Journal of Ethnopharmacology and Toxicology
Research Article

In vitro antioxidant activity and cytotoxic effect of *Prosopis cineraria* seed extract in *Allium cepa* model

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Volume: 4, Issue: 2, Pages: 1-10
DOI: https://doi.org/10.37446/jet/rsa/4.2.2026.1-10
Received: 8 January 2026 / Accepted: 13 April 2026 / Published: 7 July 2026

Prosopis cineraria (L.) is a drought-tolerant leguminous species, which is native to the arid and semi-arid regions of Asia. In India, the plant is distributed across the states such as Haryana, Rajasthan, Punjab, Uttar Pradesh, Gujarat, and Tamil Nadu. The antioxidant potential of the seed extract was evaluated using different antioxidant assays, including DPPH, nitric oxide, and hydrogen peroxide (H_2O_2) radical scavenging assays. The cytotoxic potential was evaluated using the *Allium cepa* cytotoxicity assay. The estimation of total phenolic and flavonoids revealed higher phenolic and flavonoid contents in the seed extract. The extract exhibited concentration-dependent radical scavenging activity with an IC_{50} value of 432.6 µg/mL in a DPPH assay. Whereas, the nitric oxide scavenging potential of the extract shows equal activity to quercetin, with an IC_{50} value of 103.9 µg/mL. The extract exhibited the higher hydrogen peroxide scavenging activity of extract with an IC_{50}=176.2 µg/mL. Statistical analysis confirmed a significant concentration-dependent effect in all antioxidant assays ($p < 0.0001$). The extract produced a dose-dependent reduction in the mitotic index, decreasing from 51.99% at 12.5 µg/mL to 39.80% at 100 µg/mL, indicating progressive inhibition of cell division. Although the positive control exhibited potent cytostatic activity, the extract showed mild to moderate cytotoxic effects, as evidenced by increased mitotic depression rates with increasing concentration. The *P. cineraria* seeds are a rich source of antioxidant phytochemicals and possess more potent antioxidant and moderate cytotoxic properties. These results support its potential use in the management of oxidative stress-related conditions.

Keywords: *Prosopis cineraria, antioxidant activity, cytotoxicity, Allium cepa, bioactive compounds*

Introduction

Prosopis cineraria L., a plant that belongs to the family *Fabaceae* and subfamily *Mimosoideae*, which contains nearly 3,000 species. The genus *Prosopis* contains approximately 44 formally recognized species (Sudalaimuthuasari et al., 2022). The Linnaeus assigned the taxonomical name *Mimosa cineraria* to the plant and it is also known by its basionyms *Prosopis spicigera* L. and *Prosopis spicata* Burm. Across its global distribution, *Prosopis* species are known by numerous vernacular names, including *Prosopis cineraria*, referred to as Khejri or Kalpavriksha in India, Jand in Pakistan, and Ghaf in Arabic (Gonzalez -Montemayor et al., 2019). The un ripened pods, locally known as Sangari, are widely consumed as a fresh vegetable in Rajasthan and its ripened pods are eaten as a fruit as well. The plant is also the state tree of Rajasthan (Chaudhary et al., 2018). In Ayurveda literature, the plant is mentioned as *Sami tikta katu: Sita kasaya recani laghu: Kapha-Kasa-Bhrama-Shwasa-Kustha-Rasa: Krimijit smrta*, (*dravyaguna*), referring to the treatment of Kapha, cough, vertigo, dyspnea, piles, and worms. Moreover, the plant is used for the treatment of piles, poisoning, diarrhea, skin diseases, respiratory disorders, wound healing and anthelmintic. It is also mentioned for its use in fumigation (Dhoopana)

