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Phytochemical Profiling, Antioxidant Potential, and Cytotoxic Activity of Hydroethanolic Extract from *Prosopis cineraria* Seeds

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Abstract: *Prosopis cineraria* (L.) is a perennial desert tree belonging to the family Fabaceae. It is widespread in India, the United Arab Emirates, Pakistan and Iran. The study aimed to evaluate the phytochemical screening, antioxidant potential, and to assess cytotoxic activity through a Brine Shrimp Lethality Assay. *Artemia salina* was used as a biological model to detect the toxicity of bioactive constituents. The qualitative analysis of the hydroethanolic extract of *Prosopis cineraria* seeds confirmed the presence of major phytoconstituents such as alkaloids, flavonoids, phenols, cardiac glycosides, anthraquinones, coumarins, and glycosides. FTIR spectroscopy showed dominant functional groups, including hydroxyl, amine, carbonyl, alkene and aromatic. Similarly, GC-MS chromatogram reveals the abundant compounds, such as 9, 12-octadecadienoic acid (linoleic acid), n-hexadecanoic acid, 3-O-methyl-D-glucose, Methyl salicylate, campesterol, and stigmasterol. The antioxidant potential of the extract was evidenced by IC_{50} values of 46.77 µg/mL in the ABTS radical scavenging assay and 54.61 µg/mL in the hydroxyl radical scavenging assay. This indicates a significant capacity to neutralize free radicals, and the observed strong activity exhibited a concentration-dependent pattern. The brine shrimp lethality assay demonstrated significant concentration-dependent toxicity, with an LC_{50} value of 930.3 µg/mL, indicating biologically active low-to-moderate cytotoxic potential ($R^2 = 0.9972$; $F(4, 10) = 506.64$, $p < 0.0001$).

Keywords: Oxidative stress, Antioxidant, *Prosopis cineraria*, *Artemia salina*, Cytotoxicity.

1. Introduction

Prosopis cineraria (L.) Druce is a drought-tolerant leguminous species, a member of the Fabaceae family and the genus *Prosopis* contains approximately 44 formally recognized species, distributed across tropical and subtropical regions [1]. It is commonly known by various vernacular names, including Khejri, Jand, Jandi, Khijda, Sain, Saunder, Jammi, Parampu, Tambu, Vahni, and Spunge Tree. In Sanskrit, it is also referred to as Shami tree. It is native to the arid and semi-arid regions of United Arab Emirates, Pakistan, Iran and India. Moreover, in India, its natural occurrence extends across Haryana, Rajasthan, Punjab, Uttar Pradesh, Gujarat, and Tamil Nadu [2, 3]. The plant serves as food source in Rajasthan, where its green pods are boiled, dried, and consumed as vegetables. It is often combined with other desert plants to prepare *Pach-kutta*, while the ripe pods are also eaten as food. During the Rajputana famine (1868–69) the tree served as a crucial survival food [4]. The edible pods are rich sources of vitamin C, calcium, and iron that are used to prevent malnutrition and mineral deficiencies.

Traditional medicinal systems make use of all parts of *Prosopis cineraria*, including leaves, barks, pods and seeds. The plant has been mentioned in Ayurveda literature, dravyaguna as *Sami tikta katu, Sita kasaya recani laghll, Kapha-Kasa-Bhrama-Shwasa-Kustha-Rasa, and Krimijit smrta*. The plant is used to treat deranged Kapha, cough, vertigo, dyspnea, piles, and worms [5]. *Shami* is characterized by properties such as Tikta, Katu, and Kashaya rasa; Laghu and Ruksha guna; and primarily Sheeta veerya. It exhibits antidiarrheal, anti-poisonous, antimicrobial activities, and is used for the treatment of diarrhea, respiratory disorders, cough, parasitic infections, blood-purifying, and skin disorders [6]. Folk healers utilize it for managing leprosy, leucoderma, mouth ulcers, boils, blisters, rheumatism, asthma, and eye ailments. The bark possesses cooling, tonic, and anthelmintic properties and used to treat asthma, bronchitis, skin diseases, and for relief from snake and scorpion bites. The leaves possess antibacterial, anti-hyperglycemic, antioxidant, and wound healing activities. Some literature has reported that *Shami* has potential to cure the skin disorders, eye ailments, and neurological conditions. The flowers are traditionally used to prevent miscarriage. The gum has pectoral, astringent, and demulcent properties and is used in the management of respiratory and digestive disorders [7].

P. cineraria has been reported to contain various bioactive constituents, such as flavonoids, tannins, alkaloids, quinones and phenolics. Campesterol, cholesterol, β-sitosterol, stigmasterol, hentricontane and methyl docosanoate are the particular sterols. The plant seeds were reported to have unique phytoconstituents, flavonoids such as Prosogerins C, Prosogerins D, Prosogerins E, patuletin, patulitrin, luteolin, and rutin, phenolic compounds such as gallic acid, and biologically important unsaturated fatty acids, particularly linoleic and oleic acids [8, 9]. Moreover previous studies have confirmed it to exhibit potential antioxidant, hypoglycemic, anti-inflammatory, analgesic, antibacterial, anti-hypercholesterolemic, neuroprotective, antipyretic, antitumor, and anticonvulsant properties [10-14]. However, the seeds remain insufficiently investigated compared to other plant part.

