

**INVITRO EVALUATION OF PHARMACOLOGICAL ACTIVITIES OF
PAPAVER SOMNIFERUM SEEDS**

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ABSTRACT

The present study investigated the *in vitro* antioxidant, anti-inflammatory, and antimitotic activities of the aqueous extract of *Papaver somniferum* seeds to evaluate their pharmacological potential. The total antioxidant capacity (TAC) was determined using the phosphomolybdenum method, revealing a strong concentration-dependent increase in antioxidant activity, with a maximum inhibition of 91.08% at 1000 µg/mL and an IC₅₀ value of 494.5 µg/mL, comparable to ascorbic acid (IC₅₀ = 475.1 µg/mL). Anti-inflammatory activity was assessed through inhibition of albumin denaturation, where the extract exhibited significant inhibitory potential (97.22% at 1000 µg/mL) surpassing that of the standard drug diclofenac sodium (77.77%). Antimitotic activity was evaluated using the *Vigna radiata* seed germination assay, which showed a dose-dependent suppression of seed germination and root elongation, with 96.16% inhibition at 1000 µg/mL, similar to methotrexate

(98.79%). These findings demonstrate that *P. somniferum* seeds possess potent antioxidant, anti-inflammatory, and cytotoxic properties, likely due to the presence of bioactive phytochemicals such as alkaloids, flavonoids, and phenolic compounds. The study supports the traditional use of *P. somniferum* in herbal medicine and suggests its potential as a natural source of therapeutic agents against oxidative stress, inflammation and abnormal cell proliferation.

KEYWORDS: *Papaver somniferum*; antioxidant activity; anti-inflammatory activity; antimitotic assay; *Vigna radiata*; phosphomolybdenum method; albumin denaturation; methotrexate; natural phytochemicals.

INTRODUCTION

Oxidative stress, inflammation, and abnormal cell proliferation are interconnected biological processes implicated in the pathogenesis of several chronic diseases, including cancer, cardiovascular disorders, neurodegenerative conditions, and metabolic syndromes.^[1] Free radicals such as reactive oxygen species (ROS) can damage lipids, proteins, and DNA, leading to cellular dysfunction. Natural antioxidants, particularly those derived from medicinal plants, play a critical role in scavenging free radicals and enhancing endogenous defense mechanisms.^[2] Antioxidant activity is significant as free radicals contribute to various degenerative diseases and aging processes. Plant extracts with high antioxidant potential can scavenge these radicals, thereby reducing oxidative damage.^[1]

Inflammation, although a protective immune response, can become detrimental when persistent, leading to tissue injury and chronic inflammatory disorders. *In vitro* anti-inflammatory assays such as protein denaturation, membrane stabilization, and enzyme inhibition have been widely employed to screen bioactive compounds with therapeutic potential.^[3] Anti-inflammatory activity is essential since inflammation is a defense mechanism but can lead to chronic disorders when unregulated. Natural compounds with membrane-stabilizing and enzyme-inhibiting activities may serve as safe anti-inflammatory agents.^[4]

Similarly, uncontrolled mitotic activity is a hallmark of cancer. Antimitotic agents disrupt microtubule dynamics, leading to inhibition of cell division.^[5] Antimitotic activity is assessed by seed germination assays, where inhibition of germination or root elongation indicates cytotoxic or mitotic-inhibitory potential. Green gram (*Vigna radiata*) seeds are widely used because of their fast germination, sensitivity to bioactive compounds, and easy availability.^[6] Seed germination bioassay is a simple, inexpensive, and reliable *in vitro* method to evaluate the biological activities of natural extracts, particularly their antioxidant, anti-inflammatory, and antimitotic properties. Plant-derived phytochemicals such as phenolics, flavonoids, alkaloids, and tannins play crucial roles in protecting cellular systems against oxidative stress, inflammation, and uncontrolled cell proliferation.

Papaver somniferum L. (family: Papaveraceae), commonly known as the opium poppy, is a medicinally important plant traditionally used for its analgesic, sedative, and antispasmodic properties.^[7] Besides its alkaloid content, the plant is also a source of phenolic compounds, flavonoids, and other secondary metabolites with potential antioxidant and pharmacological activities.^[8] Therefore, the present study aims to evaluate the *in vitro* antioxidant, anti-inflammatory, and antimitotic activity of the aqueous extract of *Papaver somniferum*, using standard assays.

MATERIALS AND METHODS

Collection and Preperation of plant material

Plant material

Seeds of *Papaver somniferum* were collected from an authenticated source and identified by a botanist.