(Gupta, 2024). The plant has been used to treat miscarriage, rheumatism, and dysentery and regulate the astringent, demulcent, and pectoral properties (Ukani et al., 2000). The plant reported numerous pharmacological activities, such as analgesic, antipyretic, antihyperglycemic, antioxidant, antihypercholesterolemic, antitumor, nootropic, respiratory protective, gastrointestinal protective and anticonvulsant activities (Garg and Mittal, 2013). According to the previous study, the pods are reported to contain the 3-benzylursolate, maslinic acid-3 glucoside, linoleic acid, prosophylline, 5,5′-oxybis-1,3-benzenediol, 3,4,5-trihydroxycinnamic acid 2-hydroxyethyl ester, 5,3′,4′- trihydroxyflavanone 7-glycoside (Zhong et al., 2022). Moreover, luteolin, patuletin, patulitrin, prosogerin-E, rutin, 5,3′,4′-trihydroxyflavanone 7-glycoside, gallic acid, 5,5′-oxybis-1,3-benzenediol, 3,4,5-trihydroxycinnamic acid 2-hydroxyethyl ester, 1-O-coumaroylglycerol, campesterol, stigmasterol, stigmasta-5,24(28)-dien-3-ol, stigmasta-1,3,5-triene, stigmasta-4,6-dien-3-one, cardenolide 3β,14-dihydroxy-5β-card-20(22)-enolide, 3β-O-β-glucopyranosyl-(1→4)-α-rhamnopyranoside, linoleic acid, oleic acid, methyl 12-hydroxystearate, (Z)-12-hydroxyoctadec-9-enoic acid, palmitic acid, and stearic acid (Chaudhary et al.,2018). The seeds contain numerous bioactive compounds, including flavonoids, alkaloids, phenols, phytosterol, protein, saturated and unsaturated fatty acids, lipids.

Although *Prosopis cineraria* has been extensively utilized in traditional and ayurvedic medicine, scientific evidence regarding the pharmacological properties of its seeds remains limited. The seeds have received comparatively less research attention than other parts of the plant, resulting in a lack of comprehensive experimental data. The *Allium cepa* assay is widely recognized as a reliable and versatile model for assessing cytotoxicity. Its advantages, including high sensitivity, cost-effectiveness, simple experimental procedures, and easy accessibility, have made it widely used for toxicity screening. The assay has been extensively employed to evaluate the effects of plant toxicity, environmental pollutants, pesticides, heavy metals, fertilizers and pharmaceutical residues. It provides an ethical alternative to animal-based testing (Torres-Bugarín et al., 2026). The present study aimed to evaluate the phytochemical estimation, different free radical scavenging activity and toxicological profile through the *Allium cepa* of the *Prosopis cineraria* seed extract. The results providing baseline data for its potential therapeutic scope and future pharmacological applications.

METHODOLOGY

Plant Collection, authentication, and crude extraction

Mature pods of *Prosopis cineraria* were collected from the local region of Coimbatore district, Tamil Nadu, India (N 10°53.013′, E 77°0.074′) and taxonomically authenticated by the Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore (Ref. No. BSI/SRC/5/23/2025-26/TECH./1097). After drying, the seeds were isolated from pods, washed, shade-dried, and were pulverized into a fine powder. A hydroethanolic solvent consisting of 75% ethanol and 25% distilled water was prepared. The seed powder was subjected to Soxhlet extraction using this solvent, maintaining a consistent solid-to-solvent ratio of 1:10 (Sumathi et al., 2013). The resulting extract was initially filtered through double-layered muslin cloth, followed by further clarification using Whatman No. 1 filter paper (Wasihun et al., 2023). The filtrate was then lyophilized using a freeze dryer to obtain a dry extract suitable for subsequent experimental analyses. (Feng et al., 2018)

Quantitative analysis of phytochemicals

The total flavonoid content (TFC) and total phenolic content (TPC) of *Prosopis cineraria* seed extract were quantified. Flavonoid content was quantified using the Aluminum Chloride ($AlCl_3$) colorimetric method proposed by Christ and Müller (1960) for flavonoid determination (Sultana et al., 2024). The Folin–Ciocalteu method developed by Singleton and Rossi (1965) for the estimation of phenolic compounds (Pérez et al., 2023).

Evaluation of antioxidant actions

DPPH radical scavenging assay

2,2-Diphenyl-1-picrylhydrazyl assay to detect the antioxidant properties of the extract, based on the donation of a hydrogen atom (H^+) from the extracts to 2,2-Diphenyl-1-picrylhydrazyl. The reduction of DPPH reflects the antioxidant potential of the sample. 0.024 g of DPPH were dissolved in 100 mL of methanol for making the stock solution. 100 µL of different concentrations 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500 µg/ml of the extract, were mixed with 3 ml of DPPH reagent and kept in a dark place with rapid stirring at room temperature for 10 minutes. The reaction absorbance was measured at 517 nm. Ascorbic acid was used as the standard in the concentration of the (10 µg/ml, 11 µg/ml, 12 µg/ml, 13 µg/ml, 14 µg/ml). The IC_{50} value was determined through linear regression analysis and a lower IC_{50} value indicates better antioxidant activity (Pandey et al., 2023) (Baliyan et al., 2022). The percentage of inhibition of the reaction mixture was calculated using the following formula.