In spite of the extensive traditional use of *Prosopis cineraria*, limited scientific evidence is available. Hence, there is need to characterize and identify the secondary metabolites present in the seed crude extract of seeds and to assess its toxicity using biological models. The bioassay is commonly applied in toxicity assessments on a simple zoological organism-brine shrimp model and has recently been validated as a sensitive and reliable method for detecting toxicity of phyto-medicines [15]. It serves as a preliminary toxicity assessment prior to conducting studies in mammalian animal models. The method proposed by Michael *et al.,* (1956), determines the lethal effects of test compounds on a simple zoological organism, the brine shrimp (*Artemia salina*) [16, 17]. The present study was aimed to investigate the phytochemical composition, antioxidant potential and the toxicity profile of the hydroethanolic seed extract of *Prosopis cineraria*, thereby providing baseline data for its potential therapeutic scope and future pharmacological applications.

2. Methodology

2.1 Plant Material and Crude Extraction

Prosopis cineraria plant pods were collected (N 10°53.013′, E 77°0.074′) local region of Coimbatore district, Tamil Nadu, India. The seeds were isolated from pods, shade-dried and ground into a fine powder [10]. The extraction solvent was prepared by mixing 75% ethanol with 25% distilled water. Solvent extraction using a Soxhlet apparatus with a hydroethanolic solvent in standard condition. A solute-to-solvent ratio of 1:10 was consistently maintained throughout the extraction process [18]. The extract was first filtered using a double-layered muslin cloth and clarified with Whatman No.1 filter paper [19]. The extract was then subjected to lyophilisation using a freeze dryer to obtain a solid which will be residue suitable for further experiments [20].

2.2 Qualitative Analysis of Phytochemicals

Qualitative phytochemical analysis of the hydroethanolic extract was carried out using standard analytical test procedures [21]. Alkaloids were tested using Mayer's and Dragendorff's reagents. Flavonoids were screened through alkaline, lead acetate, H_2SO_4, and Shinoda tests. Sterols were tested using the Liebermann–Burchard reaction, and terpenoids were also assessed using the Liebermann–Burchard test. Anthraquinones were tested using Bornträger's method. Anthocyanins were detected through the HCl test. Protein was assessed using the Ninhydrin test, Biuret test, and KOH pellet methods. Phenolic compounds were tested using ferric chloride, gelatin precipitation, and the Ellagic acid test. Quinones were detected using alcoholic KOH, and carbohydrates were determined by Molisch's and Fehling's tests. Tannins were analysed through various tests such as Brayer's test, gelatin precipitation, and the alkaline test. Saponins were assessed using the frothing test and Baljet reagent, while cardiac glycosides were tested using Keller–Killiani and Bornträger's tests. The aqueous NaOH test was used to analyse glycosides. Screening of lignin, coumarins, and volatile oils were done with the Gallic acid test, sodium hydroxide test, and fluorescence test, respectively. The results were recorded based on the formation of characteristic colour transformation, and precipitates, indicating the presence (+) or absence (–) of specific secondary metabolites.

2.3 UV- Spectroscopy

The UV-Vis spectra of hydroethanolic extract was observed between 200 nm and 800 nm using the UV-1700 model spectrophotometer (Shimadzu, Japan), through a sampling interval of 0.5 nm. The UV wavelengths range between 200 nm and 400 nm, and the visible regions range from 400 nm to 800 nm. Specific absorbance peaks within these ranges suggest the key phytochemicals such as flavonoids, alkaloids, and phenolic constituents [22]. These spectral features thus serve as preliminary indicators of the phytochemical richness of *Prosopis cineraria* seeds and provide a preliminary analytical support to the qualitative analysis.

2.4 Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopic test was carried out to identify the functional groups of the phytochemical constituents. The spectra were analysed out using a SHIMADZU IRSpirit FTIR spectrometer ATR module. The samples were directly placed into the ATR crystal without further preprocessing to support rapid and non-destructive analysis. Spectral data were recorded in the wavenumber range of 400–4000 cm⁻¹, with a spectral resolution of 8 cm⁻¹ under % transmittance intensity mode. The Happ–Genzel apodization function was applied at the time of signal processing to improve the signal-to-noise ratio and improve peak definition. The functional group identification was performed as per the characteristic stretching and bending vibrational frequencies with respect to specific chemical linkages. Standard interpretations and previously established spectral assignments were utilized for spectral analysis [23].

