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Original Article

In vitro Assessment of Antioxidant and Cytotoxic Effects of *Argemone mexicana* and *Prosopis cineraria* Methanolic Extracts against Hepatocellular Carcinoma and Glioblastoma Cells

Divya Vashishth, Sudhir Kumar Kataria*

Department of Zoology, Maharshi Dayanand University, Rohtak, Haryana, INDIA.

ABSTRACT

Objectives: The antioxidant and antiproliferative properties of *Argemone mexicana* and *Prosopis cineraria* were investigated against Hepatocellular carcinoma (HepG2) and glioblastoma (U87MG) cells. **Materials and Methods:** Methanolic extracts of the plants were tested for their antioxidant activity using the DPPH and FRAP assays with ascorbic acid as a positive control. The phenolic and flavonoid content of both the plant extracts were evaluated. The anti-proliferative activity of hepatocellular carcinoma and glioblastoma cell lines were estimated by treating the cells with plant extracts by MTT assay. **Results:** Ascorbic acid showcased the lowest IC_{50} value at 32.61±4.10 µg/mL, followed by *A. mexicana* (84.02±2.46 µg/mL) and *P. cineraria* (212.65±4.77 µg/mL). Additionally, the FRAP assay revealed the presence of ascorbic acid in both plant extracts, with concentrations of 29.17±0.30 µmol AAE/g in *A.mexicana* and 19.83±0.55 µmol AAE/g in *P. cineraria*. The Total Phenolic Content (TPC) in the stem of *Argemone mexicana* was determined to be 30.44±3.73 mg gallic acid equivalents (GAE) per gram, while that in the leaves of *Prosopis cineraria* was measured at 6.67±0.69 mg of GAE/g. Similarly, the total flavonoid content (TFC) in the stem of *A. mexicana* was quantified at 16.67±1.19 mg of Quercetin Equivalents (QE) per gram, contrasting with the TFC in the leaves of *P. cineraria*, which registered at 2.17±0.59 mg of QE/g. *In vitro* cytotoxicity was evaluated using the MTT assay, showing a significant decrease in cell viability in both cell lines. The IC_{50} concentrations were determined as 91.3±3.17 µg/mL and 78.43±4.16 µg/mL for *A. mexicana* and 261.43±35.16 µg/mL and 108.03±2.97 µg/mL for *P. cineraria* against HepG2 and U87MG cells, respectively. **Conclusion:** These findings suggest the capacity of both plants as source of antioxidant and carcinostatic agents against hepatocellular carcinoma and glioblastoma.

Keywords: Medicinal Plants, Antioxidant, Cytotoxic, *Argemone mexicana*, *Prosopis cineraria*.

Correspondence:
Dr. Sudhir Kumar Kataria
Professor, Department of Zoology,
Maharshi Dayanand University,
Rohtak-124001, Haryana, INDIA.
Email: sudhir.zoology24@mdurohtak.ac.in

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INTRODUCTION

Cancer, which is a group of more than 100 diseases, is the uncontrolled division of cells and their migration to other tissues. It is the major cause of concern in this century as it is propogating at a very fast pace in human population. The control of normal cell proliferation is disrupted in cancer cells which leads to malignancy and rampant growth of these cells in tissues. Mutations in the proto-oncogenes instigate them into carcinogenic oncogenes which causes abrupt multiplication of these cells and thus making them cancers.[1] These mutations can be caused by any of the factors such as exposure to carcinogens, hormones etc.[2]

According to the World Health Organization, cancer accounts for nearly about 10 million deaths in the year 2020 and more than 9 million incidences were reported in Asia only. The number of cases in the year 2020 are reported to be more than 20 million worldwide which is predicted to rise over 32 million by the year 2045.

Prevention of cancer is the only way to escape from the harrowing experiences a patient goes through during the treatment. Maintaining a healthy lifestyle such as prohibiting smoking & alcohol, avoiding direct sunlight exposure, practicing physical activities may prevent or reduce the risk of cancer.[3] However, if diagnosed the treatment involves various types of therapies viz. chemotherapy, surgery, radiotherapy and immunotherapy.