Preparation of aqueous extract

Dried seeds of *Papaver somniferum* were powdered and extracted by maceration with distilled water (1:10 w/v) for 24 hrs at room temperature. The filtrate was concentrated using a rotary evaporator and stored at 4 °C until further use.^[9]

Determination of total antioxidant capacity (TAC) of *Papaver somniferum* seeds by the phosphomolybdenum Assay

The total antioxidant capacity of crude extracts was evaluated by the phosphomolybdenum assay.^[10] The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate (Mo(V)) complex at acidic pH.^[11] One milliliter each of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate were added in 20 mL of distilled water and made up volume to 50 mL by adding distilled water. 0.3 mL of crude extracts of *P. somniferum* seeds in different concentration ranging from 100 µL to 500 µL were added to different test tubes individually containing 3 mL of reagent solution. These tubes were kept incubated at 95 °C for 90 min. Then, the absorbance of the solution was measured at 695 nm using a UV-VIS spectrophotometer against blank after cooling to room temperature. Aqueous was used as the blank for respective extracts. 3 mL of a mixture of molybdate in 20 mL of water and 0.3 mL of controls were used. Ascorbic acid was used as positive reference standard. Mean values from three trials were calculated for each extract. The antioxidant capacity was estimated using the following formula:

$$\text{Antioxidant Effect (\%)} = \frac{A_{\text{Sample}} - A_{\text{Control}}}{A_{\text{Sample}}} \times 100$$

where, A_{sample} is the absorbance of the sample and A_{control} is the absorbance of the control. The concentration of extract at which 50% inhibition is observed (IC_{50}) were calculated in $\mu\text{g/ml}$.

Assessment of *invitro* anti-inflammatory activity- Inhibition of albumin denaturation

The anti-inflammatory activity of *P. somniferum* was studied by using inhibition of albumin denaturation technique which was studied according to Mizushima *et al* and Sakat *et al*^[12,13] followed with minor modifications. The reaction mixture was consists of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The sample extracts were incubated at 37 °C for 20 min and then heated to 51°C for 20 min, after cooling the samples the turbidity was measured at 660nm. The experiment was performed in triplicate. The Percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inhibition} = (\text{Abs Control} - \text{Abs Sample}) \times 100 / \text{Abs control}$$

Invitro antimitotic activity assay

Seed Selection and Sterilization

Vigna radiata L. (green gram) seeds of uniform size and weight were selected for the assay. The seeds were surface sterilized by immersing in 5% sodium hypochlorite solution for 2 minutes, followed by rinsing 4-5 times with sterile distilled water.^[14]

Preparation of Test Solutions

Extract Solution: Stock solution of *P. somniferum* seeds aqueous extract was prepared by dissolving 1 g of extract in 10 mL of distilled water. From this, different concentrations of 200, 400, 600, 800 and 1000 $\mu\text{g/mL}$ were prepared.

Standard Drug Solution: Stock solution of methotrexate (standard anticancer drug) was prepared by dissolving 2 mg (1 mL vial) in 10 mL of sterile water.

Seed Germination Assay: Sterilized Petri dishes (90 mm diameter) lined with sterile filter paper were used for the assay. Each Petri dish was moistened with 5 mL of the respective test solution (extract or methotrexate) or distilled water (control). Six sterilized seeds were placed in each Petri dish, ensuring adequate spacing.

The experimental setup included

- Control group: Seeds treated with distilled water.
- Extract groups: Seeds treated with 200, 400, 600, 800 and 1000 µg/mL of *P. somniferum* extract.
- Standard drug group: Seeds treated with 200 µg/mL of methotrexate.
- All Petri dishes were sealed with parafilm and incubated at room temperature ($25 \pm 2^\circ\text{C}$) for 72 hours. Each treatment was performed in triplicate.

Measurement and Analysis

After the incubation period, the number of germinated seeds was counted, and the root length of each germinated seed was measured using a ruler. Seeds were considered germinated when the radicle emerged from the seed coat.^[15]

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ Inhibition} = (L_c - L_t) / L_c \times 100$$

Where, L_c = Length of root in control

L_t = Length of root in test

Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation (SD).

RESULTS AND DISCUSSION

Invitro antioxidant activity of aqueous extract of *P. somniferum* seeds

The antioxidant potential of the aqueous extract of *P. somniferum* seeds was evaluated by the phosphomolybdenum assay, and the results were compared with the standard ascorbic acid. The rate of inhibition (%) was calculated at various concentrations (200-1000 µg/mL), and the data are represented in Table 1.

Table 1: Antioxidant activity of aqueous extract of *P. somniferum* seeds by phosphomolybdenum (TAC) assay.