2.5 Gas Chromatography-Mass Spectrometry

GC–MS analysis was performed to identify the phytochemical constituents present in the extract using the protocol described by Chakraborty *et al.,* (2022), with necessary modifications [24]. The analysis was carried out using an electron impact (EI) ionization source with the Helium carrier gas at a constant flow rate in the capillary column under ambient temperature. Mass spectra were verified and recorded with an appropriate mass range to capture the characteristic ion fragments of the compounds detected. The spectral data obtained from the GC–MS analysis were interpreted using the National Institute of Standards and Technology (NIST) Mass Spectral Library (NIST 2020 version). Comparison of their mass fragmentation patterns was performed to identify the phytochemicals tentatively. Library reference, match quality and retention time consistency were taken into account for interpretation.

2.6 Antioxidant Properties

2.6.1 ABTS Radical Scavenging Assay

ABTS radical scavenging assay was used to assess the antioxidant activity of the extract. In the assay, ABTS acted as a chromogenic substrate that, upon oxidation, forms the blue–green ABTS$^{\bullet+}$ radical cation, which is measured at 745 nm. The extract reduces ABTS$^{\bullet+}$ to its colourless form, and the decrease in absorbance reflects to the radical scavenging capacity of the sample. The quercetin served as the standard and a 7 mM ABTS solution was mixed with 2.45 mM ammonium persulfate, then mixture was incubated in the dark at room temperature for 12 hours. Thereafter, 0.7 mL of different concentrations of the extract and quercetin (5, 10, 50, 100, and 200 µg/mL) was mixed with 0.3 mL of ABTS$^{\bullet+}$ solution. Ethanol served as the blank, while ABTS$^{\bullet+}$ in ethanol was used as the control. The absorbance was measured at 745 nm [25], and the percentage inhibition was calculated.

$$Inhibition(\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100 \qquad (1)$$

A control = absorbance of the control (Solution without sample)

A sample= absorbance of the test sample (Solution with extract)

The log$_{10}$ concentration-response data were subjected to linear regression analysis using OriginPro software, A regression equation of the form (y = a + bx) was generated and the fit was assessed using the coefficient of determination (R²) ((n = 3)).

2.6.2 Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging assay was performed as described by the Klein *et al.,* (1991). The varied concentrations of the extract and standard quercetin (5, 10, 50, 100, and 200 µg/mL) were mixed with 1.0 mL of iron–EDTA, 0.5 mL of 0.018% EDTA and 1.0 mL of DMSO (0.85% (v/v) in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 mL of ascorbic acid (0.22%) and the mixture was kept for incubation in water bath at 80–90°C for 15 minutes. Subsequently, the reaction was stopped by 1.0 mL of ice-cold 17.5% TCA and added 3.0 mL of Nash reagent. The mixture was allowed to stand at room temperature for 15 minutes, and the absorbance was recorded at 412 nm wavelength [26]. The concentration-response data were subjected to linear regression analysis using OriginPro software, the fit was assessed using the coefficient of determination (R²) ((n = 3)) and A regression equation of the form (y = a + bx) was generated.

2.7 Brine shrimp cytotoxicity study

Brine shrimp (*Artemia salina*) lethality bioassay was conducted to assess the cytotoxicity effect of crude hydroethanolic extract. The procedures described by Moshi *et al.,* (2010) and Meyer *et al.,* (1982) were slightly modified [27, 28]. The stock concentration prepared by dissolving 100 mg of extract in 10 mL of distilled water to obtain a concentration of 10 mg/mL (10,000 µg/mL). Working solutions of 100, 250, 500, 1000, and 1500 µg/mL were prepared by appropriate dilution of the stock solution with distilled water to a final volume of 10 mL. The different concentrations of extract were administered in to the 25 mL of saline solution containing newly hatched 30 *Artemia salina* test vessel. A negative control consisted of saline solution and a positive control contained potassium dichromate (1 mg/mL). All treatments were performed under identical experimental conditions. The behaviour and mortality of the shrimps were monitored at 1 h, 2 h, 4 h, 6 h, and 24 h following exposure. Moreover, the total number of shrimps in each group was counted and recorded in the monitoring hours. Mortality was recorded after the 24 h exposure period in both treated and control groups of the shrimps. The toxicity of different concentrations of the sample solution was evaluated using a shrimp lethality assay.

The experiments were performed in triplicate, and the results were presented as mean ± standard deviation. The median lethal concentration (LC_{50}) of the *P. cineraria* extract against brine shrimp was determined using probit analysis by plotting the $\log_{10}$ concentration of the extract against the corresponding mortality % converted to probit values. The LC_{50} values were calculated by using the regression line obtained by plotting the concentration against the % Mortality on a probit scale [29]. All experimental procedures were carried out in accordance with standard scientific protocols to ensure accuracy, reproducibility and reliability, with due consideration given to the ethical handling and welfare of the test organisms.

$$\%\,Mortality = \left(\frac{Total\ nauplii - Live\ nauplii}{Total\ nauplii}\right) \times 100 \qquad (2)$$

2.8 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), was applied to evaluate differences among control and treatment groups in antioxidant assays (ABTS and hydroxyl radical scavenging assays) and the brine shrimp lethality assay, with statistical significance set at $p < 0.05$.