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India is a country where the role of plants in curing and preventing diseases is conventional since time immemorial. Here the variety of flora is plentiful which is being utilized from ancient times in ayurvedic medicines and surgeries for the treatment of several afflictions.[4] Local people used to prepare medicines using the flora available in their region. As herbal medicines are prepared by using raw materials from plants and their constituents are a part of the physiological functioning of the living plants, they have a better compatibility with human systems and thus are in huge demand now-a-days.[5] The use of herbal medicines is accelerating in this era as they mitigate the symptoms of the disease, reduce the side effects caused by the chemo and radiotherapies and also help in sustaining the emotional and mental discomfort caused by the treatment.[6]

The flora present in the desert regions increase the accumulation of secondary metabolites to combat the drought, heat and other several harsh conditions. This increase results in augmenting the antioxidant potential of the plant as there is more ROS production in the absence of water and other essential nutrients. The presence of these secondary metabolites is proved to be of therapeutic use and are also being explored immeasurably currently.[7,8] *Argemone mexicana* L. also referred as 'Mexican prickly poppy' belongs to the family Papaveraceae. It is an annual herb which can grow in harsh environment such as drought and heat.[9,10] *Argemone mexicana* is found across various dry regions of the country and world due to its status as an invasive alien species. This weed is known to possess anti-oxidant, anti-microbial, anti-diabetic, anti-cancer, larvicidal and many other pharmacological properties due to the presence of alkaloids and phenols in it.[11] *Prosopis cineraria* (L.) Druce is a tree of the family Leguminosae found in hot desert areas. It is commonly known as khejri or janti in the Thar desert region of India and is tolerant to drought and harsh environment. This tree is very important mythologically as it is worshipped in the western part of Rajasthan. It is also used as food and fodder as it is a source of protein for humans and animals.[12] Therefore, the aim of this study was to assess the antioxidant and anticancer properties of raw/crude methanolic extracts from two desert plants, namely *Argemone mexicana* and *Prosopis cineraria*, against human Hepatoma (HepG2) and glioblastoma (U87MG) cell lines.

MATERIALS AND METHODS

Plant material collection and verification

The stem of *Argemone mexicana* and leaves of *Prosopis cineraria* were carried from nearby area of Jaipur, Rajasthan, India. The collected plant materials were authenticated and certified from Raw Materials Herbarium and Museum, Delhi, CSIR-RHMD, NIScPR with NIScPR/RHMD/Consult/ 2022/ 4019-20-1 (*Argemone mexicana*) and NIScPR/RHMD/Consult/ 2022/ 4019-20-3 (*Prosopis cineraria*) authentication numbers.

Crude methanolic extract preparation

The stems and leaves were kept under air in the shade, then ground into a fine powder using an electric grinder. A quantity of 250 g of plant material was combined with 2.5 L of methanol and placed on a shaker for 72 hr. Following this, the extract underwent filtration utilizing Whatman Filter Paper No. 1 and a slurry of the filtrate was prepared using a Rotatory Vacuum Evaporator. The slurry was subsequently dried in a hot air oven at 60°C to yield dried crude methanolic extract. These extracts were kept at 4°C for further use.

Qualitative tests

The qualitative analysis aimed to detect the presence or absence of certain active chemical components, including alkaloids, terpenoids, phenols, flavonoids, reducing sugars, tannins and saponins employing the following methodologies with some modifications.[13]

Quantitative tests

Determination of TPC (Total phenolic content)

The quantification of the TPC in the methanolic extracts was conducted utilizing the Folin-Ciocalteu (FC) reagent method,[14] with slight adjustments. Specifically, 1 mL of plant extract was combined with 2 mL of FC reagent (diluted 1:10 v/v with distilled water) and 1 mL of 20% Sodium Bicarbonate (Na_2CO_3). Subsequently, the mix was incubated for 30 min at 40°C, followed by the measurement of absorbance at 765 nm. A standard curve was generated using various Gallic acid concentrations and the TPC in the sample was quantified in mg of GAE (Gallic Acid Equivalents) per gram. Triplicate experiments were conducted for accuracy.

Determination of TFC (Total Flavonoid Content)

The determination of the TFC in the methanolic extracts was carried out using the aluminum chloride method,[15] with minor adjustments. Initially, 1 mL plant extract was mixed with 4 mL distilled water and 0.3 mL 5% Sodium Nitrite ($NaNO_2$). Following a 5-min interval, 0.3 mL 10% Aluminum Chloride ($AlCl_3$) was introduced and the mixture was further incubated for 5 min. Subsequently, 2 mL of 1M Sodium Hydroxide (NaOH) was added and the solution was maintained at room temperature for 30 min before measuring absorbance at 510 nm. A standard curve was established using various concentrations of Quercetin and the total flavonoid content in the sample was expressed as mg of QE (Quercetin Equivalents) per gram. Triplicate experiments were conducted to ensure reliability.