Concentration (µg/ml)	Aqueous extract of <i>P. somniferum</i> seeds			Ascorbic Acid		
	A	%	IC ₅₀	A	%	IC ₅₀
200	0.136	34.65	494.5	0.133	31.68	475.1
400	0.143	41.58		0.147	45.54	
600	0.161	59.40		0.159	57.42	

800	0.182	80.19		0.168	66.33	
1000	0.193	91.08		0.172	70.29	

Absorbance, %- Percentage

Phosphomolybdate assay measures the capacity of an extract to destroy a free radical by transferring an electron.^[16,17] The total antioxidant capacity (TCA) of seed extract of *P. somniferum* was measured based on the reduction of molybdate (VI) to molybdate (V).^[11] The antioxidant activity of aqueous extract at various concentrations were given in the Table 1. The TCA of aqueous extract compared with the reference standard with the total antioxidant value 70.29%. TAC was found to be higher in aqueous extract (91.08%) at a concentration of 1000 µg/ml.

The IC₅₀ value of aqueous extract was 494.5 µg/ml. Ascorbic acid was used as a reference standard with IC₅₀ value of 475.1 µg/ml. The aqueous extracts have the IC₅₀ value higher to the standard which corresponds to high antioxidant activity.

Invitro anti-inflammatory activity of aqueous extract of *P. somniferum* seeds

The anti-inflammatory potential of the aqueous extract of *P. somniferum* seeds was evaluated by the protein denaturation assay, and the results were compared with the standard anti-inflammatory drug Diclofenac sodium. The rate of inhibition (%) was calculated at various concentrations (200 – 1000 µg/mL), and the data are represented in Figure 1.

From the results, it was observed that the percentage of inhibition increased with concentration in both the test extract and the standard drug. At 200 µg/mL, the extract exhibited 61.11% inhibition, while the standard showed only 13.88%. At the maximum concentration (1000 µg/mL), the extract showed 97.22% inhibition, which was higher than the standard (77.77%). This indicates a dose-dependent increase in anti-inflammatory activity. The extract demonstrated a significant inhibition of protein denaturation, suggesting the presence of bioactive constituents capable of stabilizing protein structures against heat-induced denaturation.

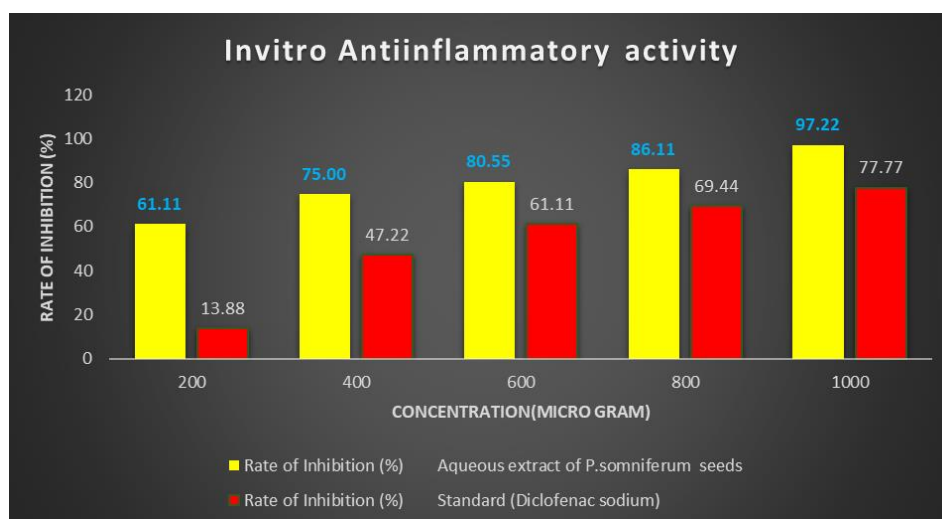


Figure 1: *Invitro* anti-inflammatory activity of aqueous extract of *P. somniferum* seeds.

Protein denaturation is a well-documented cause of inflammation. Denatured proteins may act as antigens that trigger tissue injury and inflammation. Agents that inhibit protein denaturation therefore serve as anti-inflammatory compounds.^[12] The high inhibitory effect of *P. somniferum* seed extract could be attributed to the presence of alkaloids, phenolic compounds, flavonoids, and other polar constituents. These phytochemicals are known for their ability to inhibit inflammatory mediators such as prostaglandins and cytokines, or to stabilize lysosomal membranes, thereby preventing further tissue damage.^[13]

Moreover, the aqueous extract performed remarkably well, implying that water-soluble bioactives such as polyphenols and glycosides may play a crucial role in the observed activity. Similar trends were reported in other studies using aqueous extracts of medicinal plants with comparable phytochemical composition.^[18,19]

***Invitro* antimitotic activity of aqueous extract of *P. somniferum* seeds**

The antimitotic activity of the aqueous extract of *P. somniferum* seeds was evaluated using the seed germination inhibition assay. Green gram (*Vigna radiata*) seeds were used as the experimental model. The parameters studied included percentage of germination, root length, and percentage inhibition, compared with the standard antimitotic drug Methotrexate (200 µg/mL). The results are summarized in Table 2.

