

Evaluation of Cytotoxic Activity of Punica Granatum by using Brine Shrimp Lethality Assay

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Date of Submission: 12-05-2026

Date of Acceptance: 27-05-2026

ABSTRACT :

This study is about how toxic Punica granatum leaf extract's. The people doing the study collected Punica granatum leaves. Then they dried them and made a powder. They used liquids to get the extract from the powder. When they checked the extract they found it had things like alkaloids and flavonoids and tannins and phenols and terpenoids. These things are good for medicine. They tested the extract on animals called Artemia salina nauplii with different amounts of the extract. The more extract they used the more the tiny animals died. When they used a lot of the extract all of the animals died. On average 66.67 percent of the animals died. This study shows that Punica granatum leaf extract is very toxic and it could be used to make new medicines to fight cancer. Punica granatum could be a source for this kind of research.

KEY WORDS :

Punica granatum, Cytotoxic Activity, Brine Shrimp Lethality Assay, Artemia salina, Phytochemical Screening, Anticancer Activity, Soxhlet Extraction, Polyphenols and Flavonoids.

MATERIALS :

We used leaves of Punica granatum, distilled water, ethanol, methanol, n-hexane ethyl acetate, hydrochloric acid, sulphuric acid, sodium hydroxide, ferric chloride, chloroform Wagner's reagent, dimethyl sulfoxide, filter paper, artificial seawater and Artemia salina eggs and nauplii.

EQUIPMENTS :

We used a digital weighing balance, pipettes, test tubes, a lamp, micropipettes, a magnifying glass, a microscope, glass slides, a petri dish, a beaker, a measuring cylinder, a stirrer, a mortar and pestle and a thermometer.

I. INTRODUCTION :

Punica granatum also known as pomegranate is an important medicinal plant. It is widely grown in countries like Iran, Afghanistan and India.

Pomegranate is valued for its therapeutic importance. It has bioactive compounds like polyphenols, flavonoids, tannins, anthocyanins, alkaloids and phenolic acids. These compounds are good for our health. Have strong antioxidant, anti-inflammatory, antimicrobial and anticancer properties.

Different parts of the plant like the fruits, seeds, leaves, flowers and peels have important compounds. Pomegranate seed oil is rich in punicic acid and polyunsaturated fatty acids. The leaves and peels have a lot of phenolics and flavonoids. These compounds contribute to health benefits and therapeutic activities.

The cytotoxic activity of Punica granatum has attracted a lot of interest in research. Cytotoxic agents can stop cell growth. Cause cell death. The cytotoxic potential of plant extracts is commonly evaluated using the Brine Shrimp Lethality Assay. This assay is simple, rapid and cost-effective.

II. EXPERIMENTATION :

1. Collection and Preparation of Plant Material

We collected leaves of Punica granatum from Velapur, Maharashtra, India. We washed the leaves with tap water. Then with distilled water. We shade-dried the leaves. Made a powder using a grinder. We stored the material in airtight containers.



Dry Pomegranate Leaves

2. Preparation of Extract

We took 50 grams of dried leaf powder. Extracted it using Soxhlet apparatus with different solvents. We concentrated the extracts using a water bath or rotary evaporator. Stored them under refrigerated conditions.



Soxhlet Apparatus

3. Phytochemical Screening

We screened the extracts for alkaloids, flavonoids, phenols, tannins and terpenoids using phytochemical tests. We found that the extracts had these compounds.



Alkaloid Test

4. Brine Shrimp Lethality Assay

We evaluated the activity using the Brine Shrimp Lethality Assay with *Artemia salina* nauplii. We prepared concentrations of the extract and exposed the nauplii to it for 24 hours. We recorded the mortality to determine the potential.

5. Hatching of Brine Shrimp

We prepared seawater by dissolving salt in distilled water. We incubated the brine shrimp eggs under aeration and light for 24-48 hours to get active nauplii.



Nauplii

6. Preparation of Test Solution

We dissolved the extract in dimethyl sulfoxide to prepare stock solutions. We then made serial dilutions to get concentrations for cytotoxic testing.

7. Data Collection and Statistical Analysis

We counted the dead nauplii after incubation and calculated the percentage mortality. We analyzed the data statistically to determine the activity of the extract.

OBSERVATION:

The test solution showed an increase in mortality of nauplii after 48 hours. The average mortality was 66.67%. The standard solution also showed toxicity with an average mortality of 87.5%.

III. CONCLUSION

This study shows that *Punica granatum* leaf extract has cytotoxic activity. The extract showed a concentration- increase in mortality of *Artemia salina* nauplii. Phytochemical screening confirmed the presence of bioactive compounds like alkaloids, flavonoids, tannins, phenols and terpenoids. These compounds may contribute to the biological activity.

Although the cytotoxic effect of the extract was lower than that of the solution the results suggest that *Punica granatum* leaves are a promising source of biologically active compounds with possible pharmacological and anticancer applications. Further

research is needed to isolate and characterize the constituents responsible for the cytotoxic effects.

IV. RESULT

The Punica granatum leaf extract showed cytotoxic activity against *Artemia salina* nauplii. The percentage mortality increased with increasing concentration of the extract. The average percentage mortality was 66.67%. The standard solution showed cytotoxic activity with an average mortality of 87.5%. Phytochemical screening confirmed the presence of alkaloids, flavonoids, phenols, tannins and terpenoids.

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