Antioxidant tests

2,2-diphenyl-2-picrylhdrazyl (DPPH) assay

The radical scavenging adequacy of the plant extracts was assessed using the DPPH method,[16] with some modifications. Specifically,

0.4 mL of varying concentrations of plant extract was mixed with 3.6 mL of 0.1 mM methanolic DPPH. The resultant blend was left to incubate in darkness at ambient temperature for 30 min, following which the absorbance was gauged at 517 nm. Methanol served as the control instead of plant extract, while ascorbic acid was employed as the standard. Each experiment underwent three repetitions. Radical Scavenging Activity was determined using the following formula:

$$\%\ \text{RSA (Radical Scavenging Activity)} = \frac{\text{Absorbance}_{(\text{Control})} - \text{Absorbance}_{(\text{Sample})}}{\text{Absorbance}_{(\text{Control})}}$$

FRAP assay (Ferric Reducing Antioxidant Power)

The FRAP assay assessed the antioxidant capacity of the plant extracts,[17] with certain adaptations. In summary, varying concentrations of plant extract were combined with 0.2 M phosphate buffer saline (pH 6.6) and 1% Potassium Ferricyanide ($K_3Fe(CN)_6$). Following a 20-min incubation at 50°C, 10% trichloroacetic acid ($C_2HCl_3O_2$) was introduced, followed by centrifugation at 3000 rpm for 10 min. Following this, 1.5 mL supernatant was combined with 1.5 mL distilled water and 0.1 mL 0.1% Ferric Chloride ($FeCl_3$) and the absorbance was subsequently determined at 700 nm. Methanol served as the control instead of plant extract, while ascorbic acid was employed as the standard. Each experiment was performed in triplicate.

Cell culture

The Hepatocellular Carcinoma (HepG2) and Glioblastoma (U87MG) cell lines were purchased from NCCS, Pune, India. The cells were cultured in MEM (Minimum Essential Medium) and 10% FBS (Fetal Bovine Serum) (v/v) and 1% penicillin/streptomycin antibiotic solution (v/v) were supplemented. Growth conditions included a 5% CO_2 atmosphere, 95% humidity and a temperature of 37°C.

Cell cytotoxicity MTT assay

The antiproliferative activity of HepG2 and U87MG cells was determined by (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) MTT assay with some modifications.[18] The cells in their exponential growth stage were seeded at a concentration of $5x10^4$ cells in 100 µL in 96 well plate for 24 hr. The growth conditions were 5% CO_2, 95% humidified atmosphere air and 37°C temperature. After 24 hr different concentrations of crude plant extracts (3.125, 6.25, 12.5, 25, 50, 100 µg/mL) in medium were given and the cells were again incubated for 24 hr. Fresh culture medium was used as negative control and paclitaxel (1 µM) was used as positive control. After 24 hr medium was removed and 10 µL MTT dye was added and incubated in dark. After 4 hr 100 µL solubilizing solution was added to solubilize formazan crystals. The absorbance was read at 570 nm on UV-vis ELISA plate reader within 1 hr. The experiment was done in triplicates and optical densities were used to calculate the %viability of cells as:

$$\%\ \text{Cell Viability} = \frac{\text{Absorbance}_{(\text{Sample})} - \text{Absorbance}_{(\text{Blank})}}{\text{Absorbance}_{(\text{Control})} - \text{Absorbance}_{(\text{Blank})}}$$

Statistical analysis

Results are expressed as Mean ± Standard Error Mean (Mean±SEM). Statistical analyses were carried out employing Student's t-test and One-way ANOVA, followed by Dunnette's test, utilizing Graphpad Prism software 8.0.

RESULTS

Qualitative tests

The crude alcoholic extract of *A. mexicana* and *P. cineraria* underwent phytochemical screening, indicating the presence of alkaloids, phenols, flavonoids and tannins (Table 1). Notably, the leaf extract of *P. cineraria* lacked terpenoids and saponins, while the stem extract of *A. mexicana* showed an absence of reducing sugars.