$$\text{Inhibition (\%)} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

$A_{control}$ = absorbance of the control (Solution without sample)
A_{sample} = absorbance of the test sample (Solution with extract)

Nitric oxide radical scavenging assay

The Nitric Oxide (NO) radical scavenging activity of the *Prosopis cineraria* seed extracts was evaluated following the method described by Sreejayan and Rao (1997). The assay is based on the principle that sodium nitroprusside, in aqueous solution at physiological pH, spontaneously releases nitric oxide, which subsequently reacts with oxygen to form nitrite ions. These nitrite ions can be quantified spectrophotometrically at 546 nm following their diazotization using Griess reagent. For the experiment, sodium nitroprusside (5 mM) prepared in phosphate buffer saline (0.025 M, pH 7.4) was incubated with varying concentrations of the extract and standard quercetin (5, 10, 50, 100, and 200 µg/mL) at 25°C for 5 hours. After incubation, 0.5 mL of the reaction mixture was mixed with 0.5 mL of Griess reagent, consisting of 1% sulphanilamide, 2% orthophosphoric acid, and 0.1% naphthyl ethylenediamine dihydrochloride. The absorbance of the resulting chromophore was measured at 546 nm (Sreejayan and Rao, 1997).

Hydrogen peroxide scavenging assay

The hydrogen peroxide (H_2O_2) scavenging activity of the *Prosopis cineraria* seed extracts was determined according to the method described by Ruch et al., (1989). This assay is based on the principle that hydrogen peroxide exhibits a characteristic absorbance at 230 nm, and the ability of antioxidant compounds to scavenge H_2O_2 can be measured by the decrease in absorbance at this wavelength. A 4 mM solution of hydrogen peroxide was prepared in 0.1 M phosphate buffer (pH 7.4), and 2.4 mL of the extract or standard quercetin at varying concentrations (5, 10, 50, 100, and 200 µg/mL) was mixed with 0.6 mL of the H_2O_2 solution. A blank containing only phosphate buffer without hydrogen peroxide was used for baseline correction, while the reaction mixture without sample served as the control. The absorbance of each reaction mixture was recorded at 230 nm (Ruch et al., 1989).

***Allium cepa* cytotoxicity assay**

The *Allium cepa* cytotoxicity assay was conducted using healthy, uniform-sized onion bulbs (30–40 g) grown at room temperature. The extract and positive control ($K_2Cr_2O_7$) were freshly prepared to different concentrations of 12.5, 25, 50, and 100 µg/mL. When the roots reached a length of 2–3 cm, they were treated with the extract and control for a duration of 4 hours. After that 2 cm of the distal root tips were excised and fixed in a glacial acetic acid and ethanol in a 1:3 ratios for 1 hour under refrigeration. The fixed meristematic regions were then hydrolyzed with 1N HCl for 5 minutes and stained with acetocarmine. The stain was observed under a light microscope to assess cytological and mitotic changes. Distilled water served as the negative control, while potassium dichromate functioned as the positive control, n=3 was made for each concentration to ensure that our results were reproducible. No human subjects or experimental animals were used in this study. The mitotic index (MI) and mitotic depression rate (MDR) were calculated through the formula to assess cell proliferation and the extent of mitotic inhibition. % MDR was determined to assess the extent of mitotic inhibition in treated groups compared with the control. (Mangalampalli et al., 2018; Fiskesjo, (1997)).

$$\text{MI (\%)} = (\text{Number of dividing cells} / \text{Total number of cells observed}) \times 100$$

$$\text{MDR (\%)} = [(\text{MI of control} - \text{MI of treatment}) / \text{MI of control}] \times 100$$

Statistical analysis

Statistical analysis was performed using OriginPro, and the data were expressed as mean ± standard deviation (SD) from triplicate experiments (n = 3). One-way analysis of variance (ANOVA) with OriginPro 2026 (64-bit) SR1 (Version 10.3.0.197, OriginLab Corporation, Northampton, MA, USA). The ANOVA was applied to evaluate differences among control and treatment groups in antioxidant assays and the *Allium cepa* cytotoxicity assay with statistical significance set at $p < 0.05$.

