresh, healthy leaves of *Argemone mexicana* were collected from agricultural land in Dighanchi, Maharashtra, India (17.516481° N, 74.909655° E). The plant material was authenticated by the Department of Botany at Ishwarrao More-Patil College, and a voucher specimen was deposited (Voucher No. FRB 2026).



**Fig. 1: Collection from Field and Shade Drying of *Argemone mexicana* Leaves at Room Temperature.**

The collected leaves were washed thoroughly under running tap water to remove surface impurities and dust particles. They were then shade-dried at ambient temperature until a constant weight was achieved to preserve heat-sensitive phytoconstituents. The dried leaves were subsequently ground into a coarse, uniform powder using a mechanical grinder and stored in airtight containers for further use.

## 2. Extraction of Plant Material

Two different extraction techniques were employed to obtain bioactive constituents.

### a) Soxhlet extraction (hot continuous method)

Approximately 50 g of powdered leaf material was placed in a filter paper thimble and extracted in a Soxhlet apparatus using 250–300 mL of ethanol. The extraction was carried out continuously for 6–8 hours (around 10–15 cycles) until the siphoned solvent became clear, indicating exhaustive extraction.

### b) Maceration (cold and warm method)

About 20–50 g of powdered sample was soaked in 150–250 mL of ethanol in a closed container and kept for 24–48 hours with occasional stirring. This was followed by mild warming in a water bath at 40–50°C for 30–60 minutes to enhance extraction efficiency. For both methods, the extracts were filtered using Whatman No. 1 filter paper. The filtrates were then concentrated at 40–50°C using a water bath until a semi-solid crude extract was obtained. The final extracts were stored in sterile vials at 4°C for further analysis.

## 3. Qualitative Phytochemical Analysis

Preliminary phytochemical screening of the crude extracts was carried out using standard qualitative tests to detect major classes of bioactive compounds.

- a. **Alkaloids:** Dragendorff's, Mayer's, Wagner's, Hager's, iodine, and picric acid tests
- b. **Cardiac glycosides:** Keller–Killiani test, Kedde's test, and cardenolide-specific assays (using glacial acetic acid,  $\text{FeCl}_3$ , and concentrated  $\text{H}_2\text{SO}_4$ )
- c. **Flavonoids:** Alkaline reagent test ( $\text{NH}_4\text{OH}$ ), lead acetate test, ferric chloride test, Pew's test, and zinc-

hydrochloride reduction test

- d. **Tannins:** Gelatin test, Braymer's ( $\text{FeCl}_3$ ) test, NaOH test, and lead sub-acetate test
- e. **Terpenoids and steroids:** Salkowski reaction using chloroform and concentrated sulfuric acid
- f. **Saponins:** Froth formation test and emulsion test using olive oil.

## 4. In-Vitro Pharmacological Evaluation

### 4.1 Anti-inflammatory Activity by Protein Denaturation Assay

- a. **Principle:** Protein denaturation is closely associated with inflammatory processes. This assay evaluates the ability of plant extracts to prevent heat-induced denaturation of proteins.
- b. **MATERIALS:** 1% bovine serum albumin (BSA) or egg albumin prepared in phosphate-buffered saline (PBS, pH 6.4). Diclofenac sodium or ibuprofen was used as the standard reference drug.
- c. **METHOD:** Different concentrations of the extract (250, 500, and 1000  $\mu\text{g}/\text{mL}$ ) were prepared. Each test sample (0.5 mL) was mixed with 0.5 mL of 1% BSA solution. The control contained BSA and phosphate buffer without extract. The mixtures were incubated at 37°C for 15–20 minutes and then heated at 70°C for 5–10 minutes to induce protein denaturation. After cooling, absorbance was recorded at 660 nm using a UV-Visible spectrophotometer.
- d. **Calculation:** Percentage inhibition of protein denaturation was determined using the standard comparative absorbance formula.