Quantitative tests for TPC and TFC

The TPC in *A. mexicana* stem was found to be 30.44±3.73 mg of GAE/g and that of *P. cineraria* leaves was 6.67±0.69 mg of GAE/g. The TPC in *A. mexicana* was found to be significantly higher to that of TPC in *P. cineraria*. The TFC in *A. mexicana* stem was found to be 16.67±1.19 mg of QE/g and that of *P. cineraria* leaves was 2.17±0.59 mg of QE/g. The TFC in *A. mexicana* was found to be significantly higher to that of TFC in *P. cineraria* (Table 2 and Figure 1).

DPPH Assay

The antioxidant capacity of both plants was examined by measuring the %RSA based on the absorbance of plant extracts and ascorbic acid across various concentrations. It was observed that the *A. mexicana* extract exhibited significantly greater RSA compared to *P. cineraria* extract across all concentration. Ascorbic Acid showed maximum RSA as compared to both the plants (Table 3 and Figure 2). The half maximum Inhibitory Concentration (IC_{50}) was determined by the regression curve analysis and it was found that the IC_{50} value of Ascorbic Acid 32.61±4.10 µg/mL was lowest followed by *A. mexicana* 84.02±2.46 µg/mL and *P. cineraria* 212.65±4.77 µg/mL (Table 4).

FRAP Activity

In the FRAP assay, reduction of Fe^{3+} (Ferric) ion to Fe^{2+} (Ferrous) ion occurs by the antioxidants present in the sample. The assay can indeed be used to measure the ascorbic acid content in a sample. Ascorbic acid, also known as vitamin C, is a powerful antioxidant that can reduce Ferric ions (Fe^{3+}) to Ferrous ions (Fe^{2+}) in the FRAP assay. To determine the ascorbic acid content using the FRAP assay a calibration curve using known concentrations of ascorbic acid was prepared. The reducing power of *A. mexicana* and *P. cineraria* extracts was evaluated by determinining the

concentration of ascorbic acid in both the extracts which was found to be 29.17±0.30 and 19.83±0.55 µmol AAE/g respectively (Table 5 and Figure 3).

Cytotoxicity Assay

The MTT assay was employed to assess cell viability after treatment with varying concentrations of methanolic extracts from *A. mexicana* and *P. cineraria*. Paclitaxel was taken as positive control. The results revealed concentration-dependent cytotoxic effects of both *A. mexicana* and *P. cineraria* methanolic extracts on HepG2 (Table 6 and Figure 4) and U87MG cells (Table 7 and Figure 5).

The IC_{50} values of both the plant extracts were determined against HepG2 and U87MG cells and were found to be 91.3±3.17 µg/mL; 78.43±4.16 (*A. mexicana*) and 261.43±35.16 µg/mL; 108.03±2.97 µg/mL (*P. cineraria*).

DISCUSSION

The preliminary phytochemical analysis of the methanolic extracts from *A. mexicana* and *P. cineraria* revealed the presence of alkaloids, phenols, flavonoids and tannins. Noteworthy differences were observed, with the leaf extract of *P. cineraria* lacking terpenoids and saponins, while the stem extract of *A. mexicana* showed an absence of reducing sugars. These findings are consistent with prior investigations.[19,20]

In this study, we investigated the antioxidant capacity of extracts from *A. mexicana* and *P. cineraria* by assessing their % Radical Scavenging Activity (%RSA) and determining the half-maximal Inhibitory Concentration (IC_{50}) values. Our findings are consistent with previous research indicating the antioxidant activity of *A. mexicana* due to its rich phenolic and flavonoid content. For instance, the IC_{50} values for DPPH and superoxide radicals in different extracts of *A. mexicana* gathered from the arid regions of the Western Ghats in Maharashtra, was reported with the lowest IC_{50} values observed in methanol (50.66±3.48 µg/mL) extract.[21]

Likewise, notable antioxidant potency was found in extracts of *A. mexicana*, noting the lowest IC_{50} value (65.56 µg/mL) for DPPH in the methanolic extract sourced from specimens collected in Bangladesh. These results underscore the robust antioxidant attributes present in *A. mexicana* extracts across different solvent formulations.[22]

The chloroform fraction of *Argemone mexicana* obtained from the Punjab region of the country exhibited a remarkably low IC_{50} value of 26.12 µg/mL at a concentration of 25 µg/mL for the extract.[23]

In contrast, previous studies have reported relatively lower antioxidant activity in *Prosopis cineraria* extracts. % RSA of 31.80 was reported in the bark of *P. cineraria* collected from the arid regions of Rajasthan, indicating moderate antioxidant activity

Table 1: Presence and absence of phytochemicals in *A. mexicana* and *P. cineraria* methanolic extracts.