3. Results and Discussion

3.1 Qualitative Analysis of Phytochemicals

Hydroethanolic extraction of 60 g of seed powder yielded 5 g of freeze-dried extract. Extraction yield was recorded as 8.33%. The preliminary phytochemical analysis revealed the presence of various important bioactive metabolites, as presented in Table 1. The results showed the presence of the following metabolites alkaloids, flavonoids, sterols, saponins, cardiac glycosides, anthraquinones, proteins, carbohydrates, coumarins, and glycosides.

Table 1. '+' and '-' denotes the presence and absence of particular phytoconstituents, respectively

Metabolite	Result	Metabolite	Result
Alkaloids	+	Carbohydrates	+
Flavonoids	+	Tannin	-
Sterols	+	Saponins	+
Terpenoids	-	Cardiac Glycosides	+
Anthraquinone	+	Glycoside's test	+
Anthocyanin	-	Lignin	-
Proteins	+	Coumarins	+
Phenolic Compounds	+	Volatile oil	-
Quinones	-		

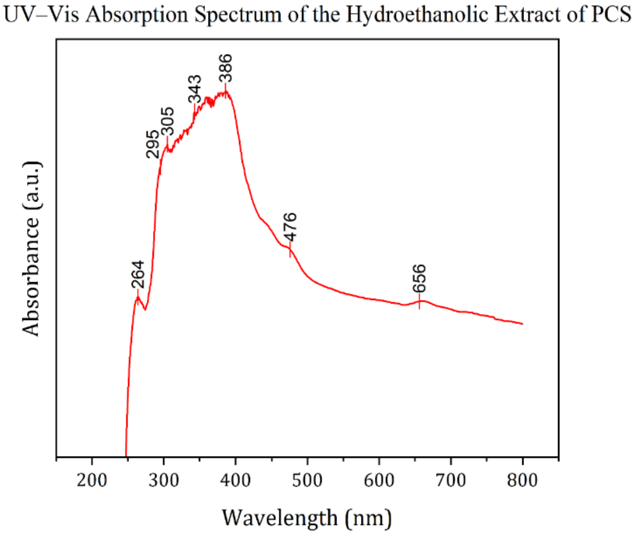
UV–Vis Absorption Spectrum of the Hydroethanolic Extract of PCS

Figure 1. Represent the UV- spectrum and peaks

Tannin, lignin, Terpenoids, Quinones and Quinones were not present in the extract. These phytochemical constituents present in extract are responsible for its therapeutic potentials. Flavonoids of *Prosopis cineraria* possess neuroprotective property as discovered by Aslam *et al.,* (2025). Many therapeutic efficacies are attributed to the presence of secondary metabolites recorded [30].

3.2 UV–Visible Spectroscopy

UV–Visible spectroscopy results reveal multiple distinct absorption peaks (Figure 1). Generally, Band II, appearing in the range of approximately 240–290 nm, corresponds to the benzoyl system (A-ring) and is mainly associated with simple flavonoids and other aromatic compounds. The extract exhibited strong peaks at 264 nm and 295 nm represent aromatic rings, which suggest the presence of phenolic compounds, simple flavonoids, and alkaloids. The peaks at 305 nm, 343 nm and 368 nm in Band I within the range of 300–380 nm, corresponds to the cinnamoyl system (B-ring conjugation) and is typically associated with flavones and flavonols. In addition, the peak at 476 nm indicates presence of extended conjugation and anthraquinones, which substantiate the phytochemical screening results [31-34].

3.3 Fourier Transform Infrared Spectroscopy

FTIR spectrum ranging between 4000 cm⁻¹ and 1500 cm⁻¹ shows peaks corresponding to the functional groups of organic compounds and the region below 1500 cm⁻¹ is referred to as the fingerprint region. The spectrum of the hydroethanolic extract has shown multiple characteristic absorption bands corresponding to different functional groups (Table 2, Figure 2). A broad and strong band was observed at 3294 cm⁻¹ corresponding to O–H stretching vibrations of alcohols, phenols, and carboxylic acids. In addition to N–H stretching of amines and amides were recorded. Peaks at 3008 cm⁻¹ and 2923 cm⁻¹ are typical of alkanes and represent C–H stretching vibrations. Also, the band at 2852 cm⁻¹ is representative of aldehydic C–H stretching. In the spectrum a sharp peak at 1710 cm⁻¹ corresponds to C=O stretching vibrations of carboxylic acids and ketones. The peak recorded at 1619 cm⁻¹ corresponds to C=C stretching vibrations of alkenes. Furthermore, the several peaks at 1245, 1045, 992, and 924 cm⁻¹ indicates C–O stretching and bending vibrations of alcohols, ethers, esters, carboxylic acids, and anhydrides. Peak at 1460 cm⁻¹ is corresponds to CH_2 bending vibrations and 827 cm⁻¹ corresponds to C–H bending of aromatic rings. These spectral characteristics confirm the presence of phenolics, flavonoids, carboxylic acids, aldehydes, ketones, alkanes, and aromatic compounds [35, 36]. FTIR analysis confirmed the presence of hydroxyl, carbonyl, alkene, and aromatic functional groups. These findings corroborate the preliminary phytochemical screening and are consistent with previous studies, which reported distinct FTIR absorption bands corresponding to major functional groups such as O–H, N–H, C–H, and C–Br in the aqueous pod extract of *Prosopis cineraria*. The observed spectral peaks within the range of 3821.04–678.43 cm⁻¹ further confirm the presence of biologically relevant chemical moieties and support the phytochemical richness of the seeds [37].