The percentage of seed germination and root length decreased markedly with increasing concentrations of the aqueous extract. The control seeds showed 97.22% germination with a mean root length of 82.67 mm. In contrast, the extract-treated seeds showed a gradual

reduction in both parameters, indicating a strong antimitotic (cell division–inhibitory) effect. At the highest concentration (1000 µg/mL), germination dropped to 33.33% with an average root length of only 1.00 mm, corresponding to 98.79% inhibition, which was comparable to the standard Methotrexate (98.79%). This demonstrates that *P. somniferum* seeds possess potent cytotoxic or mitotic inhibitory compounds capable of suppressing cell division in the root meristem.

Table 2: Effect of *P. somniferum* extract and methotrexate on seed germination and root length.

Treatment	Concentration(µg/ml)	Germination(%)	Root length(mm)	Inhibition (%)
Control		97.22 ± 2.43	82.67 ± 3.93	0.00%
Aqueous extract of <i>P. somniferum</i> seeds	200	83.33 ± 0.53	23.33 ± 12.71	71.77%
	400	83.33 ± 3.14	14.71 ± 7.06	82.20%
	600	66.66 ± 2.98	11.67 ± 3.56	85.88%
	800	50.00 ± 0.43	3.33 ± 1.97	95.97%
	1000	33.33 ± 1.93	3.17 ± 1.47	96.16%
Methotrexate	200	33.33 ± 0.17	1.00 ± 1.26	98.79%

Values expressed as Mean±S.D



A. Negative Control (Distilled water)



B. Positive Control (Methotrexate)



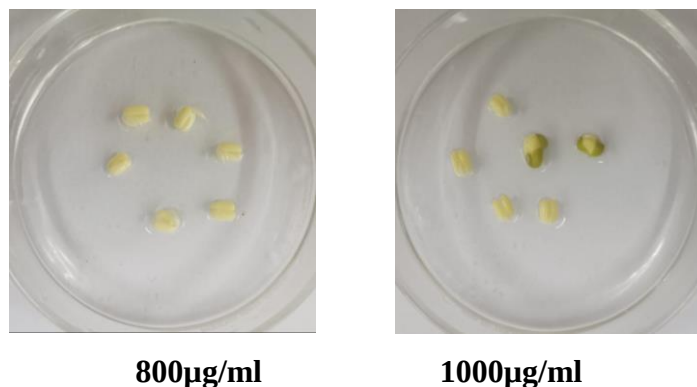
200µg/ml



400µg/ml



600µg/ml



C. Aqueous extracts of *Papaver somniferum* seeds

Plate 1: *Invitro* antimitotic activity of *P.somniferum* seeds against Methotrexate by seed germination assay.

The seed germination inhibition assay is a reliable and simple method for evaluating antimitotic and cytotoxic potential of plant extracts. The reduction in germination and root elongation directly reflects the inhibition of mitotic cell division in meristematic tissues.^[5] The aqueous extract of *P. somniferum* showed a dose-dependent inhibitory effect, suggesting that its bioactive compounds interfere with microtubule formation or DNA replication, similar to the action of known antimitotic drugs like Methotrexate or Colchicine.^[20] The strong inhibition (>95%) observed at concentrations above 600 µg/mL indicates a significant cytostatic effect.

CONCLUSION

The study concludes that the aqueous extract of *Papaver somniferum* seeds possesses remarkable *invitro* biological activities, confirming its potential as a source of natural therapeutic agents. The strong antioxidant activity indicates its ability to counteract oxidative stress and prevent free radical-induced cellular damage. The significant anti-inflammatory effect supports its traditional use in managing inflammatory disorders. The potent antimitotic activity demonstrates its ability to inhibit cell division, suggesting possible applications in anticancer or cytotoxic research. These results collectively validate the medicinal importance of *Papaver somniferum* and provide scientific evidence supporting its pharmacological relevance. However, further studies are recommended to isolate and characterize the active phytoconstituents, evaluate their mechanisms of action, and confirm the efficacy and safety through *in vivo* and clinical investigations.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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