Sl. No.	Test for	*Argemone mexicana*	*Prosopis cineraria*
1.	Alkaloids	+	+
2.	Terpenoids	+	-
3.	Phenols	+	+
4.	Flavonoids	+	+
5.	Reducing Sugars	-	+
6.	Tannins	+	+
7.	Saponins	+	-

Table 2: TPC and TFC in *Argemone mexicana* and *Prosopis cineraria*.

Sl. No.	Sample	TPC (mg GAE/g)	TFC (mg QE/g)
1.	*Argemone mexicana*	30.44±3.73*	16.67±1.19**
2.	*Prosopis cineraria*	6.67±0.69*	2.17±0.59**

Values are expressed as Mean±SEM. Means are statistically significant via student's *t*-test *($p<0.05$) and **($p<0.01$).

Table 3: % Radical Scavenging Activity (%RSA) of Ascorbic acid, *Argemone mexicana* and *Prosopis cineraria* at different concentrations.

Sl. No.	Sample	Concentration (µg/mL)				
		62.5	125	250	500	1000
1.	Ascorbic Acid	45.56±0.44	54.98±0.43	66.11±0.24	79.40±0.22	98.98±0.05
2.	*Argemone mexicana*	40.73±0.12*	52.61±0.25*	64.20±0.16*	76.79±0.07*	92.40±0.15*
3.	*Prosopis cineraria*	39.89±0.50*	45.79±0.24*	56.71±0.23*	63.12±1.11*	77.57±0.38*

Values are expressed as Mean±SEM. Means are statistically significant via One-way ANOVA.*($p<0.05$).

compared to *A. mexicana*.[24] Additionally, a relatively high IC_{50} value of 236.24 µg/mL was reported for DPPH radicals in methanol extracts of *P. cineraria*, suggesting lower antioxidant potency compared to *A. mexicana*. Our findings also showed high IC_{50} value of 212.65±4.77 µg/mL in *P. cineraria* methanolic extracts.[25]

FRAP assay was also employed to estimate the reducing power of extracts from *A. mexicana* and *P. cineraria*. Our results showed consistency with previous studies that have utilized the FRAP assay to assess the antioxidative potential of plant extracts. For instance, a FRAP value of 22.43 µg/mL was determined in the ethanolic extract of *A. mexicana*, further supporting the antioxidant potential of this plant species.[26]

Similarly, The value was determined for the aqueous extract of *P. cineraria* to be 17.951±0.026 mg Fe(II) eqv/g, indicating its reducing power in terms of ferrous ion equivalents. Furthermore, the reducing capacity of *P. cineraria* extracts was also assessed using the ferrozine assay. The reducing capacity of ethanolic and aqueous extracts of *P. cineraria* was found to be 4.767±0.4877 and 10.370±0.005 mg AE/g, respectively. These results provide additional evidence of the antioxidant potential of *P. cineraria* extracts.[27]

Table 4: IC_{50} concentrations of Ascorbic acid, *Argemone mexicana* and *Prosopis cineraria*.

Sl. No.	Sample	IC_{50} (µg/mL)
1.	Ascorbic Acid	32.61±4.10
2.	*Argemone mexicana*	84.02±2.46**
3.	*Prosopis cineraria*	212.65±4.77****

Values are expressed as Mean±SEM. Means are determined to be statistically significant via One-way ANOVA.**(p<0.01) and **** (p<0.0001).

Table 5: Ascorbic acid content in *Argemone mexicana* and *Prosopis cineraria* determined by FRAP assay.

Sl. No.	Sample	FRAP (µmol AAE/g)
1.	*Argemone mexicana*	29.17±0.30**
2.	*Prosopis cineraria*	19.83±0.55**

Values are expressed as Mean±SEM. Means are determined to be statistically significant via student's t-test **(p<0.01).