3.4 Gas Chromatography-Mass Spectrometry

The GC-MS analysis of the extract confirmed 14 bioactive compounds with different retention times and peak intensities were identified (Figure 3, Table 3). These are possibly phenols, fatty acids, sterols and aromatic compounds. A dominant peak corresponds to 9, 12-Octadecadienoic acid (Z, Z)- with the highest relative abundance (Relative Peak Area % - 100%), and n-Hexadecanoic acid (31.43%).

Table 2. FTIR spectral peaks and their corresponding functional groups of the hydroethanolic Extract of *Prosopis cineraria*

Peak	Range	Functional Groups	Vibration
3294	3200-3200 cm^{-1}	Alcohols, phenols Carboxylic acids (O-H), amines and amides (N-H)	Stretch
3008, 2923	3000 +- 150 cm^{-1}	Alkanes (C-H)	Stretch
2852	2800-2900cm^{-1}	Aldehyde (C-H)	Stretch
1710	1700- 1725cm^{-1}	Carboxylic acid, Ketone (C=O)	Stretch
1619	1600-1680cm^{-1}	Alkene (C=C)	Stretch
1460	1460 cm^{-1}	CH_2	Bend
1245,1045, 992, 924	900- 1300cm^{-1}	Alcohols, ethers, esters, carboxylic acids, anhydrides (-C-O)	Bend
827	690- 900cm^{-1}	Aromatics (C-H)	Bend