RESULT AND DISCUSSION

Quantitative analysis of phytochemicals

The total phenolic content (TPC) and total flavonoid content (TFC) of the *Prosopis cineraria* seed extract results revealed that the extract contained 3.10 g/100 g of total phenolic compounds and 1.95 g/100 g of total flavonoids (Figure 1). The relatively higher phenolic content compared to flavonoid content suggests predominant antioxidant phytochemicals present in the seed extract.

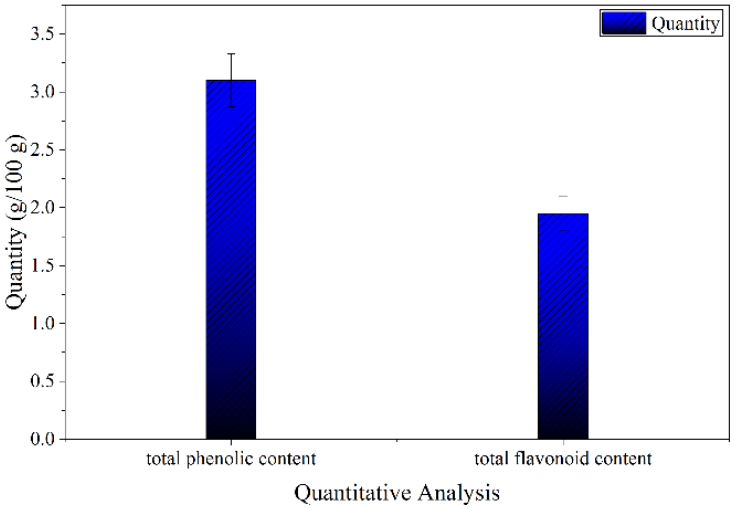


Figure 1. Quantitative Analysis of total phenolic content and total flavonoid content

DPPH radical scavenging effect

The study shows the DPPH radical scavenging potential of the extract. Ascorbic acid exhibited an IC_{50} value of 10.99 µg/mL and extract showed an IC_{50} value of 432.6 µg/mL (Table 1, Figure 2). The concentration of the extract, 100 µg/ml and 500 µg/ml of sample indicates 24.53 ± 1.66 % and 54.62 ± 0.46 % percentage of inhibition against the free radicals respectively (Figure 2). The standard shows the higher antioxidant potential with percentage of inhibition for 39.54 ± 0.88% at 10 µg/mL to 73.27 ± 1.45% at 14 µg/mL. The standard exhibited a highly significant concentration-dependent effect ($F(4,10) = 138.19$, $p < 0.0001$; $R^2 = 0.9822$) compared with the extract, which showed a significant concentration-dependent effect ($F(4,10) = 84.48$, $p < 0.0001$; $R^2 = 0.9713$) on DPPH radical scavenging activity. Therefore, the standard and extract exhibited significant concentration-dependent DPPH radical scavenging activity. While, the antioxidant activity of the extract was lower than that of the standard, the observed dose-dependent increase in radical scavenging activity confirms the presence of bioactive principles capable of neutralizing DPPH free radicals. The *P. cineraria* seed extract possesses appreciable antioxidant potential and may serve as a natural source of antioxidant.

Nitric oxide radical scavenging effect

The nitric oxide radical scavenging activity of the extract was evaluated at concentrations ranging from 5 to 200 µg/mL and the calculated IC_{50} value for the extract 103.9 µg/mL, was lower than that of quercetin 159.1 µg/mL (Table 1, Figure 2). The result indicates that the extract possesses nitric oxide scavenging activity like standard. The extract shows an antioxidant potential at the lowest concentration 5 µg/mL as 24.16 ± 2.07% inhibition, which progressively increased to 57.99 ± 0.65% at 200 µg/mL. As well as quercetin exhibited 11.61 ± 1.39% inhibition at 5 µg/mL and reached 53.26 ± 0.83% at 200 µg/mL. One-way ANOVA of the extract showed a significant effect in a concentration-dependent manner on nitric oxide radical scavenging activity ($F(4,10) = 113.37$, $p < 0.0001$) ($R^2 = 0.9784$) and standard also shows the significant effect on concentration ($F(4,10) = 311.35$, $p < 0.0001$) ($R^2 = 0.992$). The extract shows the substantial nitric oxide scavenging activity compare with the standard.