Our results align with previous research[28] in which high TPC (30.90 mg GAE/g in leaves and 24.69 mg GAE/g in stem) and TFC values in *A. mexicana*, particularly in methanol extracts were reported. Additionally, high TPC values in ethyl acetate and methanol extracts of *A. mexicana* leaves was also found, supporting the richness of phenolic compounds in this species.[22] The TPC of 21.42 µg/mL was also reported in *A. mexicana* ethanolic extract.[26]

In contrast, *P. cineraria* exhibited lower TPC and TFC values compared to *A. mexicana*. A relatively lower TPC and TFC values were determined in *P. cineraria* leaves and stem bark extracts, suggesting a lower concentration of phenolic and flavonoid compounds in this species compared to *A. mexicana*.[25]

Furthermore, our findings highlight the importance of solvent selection in phytochemical extraction. For *P. cineraria*, acetone extracts showed the highest TPC (6.84±0.18 mg GAE/g) and TFC (3.56±0.78 mg CE/g) values, followed by methanol (2.28±0.34 mg GAE/g, 1.69±0.47 mg CE/g) and aqueous extracts (0.71±0.79 mg GAE/g, 0.35±0.79 mg CE/g).[28] This indicates that different solvent systems may yield varying concentrations of phenolic and flavonoid compounds, emphasizing the need for standardized extraction protocols in phytochemical studies.

In a study, the TPC was found to be 6.95±0.82 mg GAE/g DW (dry weight), while the TFC was reported as 1.13±0.34 mg QE/g DW.[24] However, a lower TPC value of 3.512±0.013 mg GAE/g was reported for the aqueous extract of *P. cineraria* stem bark.[27] On the other hand, it was investigated that the TPC and TFC of

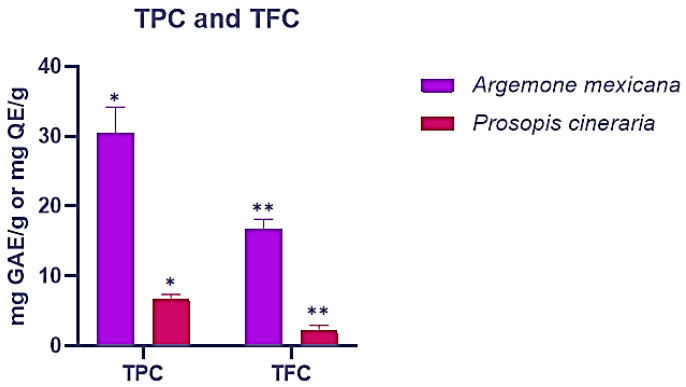

Figure 1: Histogram showing TPC and TFC in *Argemone mexicana* and *Prosopis cineraria*.

Table 6: % Viability determined by MTT assay against HepG2 cells at different concentrations of plant extract.

Sl. No.	Sample	Concentration (µg/mL)							
		Control	3.125	6.25	12.5	25	50	100	Paclitaxel 1 µM
1.	*Argemone mexicana*	100±0	96.66±0.72**	82.54±1.77**	73.51±0.50**	64.44±0.42**	56.99±0.23**	52.10±0.71**	33.65±1.18**
2.	*Prosopis cineraria*	100±0	97.69±0.15*	91.46±0.14*	86.22±1.08*	77.76±1.99*	66.66±1.22*	59.89±0.80*	33.65±1.18**

Values are expressed as Mean±SEM. Means are determined to be statistically significant via One-way ANOVA *(p<0.05) and **(p<0.01).

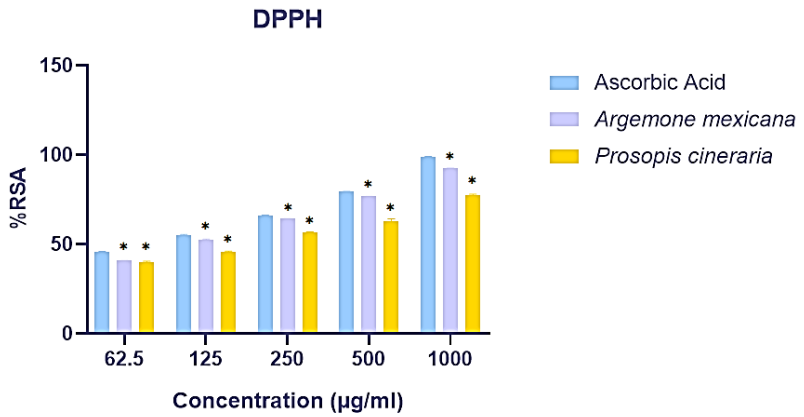

Figure 2: Histogram showing % Radical Scavenging Activity (%RSA) of Ascorbic acid, *Argemone mexicana* and *Prosopis cineraria* at different concentrations.