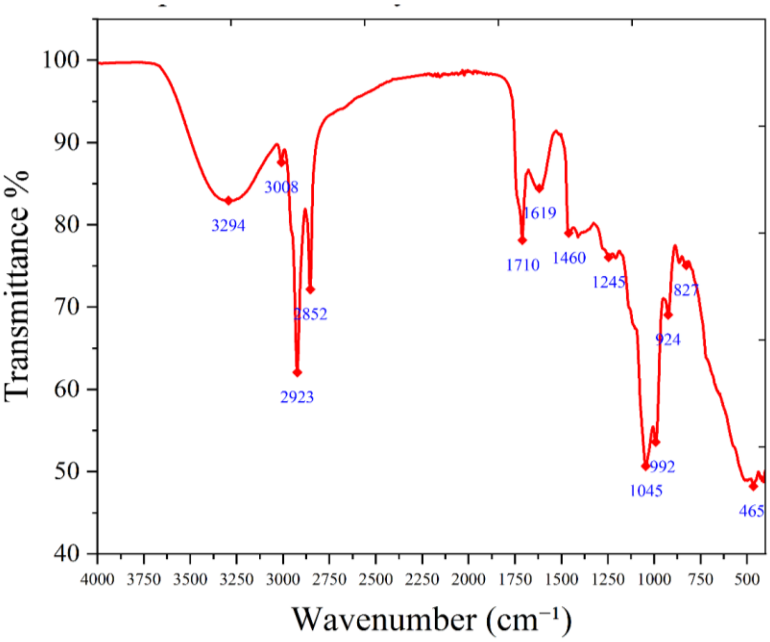

Figure 2. FTIR Spectrum of the Hydroethanolic Extract

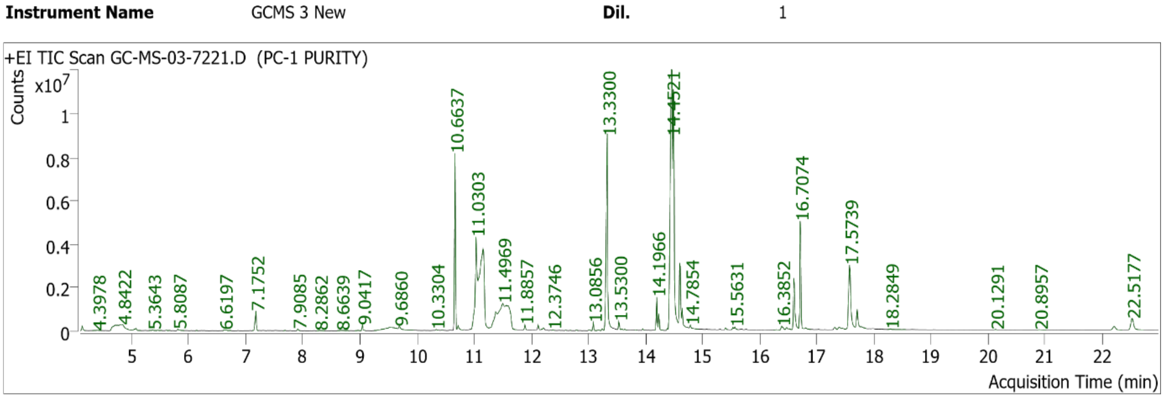

Figure 3. GC-MS peak profile of the Hydroethanolic Extract

Table 3. List of compounds detected by GC–MS in the hydroethanolic extract

S.No	RT	Component Area	Relative Peak Area (% of Maximum Peak)	Match factor	Compound Name
1	14.452	52147040.2	100	99	9,12-Octadecadienoic acid (Z,Z)-
2	13.33	16389298.8	31.43	98.3	n-Hexadecanoic acid
3	22.518	1897002.4	3.64	97.4	Stigmasterol
4	7.1752	1407523.1	2.7	96.4	Methyl salicylate
5	16.596	4579165.6	8.78	96.1	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
6	14.597	7014685.9	13.45	95.4	Octadecanoic acid
7	11.886	273677.4	0.52	95.3	Tetradecanoic acid
8	22.207	563355.8	1.08	95.2	Campesterol
9	17.707	2586645.6	4.96	93.2	Octadecanoic acid, 2,3-dihydroxypropyl ester
10	17.574	8357986.1	16.03	92.3	9-Octadecenoic acid (Z)-, 2-hydroxy-1- (hydroxymethyl)ethyl ester
11	6.6197	141326.7	0.27	83.4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
12	11.375	3859724	7.4	83.3	3-O-Methyl-d-glucose
13	13.175	78796.3	0.15	82.8	cis-9-Hexadecenoic acid
14	10.33	57063.7	0.11	80.3	Dodecanoic acid

The compounds includes, stigmasterol, campesterol, Methyl salicylate, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, octadecanoic acid, 2,3-dihydroxypropyl ester, and 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester were present at low levels in the extract. Octadecanoic acid (stearic acid), tetradecanoic acid (myristic acid), cis-9-hexadecenoic acid, dodecanoic acid (lauric acid) are found to be fatty acids. 3-O-methyl-D-glucose and 4H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl- are carbohydrate-derived compounds. The identified compounds are tentative assignments based on NIST library matching and require further analytical confirmation. The previous studies recorded the presence of 3-O-Methyl-D-Glucose in the ethanolic extract of the *Prosopis cineraria* pods [37]. Furthermore, the researchers reported that the methanolic extract of the pods contained the compounds such as 1-Heptatricontanol, 3, 8, 8-trimethoxy-3-piperidyl-2, 2´-binaphthalene-1, 1´, 4, 4´-tetron, β-sitosterol, and catechin, which were isolated and confirmed through GC–MS and NMR analyses [38]. According to the Sharma *et al*., (2021) reported the 32 bioactive compounds in the ethanolic extraction of the *Prosopis cineraria* pods with seeds [39]. GC–MS analysis identified 14 phytochemicals in the present study, which is consistent with previous findings. The detection of a considerable number of compounds in the seed extract suggests that *P. cineraria* seeds also possess substantial phytochemical diversity.

3.5 Radical Scavenging Assays

3.5.1 Free Radical Scavenging action on ABTS

The antioxidant potential of the hydroethanolic extract was determined using the ABTS radical scavenging assay. In this assay Quercetin acted as the standard antioxidant at a concentration of 1mg/ml in distilled water and the extract prepared at a concentration of 1mg/ml in ethanol. The percentage of inhibition recorded at different concentrations (5, 10, 50, 100,200 µg/mL) of the standard and extract (Table 4, Figure 4 (a) & (b)). Furthermore, the quercetin exhibited a concentration dependent increase in radical scavenging activity, ranging from 24.11 ± 2.67% at 5 µg/mL to 74.80 ± 0.55% at 200 µg/mL. The *P. cineraria* extract also showed a strong dose dependent antioxidant activity, initial with 1.24 ± 0.59% inhibition at 5 µg/mL and reaching 89.99 ± 0.44% inhibition at 200 µg/mL. The IC_{50} values of quercetin and the extract were 22.39 µg/mL and 46.77 µg/mL respectively. It indicates that although the extract exhibited high radical scavenging activity at higher concentrations, quercetin showed greater antioxidant potency. Its relatively higher IC_{50} value 46.77 µg/mL suggests that a larger concentration of the extract is required to achieve 50% inhibition. The linear regression of quercetin showed a regression equation of $y = 1.13083 + 36.15499x$ with an R^2 value of 0.90184, indicating a strong positive relationship between concentration and ABTS radical scavenging activity. *Prosopis cineraria* seed extract exhibited a regression equation of $y = - 43.35004 + 55.76838x$ with an R^2 value of 0.96931, demonstrating excellent linearity. This difference may be attributed to variations in the purity, structure, and reactivity of the phyto-constituents present in the crude extract compared to the pure standard compound quercetin. ABTS radical scavenging activity increased significantly with concentration for both the standard and *P. cineraria* extract. One-way ANOVA demonstrated significant differences among the tested concentrations for the extract ($F (4, 10) = 608.21$, $p < 0.0001$) and the standard ($F (4, 10) = 319.56$, $p < 0.0001$), indicating concentration-dependent antioxidant activity. The ABTS radical scavenging assay demonstrated notable antioxidant activity in the seed extract, consistent with previous reports on the antioxidant potential of various parts of *Prosopis cineraria*. The hydroethanolic leaf extract, fractionated into ethyl acetate, chloroform, and butanol fractions, exhibited ABTS scavenging activity with IC_{50} values of 93, 59, and 22 µg/mL, respectively [40]. Similarly, Ram H. *et al*. (2019) reported that the ethanolic pod extract of *P. cineraria* achieved a maximum ABTS radical scavenging inhibition of 75.66 ± 1.53% at a concentration of 25 µg/mL [41]. These results suggest that the seeds, like the leaves and pods, possess significant antioxidant potential attributable to their diverse phytochemical constituents.