### 4.2 Antioxidant Activity

The antioxidant potential of the extract was assessed using multiple in vitro assays

- a. **DPPH and nitric oxide scavenging assays:** These methods evaluate the ability of the extract to neutralize free radicals. The decrease in absorbance after incubation in the dark is used to calculate percentage inhibition.

### b. FRAP assay (Ferric Reducing Antioxidant Power)

This method measures the reducing ability of antioxidants present in the extract. Ferric ions ( $Fe^{3+}$ ) are reduced to ferrous ions ( $Fe^{2+}$ ), which subsequently form a blue-colored complex measurable at 700 nm using a spectrophotometer.

## RESULT AND DISCUSSION

### 1. Evaluation of Phytochemical Constituents of *Argemone mexicana* Leaf Extract

The preliminary phytochemical screening of *Argemone mexicana* leaf extract revealed the presence of several important secondary metabolites including alkaloids, cardiac glycosides, flavonoids, tannins, terpenoids, saponins, and steroids. Different qualitative chemical tests produced characteristic colour changes and precipitates confirming the presence of these phytoconstituents. Positive results for alkaloids were observed in Dragendroff's, Hager's, Mayer's, Wagner's,

picric acid, and iodine tests. Cardiac glycosides were confirmed by Keller-Killani, Kedde's, cardenolide, and bromine water tests. Flavonoids showed positive reactions in alkaline reagent, lead acetate, ferric chloride, Pew's, zinc hydrochloride reduction, ammonia, and concentrated sulfuric acid tests.

Similarly, tannins were identified by gelatin, Braymer's, NaOH, bromine water, and lead subacetate tests. The Salkowski test confirmed the presence of terpenoids, while foam, emulsification, and lead acetate tests indicated the presence of saponins. Steroids were also detected by Salkowski and sulfuric acid tests through characteristic reddish-brown and yellow colour formations. The presence of these bioactive phytochemicals suggests that *Argemone mexicana* leaf extract possesses significant pharmacological potential and may contribute to its antioxidant, anti-inflammatory, and anti-psoriatic activities.

**Table 1: Preliminary Phytochemical Screening of *Argemone mexicana* Leaf Extract with Pharmacological Significance.**

Sr. No.	Phytochemical Constituents	Result	Discussion
1.	Alkaloids	Present	May contribute to anti-inflammatory and antimicrobial activity.
2.	Cardiac Glycosides	Present	Possess potential antioxidant and therapeutic properties.
3.	Flavonoids	Present	Known for antioxidant and free radical scavenging activity.
4.	Tannins	Present	Help in reducing inflammation and oxidative stress.
5.	Terpenoids	Present	Exhibit anti-inflammatory and antimicrobial effects.
6.	Saponins	Present	Show immunomodulatory and antioxidant properties.
7.	Steroids	Present	Possess significant anti-inflammatory activity.

The phytochemical investigation of *Argemone mexicana* leaf extract confirmed the presence of various biologically active secondary metabolites, which are known to possess important therapeutic properties. Alkaloids detected in the extract are reported to exhibit antimicrobial, antioxidant, analgesic, and anti-inflammatory activities. Flavonoids and tannins are well known for their strong free radical scavenging and antioxidant properties, which help in reducing oxidative stress and inflammation associated with chronic skin disorders such as psoriasis. The presence of cardiac glycosides, terpenoids, saponins, and steroids further enhances the medicinal value of the plant due to their anti-inflammatory, immunomodulatory, antimicrobial, and membrane stabilizing effects.

The positive results obtained in different qualitative tests indicate that the therapeutic potential of *Argemone mexicana* may be due to the synergistic action of these phytoconstituents. These compounds may contribute to the observed antioxidant and anti-inflammatory activities of the extract by protecting biological tissues against oxidative damage and inflammatory responses. Therefore, the phytochemical constituents identified in the present study support the traditional medicinal use of *Argemone mexicana* and suggest its possible application in the management of psoriasis and other inflammatory

disorders.