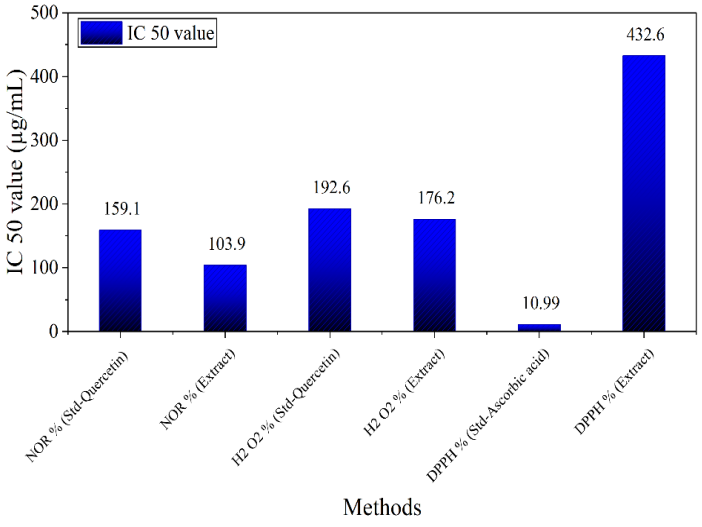
Hydrogen peroxide scavenging effect

The hydrogen peroxide scavenging potential of the extract showed with 25.71 ± 1.33% inhibition at 5 µg/mL and increasing to 51.62 ± 1.05% at 200 µg/mL. The extract exhibited an IC_{50} value 176.2 µg/mL, demonstrating moderate antioxidant activity (Table 1, Figure 2). The standard quercetin showed inhibition values increasing gradually from 30.83 ± 1.19% at 5 µg/mL to 50.28±1.09 % at 200 µg/mL, with an IC_{50} of 192.6 µg/mL. Compared with the standard, the extract

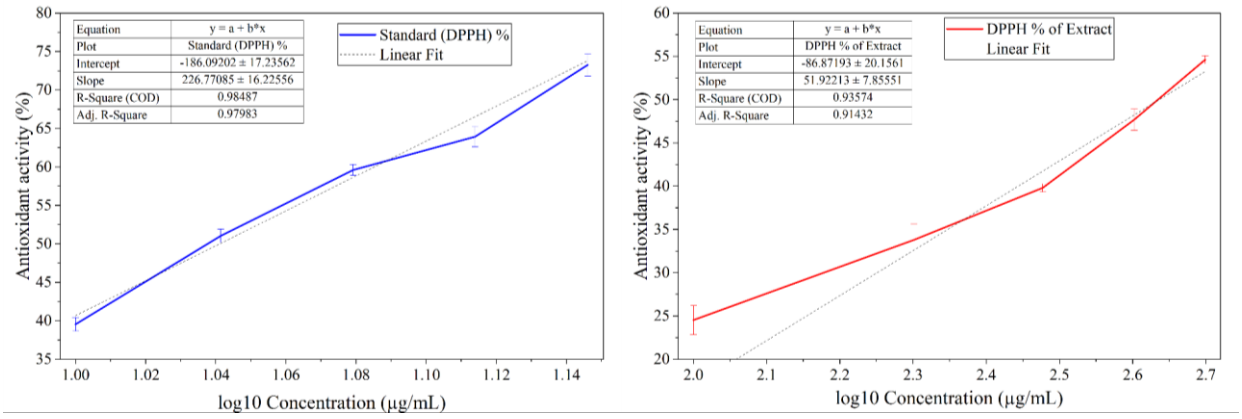
demonstrated superior antioxidant activity and also the extract showed a concentration-dependent increase in scavenging activity, similar to the standard. One-way ANOVA revealed a highly significant effect of concentration- dependant manner on H_2O_2 scavenging activity ($F(4,10) = 41.26$, $p < 0.0001$) ($R^2 = 0.9429$). The extract demonstrated a considerable effect on H_2O_2 scavenging activity ($F(4,10) = 47.53$, $p < 0.0001$ ($R^2 = 0.9500$) compared with the standard, indicating concentration-dependent antioxidant activity of the extract.