3.5.2 Radical Quenching Effect on Hydroxyl Radicals

The hydroxyl radical scavenging activity of the hydroethanolic extract determined the antioxidant potential at the concentrations ranging from 5 to 200 µg/mL and compared with quercetin as the standard. Quercetin exhibited a progressive increase in scavenging activity, rising from 32.26 ± 2.91% at 5 µg/mL to 77.82 ± 2.31% at 200 µg/mL, with an IC_{50} value of 33.80 µg/mL. The extract also exerted a concentration-dependent activity, with 36.18 ± 1.05% inhibition at 5 µg/mL, reaching 84.78 ± 2.91% at the highest concentration (Table 4, Figure 4 (c)&(d)). The IC_{50} value of the extract was observed to be 54.61 µg/mL (Figure 4, (e)). The linear regression of quercetin exhibited a equation of $y = 42.48712 + 0.22227x$ with an $R^2=0.81085$, whereas the extract showed a regression equation of $y = 34.53942 + 0.2831x$ with an $R^2 =0.9216$. Overall results clearly indicating that although the extract exhibited high radical scavenging activity at higher concentrations and standard demonstrated superior antioxidant efficacy. Both quercetin and the plant extract showed strong, and dose-dependent scavenging activity.

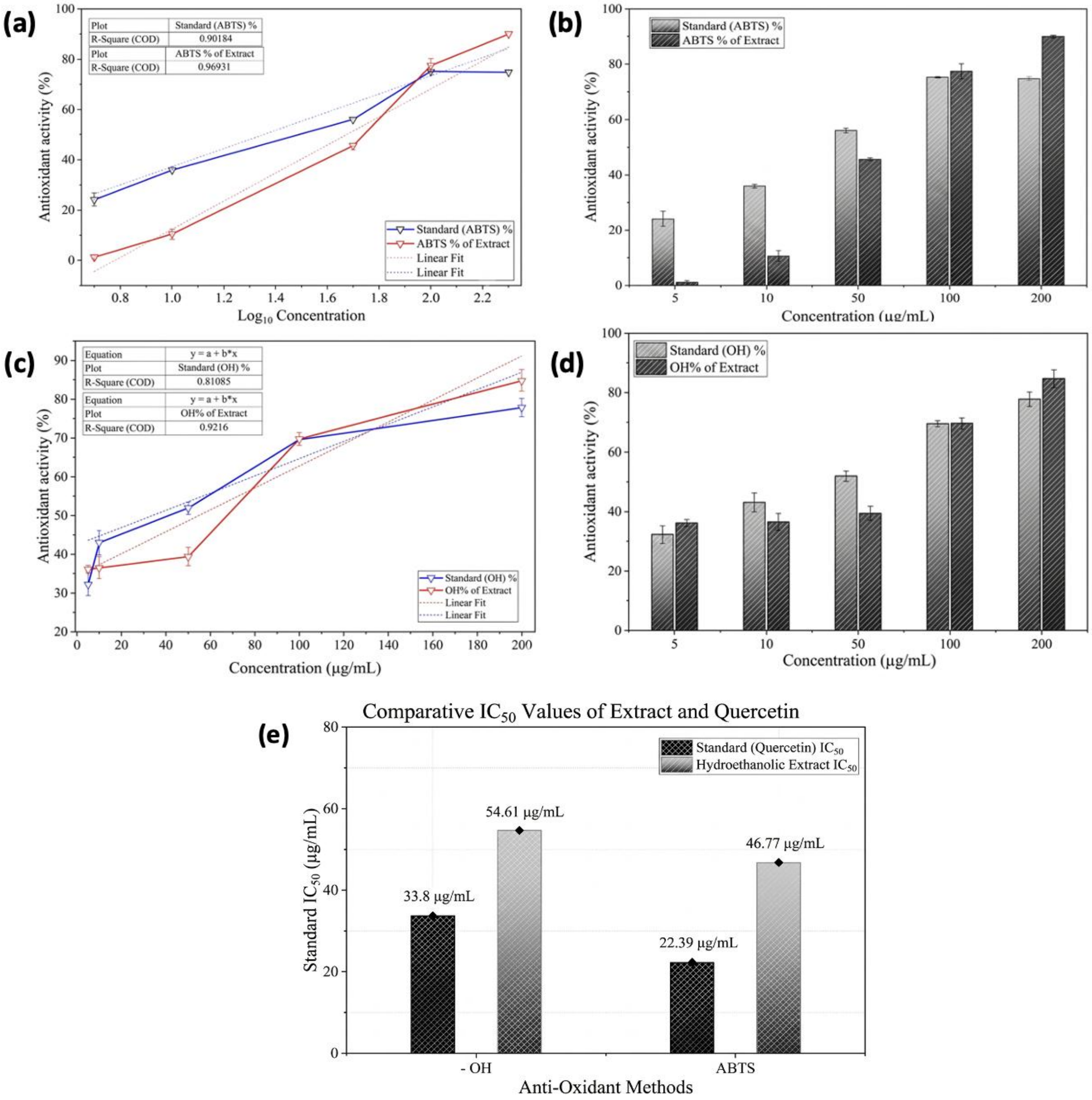

Figure 4. (a) & **(b)** - ABTS assay Dose-dependent antioxidant activity and linear regression plot of the extract and Quercetin. **(c)** & **(d)** - Hydroxyl Radical Scavenging Assay (OH %) Dose-dependent antioxidant activity and linear regression plot of the extract and Quercetin. **(e)** - Comparative IC_{50} values of the hydroethanolic extract in antioxidant assays.

Table 4. Antioxidant activity of the Hydroethanolic Extract

S. No	Method	Concentration (µg/ml)					IC50 Value (µg/ml)
		5	10	50	100	200	
1	ABTS % (Std-Quercetin)	24.11 ± 2.67	35.90 ± 0.58	56.06 ± 0.63	75.21 ± 0.18	74.80 ± 0.55	22.39 µg/ml
2	ABTS % (Extract)	01.24 ± 0.59	10.51 ± 2.03	45.58 ± 0.37	77.46 ± 2.80	89.99 ± 0.44	46.77 µg/ml
3	Hydroxyl Radical Scavenging Assay % (Std-Quercetin)	32.26 ± 2.91	43.02 ± 3.22	51.96 ± 1.68	69.56 ± 1.13	77.82 ± 2.31	33.80 µg/ml
4	Hydroxyl Radical Scavenging Assay % (Extract)	36.18 ± 1.05	36.52 ± 2.88	39.40 ± 2.33	69.78 ± 1.71	84.78 ±2.91	54.61 µg/ml