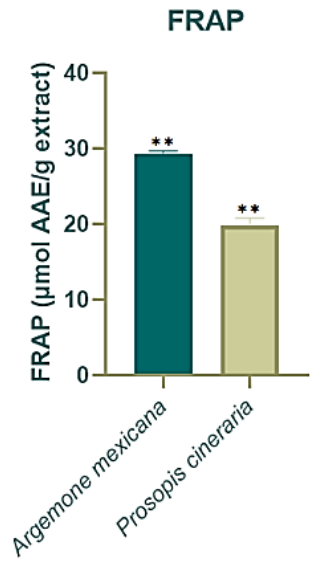

Figure 3: Histogram showing the concentration of Ascorbic acid in *Argemone mexicana* and *Prosopis cineraria* determined by FRAP assay.

Table 7: % Viability determined by MTT assay against U87MG cells at different concentrations of plant extract.

Sl. No.	Sample	Concentration (µg/mL)							
		Control	3.125	6.25	12.5	25	50	100	Paclitaxel 1 µM
1.	*Argemone mexicana*	100±0	91.88±2.42**	79.75±2.34**	73.51±1.28**	64.34±1.79**	57.17±1.33**	46.36±1.59**	35.91±0.26**
2.	*Prosopis cineraria*	100±0	95.28±0.34**	84.72±1.20**	77.35±1.24**	67.33±0.56**	59.64±1.01**	51.76±0.22**	35.91±0.26**

Values are expressed as Mean±SEM. Means are determined to be statistically significant via One-way ANOVA **(p<0.01).

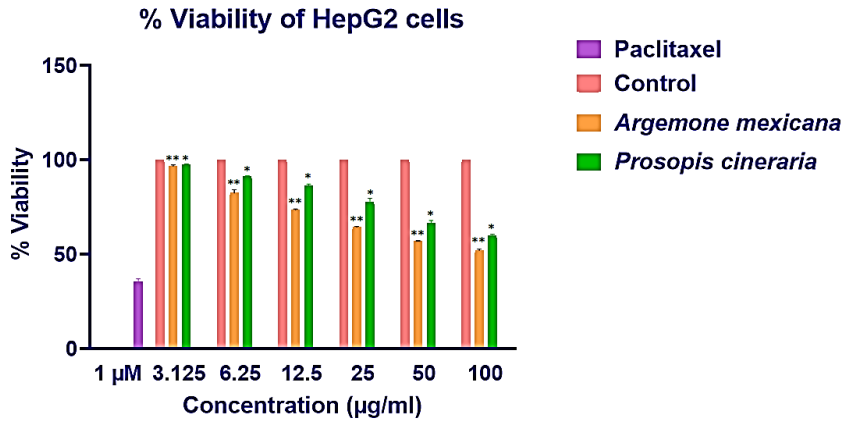

Figure 4: % Viability against HepG2 cells at different concentrations of plant extracts.

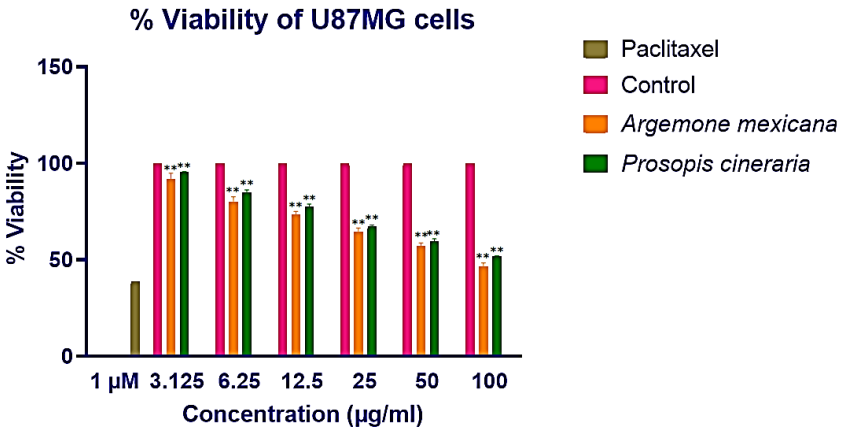

Figure 5: % Viability against U87MG cells at different concentrations of plant extracts.