Table 1. Antioxidant inhibitory potential of the extract and standards

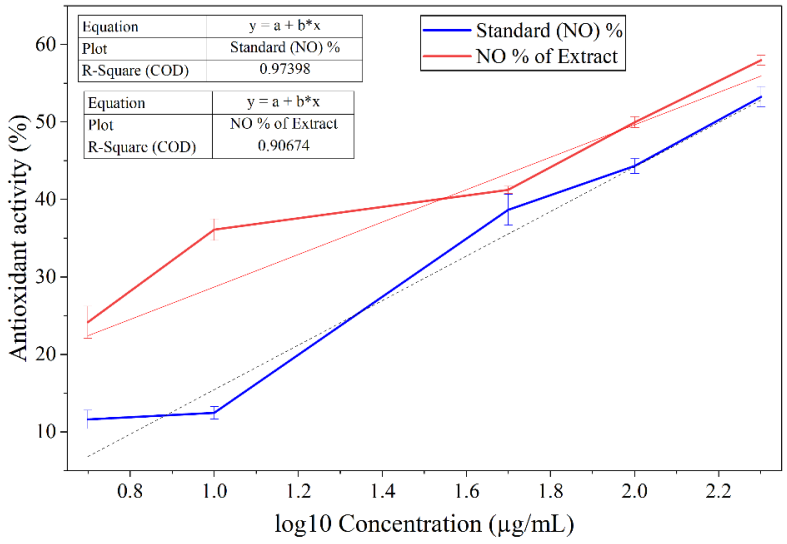
S. No	Method	Concentration (µg/ml)					IC_{50} Value (µg/ml)
		5	**10**	**50**	**100**	**200**	
1	NOR % (Std-Quercetin)	11.61 ± 1.39	12.46 ± 0.91	38.67 ± 1.04	44.34 ± 0.41	53.26 ± 0.83	159.1 µg/ml
2	NOR % (Extract)	24.16 ± 2.07	36.11 ± 1.40	41.24 ± 0.46	49.97 ± 0.71	57.99 ± 0.65	103.9 µg/ml
3	H_2O_2 % (Std-Quercetin)	30.83 ± 1.19	35.37 ± 0.81	37.48 ± 1.99	43.64 ± 0.94	50.28±1.09	192.6 µg/ml
4	H_2O_2 % (Extract)	25.71 ± 1.33	38.06 ± 1.65	45.17 ± 1.80	42.44 ± 0.97	51.62 ± 1.05	176.2 µg/ml
5	DPPH % (Std-Ascorbic acid)	**10**	**11**	**12**	**13**	**14**	10.99 µg/ml
		39.54 ± 0.88	51.04 ± 0.88	59.61 ± 0.71	63.93 ± 1.33	73.27 ± 1.45	
6	DPPH % (Extract)	**100**	**200**	**300**	**400**	**500**	432.6 µg/ml
		24.53 ± 1.66	33.79 ± 1.85	39.80 ± 0.46	47.68 ± 1.22	54.62 ± 0.46	