### 2. Evaluation of Anti-inflammatory Activity of *Argemone mexicana* by Protein Denaturation Method

The anti-inflammatory activity of *Argemone mexicana* leaf extract was evaluated by the protein denaturation method using Diclofenac Sodium as the standard reference. The extract exhibited moderate inhibition of protein denaturation in a concentration-dependent manner. At 200  $\mu\text{g/mL}$ , the extract showed 20.93% inhibition, which increased to 37.93% at 400  $\mu\text{g/mL}$ . A slight decrease was observed at 600  $\mu\text{g/mL}$  with 36.82% inhibition, followed by a further increase to 46.12% and 46.51% inhibition at 800  $\mu\text{g/mL}$  and 1000  $\mu\text{g/mL}$  respectively.



Fig. 2: Protein Denaturation Assay – Control, Standard (Diclofenac Sodium) and Sample (*Argemone mexicana*).

The results indicate that the extract possesses appreciable anti-inflammatory potential, possibly due to the presence of phytoconstituents such as flavonoids, alkaloids, phenolic compounds, and tannins. The inhibition of protein denaturation suggests the ability of the plant extract to stabilize proteins against heat-induced

denaturation, which is one of the mechanisms involved in inflammatory conditions. The gradual increase in inhibition at higher concentrations demonstrates dose-dependent anti-inflammatory activity of *Argemone mexicana* leaf extract.

Table 2: Percentage Inhibition of Protein Denaturation by *Argemone mexicana* Leaf.

Concentration ( $\mu\text{g/mL}$ )	Protein Denaturation (% Inhibition)
200	20.93
400	37.93
600	36.82
800	46.12
1000	46.51

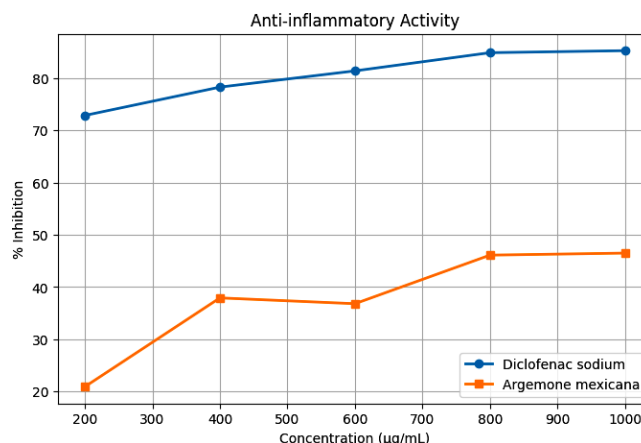


Fig. 3: Extract and Standard Diclofenac Sodium.

The protein denaturation assay revealed that *Argemone mexicana* leaf extract possesses appreciable anti-inflammatory activity by inhibiting heat-induced protein denaturation, which is one of the important mechanisms involved in inflammation. The extract showed a gradual increase in percentage inhibition from 20.93% at 200  $\mu\text{g/mL}$  to 46.51% at 1000  $\mu\text{g/mL}$ , indicating concentration-dependent activity, although a slight decrease was observed at 600  $\mu\text{g/mL}$ . The observed anti-inflammatory effect may be attributed to the presence of phytoconstituents such as flavonoids, phenolic compounds, tannins, and alkaloids, which are known for their protein stabilizing and free radical scavenging

properties.

### 3. Evaluation of In-vitro Antioxidant Potential of *Argemone mexicana* Using DPPH, Nitric Oxide and FRAP Methods

The combined antioxidant activity of *Argemone mexicana* leaf extract was evaluated using DPPH radical scavenging assay, Nitric Oxide scavenging assay, and FRAP assay at different concentrations ranging from 200–1000  $\mu\text{g/mL}$ . In the DPPH assay, the extract exhibited a concentration-dependent increase in free radical scavenging activity, showing 41.35% inhibition at 200  $\mu\text{g/mL}$  and reaching a maximum of 89.51%

inhibition at 1000  $\mu\text{g/mL}$ .