Khejri (*Prosopis cineraria*) stem bark methanol extract was found to be 1.55±0.14 mg GAE/g and TFC to be 0.23±0.07 mg EC/g.[25]

In our study, we investigated the cytotoxic effects of methanolic extracts from *A. mexicana* and *P. cineraria* on HepG2 and U87MG cancer cells using the MTT assay. The determination of half-maximal inhibitory concentration (IC_{50}) values provided further insight into the cytotoxic potential of the extracts. For HepG2 cells, the IC_{50} values of *A. mexicana* and *P. cineraria* extracts were 91.3±3.17 µg/mL and 261.43±35.16 µg/mL, respectively. Similarly, for U87MG cells, the IC_{50} values were 78.43±4.16 µg/mL for *A. mexicana* and 108.03±2.97 µg/mL for *P. cineraria*. These results indicate that *A. mexicana* extracts exhibited higher cytotoxic potency compared to *P. cineraria* extracts against both cell lines.

Our results align with previous studies indicating the cytotoxic potential of *A. mexicana* and *P. cineraria* extracts against various cancer cell lines. An IC_{50} value of 66.42 µg/mL was reported for the aqueous extract prepared from the *A. mexicana* leaves collected from the Coimbatore region of Tamil Nadu (India), against MCF-7 breast cancer cell lines.[29] Additionally, the inhibitory effects of *A. mexicana* extracts were observed that on HeLa-B75 (48.13±0.54%), HL-60 (20.15±0.33%) and PN-15 (58.11±0.32%) cell lines at 1000 µg/mL concentration.[30] Similarly, the cytotoxic activity of the extracts made from *P. cineraria* of the arid region of Rajasthan (India) was assessed against Huh7 cell line, with IC_{50} values 175 and 150 µg/mL reported for crude and flavonoid-enriched extracts.[31]

CONCLUSION

In conclusion, our study delved into the antioxidant capacity and cytotoxic effects of extracts from *A. mexicana* and *P. cineraria*. Through assessing % Radical Scavenging Activity (%RSA), determining IC_{50} values and employing various assays such as FRAP, TPC and TFC, we elucidated the antioxidant potential of these plant extracts.

A. mexicana displayed robust antioxidant activity, which is attributed to its rich phenolic and flavonoid content as per the current findings. In contrast, *P. cineraria* exhibited comparatively lower antioxidant potency. These findings corroborate the importance of solvent selection in phytochemical extraction and underscore the necessity for standardized protocols in such studies.

Furthermore, our investigation into the cytotoxic effects revealed *A. mexicana* extracts to possess higher potency against HepG2 and U87MG cancer cell lines compared to *P. cineraria* extracts. These results align with existing literature, further highlighting the potential of these plant extracts as cytotoxic agents against various cancer cell lines.

Our study also highlights the importance of considering the geographical source of plant materials in phytochemical and biological evaluations. Future research should further explore the impact of geographic variations on the bioactivity of plant extracts and elucidate the underlying mechanisms driving these differences. Such insights could contribute to the development of region-specific phytopharmaceuticals with enhanced efficacy and safety profiles.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ROS: Reactive Oxygen Species; **TPC:** Total Phenolic Content; **TFC:** Total Flavonoid Content; **GAE:** Gallic Acid Equivalent; **QE:** Quercetin Equivalent; **DPPH:** 2-2-diphenyl-2-picrylhdrazyl; **FRAP:** Ferric Reducing Antioxidant Power; **MEM:** Minimum Essential Medium; **FBS:** Fetal Bovine Serum; **MTT:** 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; ***A. mexicana*:** *Argemone mexicana*; ***P. cineraria*:** *Prosopis cineraria*; **RSA:** Radical Scavenging Activity; **IC_{50}:** Half Maximal Inhibitory Concentration; **AAE:** Ascorbic Acid Equivalents; **CE:** Catechin Equivalent.

SUMMARY

The study identified the antioxidant and cytotoxic potential of two plant extracts and both the extracts revealed significant cytotoxic effects against two cancer cell lines. The therapeutic potential of *A. mexicana* was slightly higher as compared to *P. cineraria* due to the presence of higher amount of phenols and flavonoids as revealed by TPC and TFC assays. The antioxidant activity estimated by DPPH and FRAP assays also exhibited the potent Radical Scavenging Activity of both the plant extracts. In summary, our research enhances comprehension of the antioxidant and cytotoxic attributes present in *A. mexicana* and *P. cineraria* extracts, highlighting their possible utility in pharmaceutical and medicinal domains. There is a need for additional investigations to clarify the underlying mechanisms and refine extraction methodologies, aiming to maximize efficacy and therapeutic benefits.

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