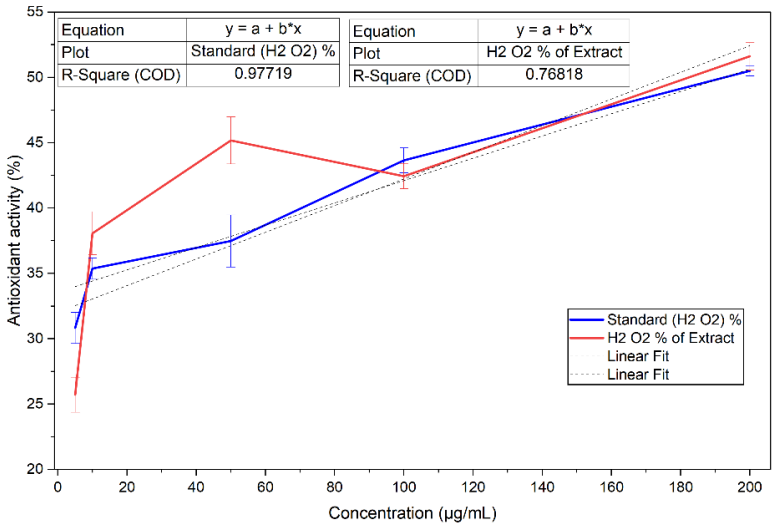
A



B



C



D

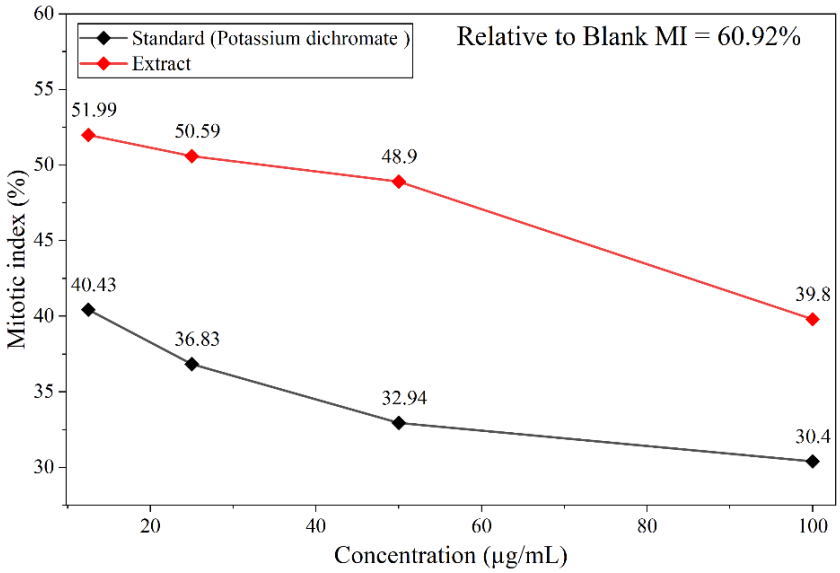
Figure 2. A – Comparative IC_{50} values of Methods. B – DPPH assay inhibition percentage graph with ascorbic acid as the standard. C, D – Nitric oxide radical scavenging activity and hydrogen peroxide scavenging activity with quercetin as the standard.

Cytotoxicity on *Allium cepa* cells

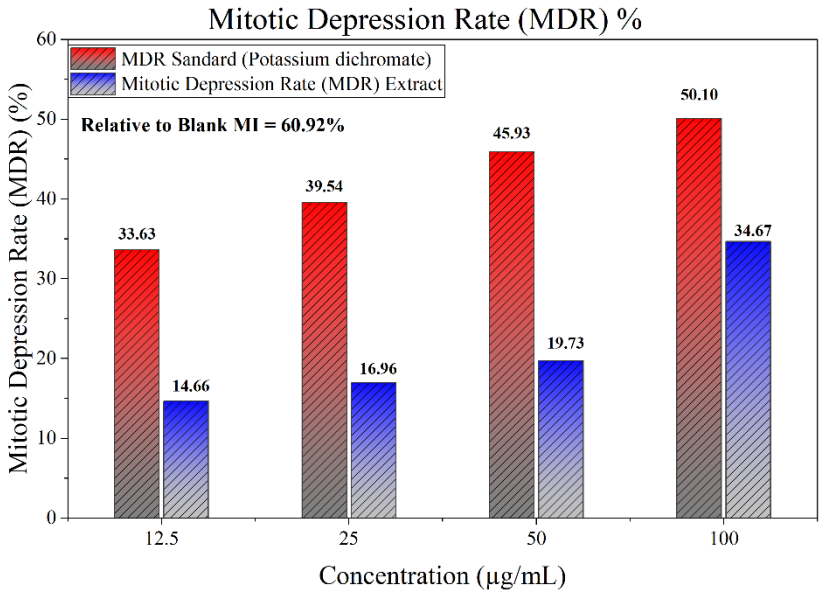
The cytotoxicity assay was evaluated through the *Allium cepa* with different concentrations of the extract. The mitotic index (MI) values are demonstrating a dose-responsive reduction in mitotic activity. The negative control displayed an MI of 60.92%, indicating a physiologically normal rate of cell division and exposure to extract resulted in a gradual reduction of MI from 51.99% (12.5 µg/mL) to 39.80% (100 µg/mL). The Positive Control, as expected, showed a more pronounced suppression of mitosis, with MI decreasing from 40.43% at the lowest concentration to 30.40% at the highest concentration (Table 2). Although mitotic division was observed in all phases at different concentration, the decreasing MI% indicates a clear cytostatic effect, which indicates that the extract possesses dose-dependent cytotoxic potential. Compared with positive control, the extract consistently exhibited a higher MI at all concentrations, demonstrating that extract has a weaker cytotoxic effect relative to the positive control. The extract possesses mild to moderate cytotoxicity. The standard and extract exhibited a concentration-dependent increase in mitotic depression, indicating progressive inhibition of cell division with increasing concentration. However, the standard showed consistently higher MDR values (33.63 to 50.09%) compared with the extract (14.65 to 34.66%), which is demonstrating a strong cytostatic effect for the positive control and a moderate cytostatic effect for the extract on *Allium cepa* root meristem cells.