Similarly, the Nitric Oxide scavenging assay demonstrated gradual antioxidant activity with values increasing from 13.52% to 23.65% inhibition at 800

$\mu\text{g/mL}$ , followed by a slight decrease to 22.85% at 1000  $\mu\text{g/mL}$ . The FRAP assay also showed a steady increase in reducing power, where the mean optical density increased from 0.212 at 200  $\mu\text{g/mL}$  to

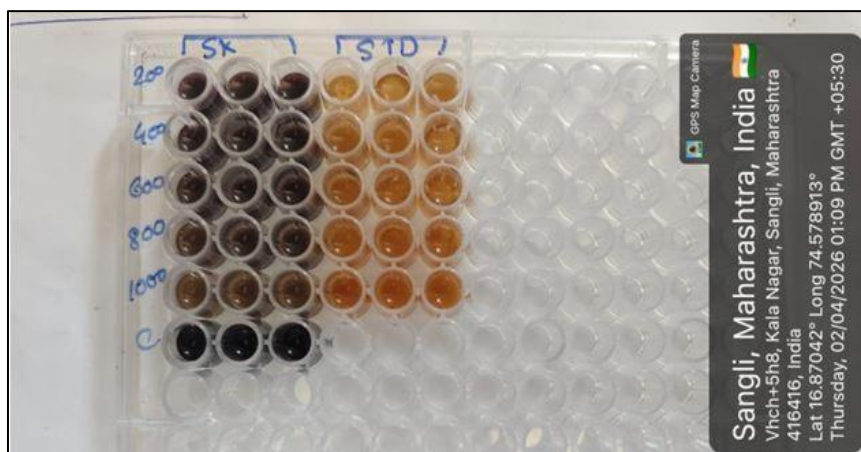


Fig. 4. In vitro Antioxidant activity by DPPH (96 well method).

0.552 at 1000  $\mu\text{g/mL}$ , indicating enhanced ferric ion reducing capacity of the extract at higher concentrations.

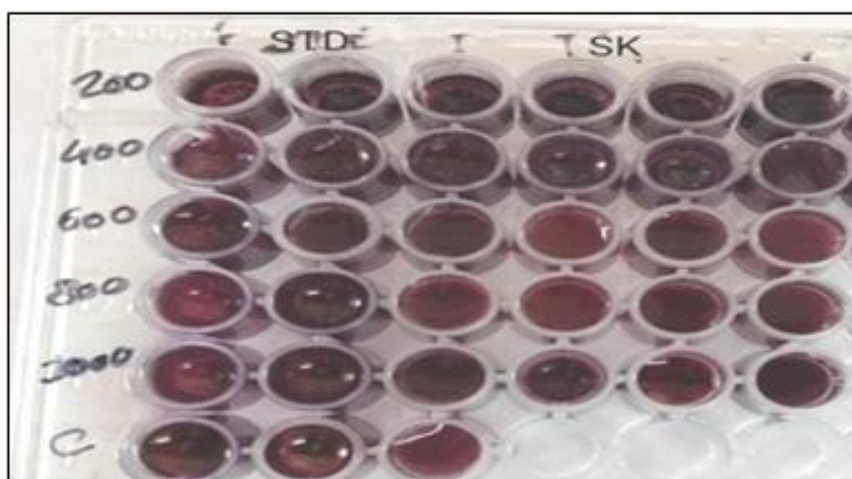
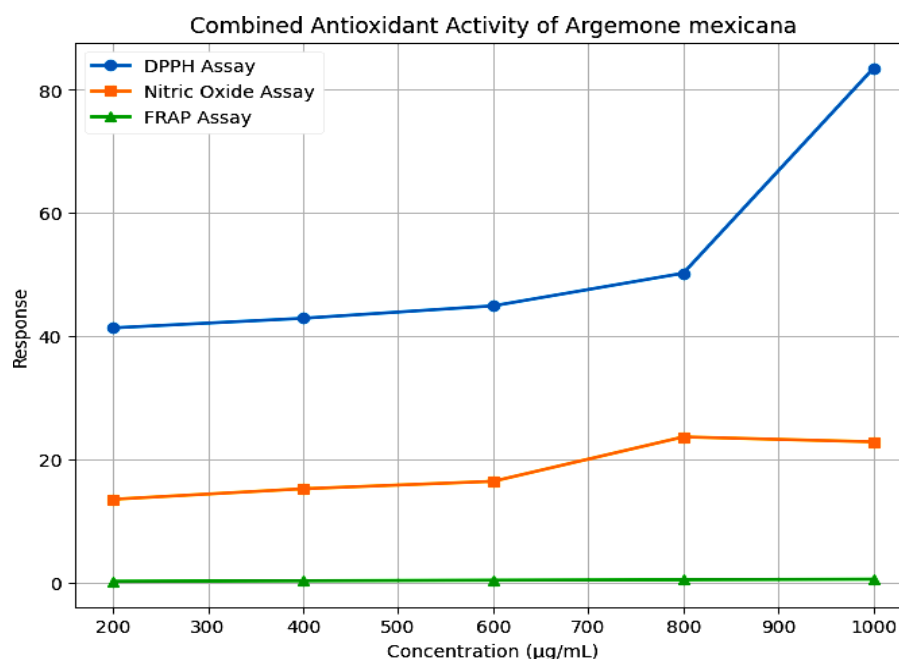