Table 2. Mitotic Depression Rate (MDR%) of Extract

S. No	Negative control	Concentration	% MI Standard (Positive control)	% MI Extract	% MDR Standard (Positive control)	% MDR sample
1	60. 92 %	12.5	40.43	51.99	33.63	14.65
2		25	36.83	50.59	39.54	16.95
3		50	32.94	48.9	45.92	19.73
4		100	30.4	39.8	50.09	34.66



A



B

Figure 3. A- Mitotic Index (MI), B- Mitotic Depression Rate (MDR) percentage of the extract in the *Allium cepa* Assay

Discussion

Prosopis cineraria has been widely utilized for various medicinal purposes and as a food source in recent years. The seeds are rich in flavonoids and phenolic compounds, which contribute significantly to their antioxidant potential. Previous studies have reported flavonoid contents ranging from 44.44 ± 7.6 to 432 mg RH/g in *P. cineraria* pods (Asati et al., 2022). The hydroethanolic seed extract of *Prosopis cineraria* exhibited a total phenolic content of 3.10 g/100 g, indicating

the presence of substantial amounts of phenolic compounds. Moreover, the researcher reported, the total phenolic content of different extracts, including ethanol (0.73 GAE 100 g^{-1}), methanolic (0.60 GAE 100 g^{-1}) and aqueous (0.40 GAE 100 g^{-1}) extracts of the pods of *P. cineraria*. In this result ethanolic extract showed higher phenolic contents than other extracts and the solvent type significantly influencing phenolic yield (Yadav et al., 2025). The flavonoid and phenolic compounds of the present seed extract is comparable to those reports that the seeds are also having a rich source of antioxidant phytochemicals. In the present study, the seed extract exhibited considerable antioxidant activity, although lower than that of the standard antioxidant. Previous studies have also reported that the pods of *P. cineraria* possess notable DPPH radical scavenging activity (Asati et al., 2021). Moreover, the researcher Pandey et al., 2023 reported that the *Prosopis cineraria* shows significant potential of DPPH scavenging activities with multiple extracts, including methanol, Hydro alcohol, and aqueous. Methanol has the least efficiency compared with the others. Furthermore, the H_2O_2 free radical scavenging activity of bark shows the IC_{50} values of the methanol, Hydro alcohol and aqueous were 135 µg/mL, 103 µg/mL, and 109 µg/mL, respectively. These results show that the seeds also have a comparable ability to scavenge hydrogen peroxide radicals (Pandey et al., 2023). Followed by the researcher Yadav et. al. (2018) reported the hydroethanolic extract of *Prosopis cineraria* leaves was fractionated into chloroform, ethyl acetate, and n-butanol fractions. Using the DPPH assay, these fractions exhibited considerable ability, whereas compared to others, n-butanol exhibits high. (Yadav et al., 2018). Nitric oxide plays a dual role in biological systems, functioning as a signalling molecule at physiological levels. It is also contributing to oxidative and inflammatory damage when produced in excess and also displays neurotoxicity. The extract is more capable of scavenging nitric oxide radicals, this property may contribute to the management of conditions associated with neuroinflammation, neurotoxicity, and oxidative stress (Subedi et al., 2021). The antioxidant activity observed in the extract is based on the presence of phenolic compounds, flavonoids, and other bioactive constituents in the extract, their electron-donating capacity, which aids in neutralizing free radicals and stabilizing reactive species. The radical scavenging action is particularly evident at higher concentrations. The concentration-dependent reduction in the mitotic index, indicating progressive inhibition of cell division by the extract. The increase in mitotic depression rate with increasing concentration further confirms the cytostatic potential of the extract. These findings suggest that the extract possesses dose-dependent cytotoxic activity shows biologically active.

Conclusion

The hydroethanolic seed extract of *Prosopis cineraria* possesses significant antioxidant activity and mild to moderate cytotoxic potential. The extract exhibited concentration-dependent free radical scavenging activity and effectively reduced mitotic activity in the *Allium cepa* assay, indicating its cytostatic properties and biologically active compounds. These results support the traditional medicinal use of *P. cineraria* and its seeds as a valuable natural source of bioactive compounds with antioxidant potential. Therefore, *P. cineraria* seeds may have promising applications in the management of oxidative stress-related disorders and require further investigation to identify their active constituents and therapeutic mechanisms.

Author contributions

All authors contributed equally.

Funding

The project was funded from the authors' personal resources.

Conflict of interest

The authors declare no conflict of interest. The manuscript has not been submitted for publication in other journal.

AI tool declaration

The authors have not used AI and it's related tools to write this manuscript. The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

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