Fig. 5: Nitric oxide scavenging Assay of *Argemone mexicana* Leaf Extract.

Table 3: In-vitro Antioxidant Activity of *Argemone mexicana* Leaf Extract by DPPH, Nitric Oxide Scavenging, and FRAP Assays at Different Concentrations (200–1000  $\mu\text{g/mL}$ ).

Conc. ( $\mu\text{g/mL}$ )	DPPH Assay (% Inhibition)	Nitric Oxide Assay (% Inhibition)	FRAP Assay (Mean O.D.)
200	41.35	13.52	0.212
400	42.91	15.23	0.301
600	44.93	16.44	0.388
800	50.23	23.65	0.466
1000	89.51	22.85	0.552



**Fig. 6: Combined In-vitro Antioxidant Activity of Argemone mexicana Leaf Extract (DPPH, Nitric Oxide, and FRAP Assays).**

The results collectively suggest that *Argemone mexicana* possesses significant antioxidant potential due to the presence of bioactive phytoconstituents such as flavonoids, phenolics, alkaloids, and tannins. The strong DPPH radical scavenging activity observed at higher concentrations indicates the ability of the extract to donate hydrogen atoms or electrons to neutralize free radicals. The Nitric Oxide scavenging activity further supports its role in reducing reactive nitrogen species involved in inflammatory and oxidative stress-related disorders. Additionally, the gradual increase in FRAP values confirms the reducing ability of the plant extract, reflecting its electron-donating potential. Overall, the study demonstrates that the antioxidant activity of *Argemone mexicana* is concentration dependent and may contribute to its therapeutic usefulness in the management of oxidative stress-mediated diseases.

## CONCLUSION

The study concludes that *Argemone mexicana* leaf extract contains important phytoconstituents such as alkaloids, flavonoids, tannins, saponins, terpenoids, cardiac glycosides, and steroids, confirmed by standard qualitative tests showing characteristic colour changes and precipitates. The extract exhibited moderate antioxidant activity in DPPH, nitric oxide, and FRAP assays with a concentration-dependent increase, though lower than standard drugs like ascorbic acid, indicating its free radical scavenging and reducing potential.

It also showed moderate anti-inflammatory activity in the protein denaturation method compared to diclofenac sodium, with inhibition increasing at higher concentrations, suggesting membrane/protein stabilization effects. Overall, the results indicate that

*Argemone mexicana* leaf extract possesses notable antioxidant and anti-inflammatory properties supporting its traditional use, but further studies such as compound isolation, toxicity profiling, and in-vivo evaluation are required to confirm its safety and therapeutic potential.