The IC_{50} values indicate that the extract contributes notably to its antioxidant strength and potential protective effects against oxidative stress. One-way ANOVA demonstrated significant differences among the tested concentrations for the standard ($F(4, 10) = 61.75$, $p < 0.0001$) and the extract ($F(4, 10) = 96.46$, $p < 0.0001$), indicating significant dose-dependent antioxidant activity. Hydroxyl radicals are highly reactive species that cause lipid, protein, and DNA damage; however, *Prosopis cineraria* seed extract demonstrated significant hydroxyl radical scavenging activity at higher concentrations, suggesting a protective antioxidant effect [42].

3.6 Brine Shrimp Lethality Assay

The hydroethanolic extract exhibited a lethal concentration (LC_{50}) in the brine shrimp lethality assay, as shown in Figure 5 (a), (b) & (c). After 24 hours of exposure, the extract exhibited a lethal concentration (LC_{50}) value of 930.3 μg/mL. The mortality increased in a concentration-dependent manner, indicating a direct relationship between extract concentration and brine shrimp lethality given in a Figure 6 (b & c). There was no effect of lethality by any of the concentrations at 2 hours as depicted in Figure 6 (a). The brine shrimp continued moving in the test materials. In the present study, extract exhibited an LC_{50} value of 930.3 μg/mL, suggesting low to moderate cytotoxic activity according to Meyer's toxicity criterion LC_{50} values less than <1000 μg/mL are considered biologically active and values above this threshold are observed as non-toxic [28]. The LC_{50} obtained in this study falls just below this limit and these indicate that the extract possesses measurable cytotoxic potential while remaining relatively safe at lower concentrations. The LC_{50} value was determined by probit analysis. The regression equation obtained was $y = -0.10208 + 1.71883x$ with an R^2 value of 0.8095. The potassium dichromate served as the positive control for this brine shrimp lethality assay at the 1 mg/mL of concertation and complete mortality of the shrimps was observed within 1 hour of exposure. The toxicity increased in a concentration-dependent manner, with mortality rising from 8.89 ± 1.11% at 100 μg/mL to 81.11 ± 1.11% at 1500 μg/mL after 24 h of exposure. One-way ANOVA revealed significant differences among the tested concentrations ($R^2 = 0.9972$; $F(4, 10) = 506.64$, $p < 0.0001$), with indication of a significant dose-dependent toxic effect of the extract on brine shrimp. The brine shrimp lethality assay demonstrated significant concentration-dependent cytotoxicity, with an LC_{50} value of 930.3 μg/mL. According to Meyer's toxicity criterion, extracts exhibiting LC_{50} values below 1000 μg/mL are considered biologically active.

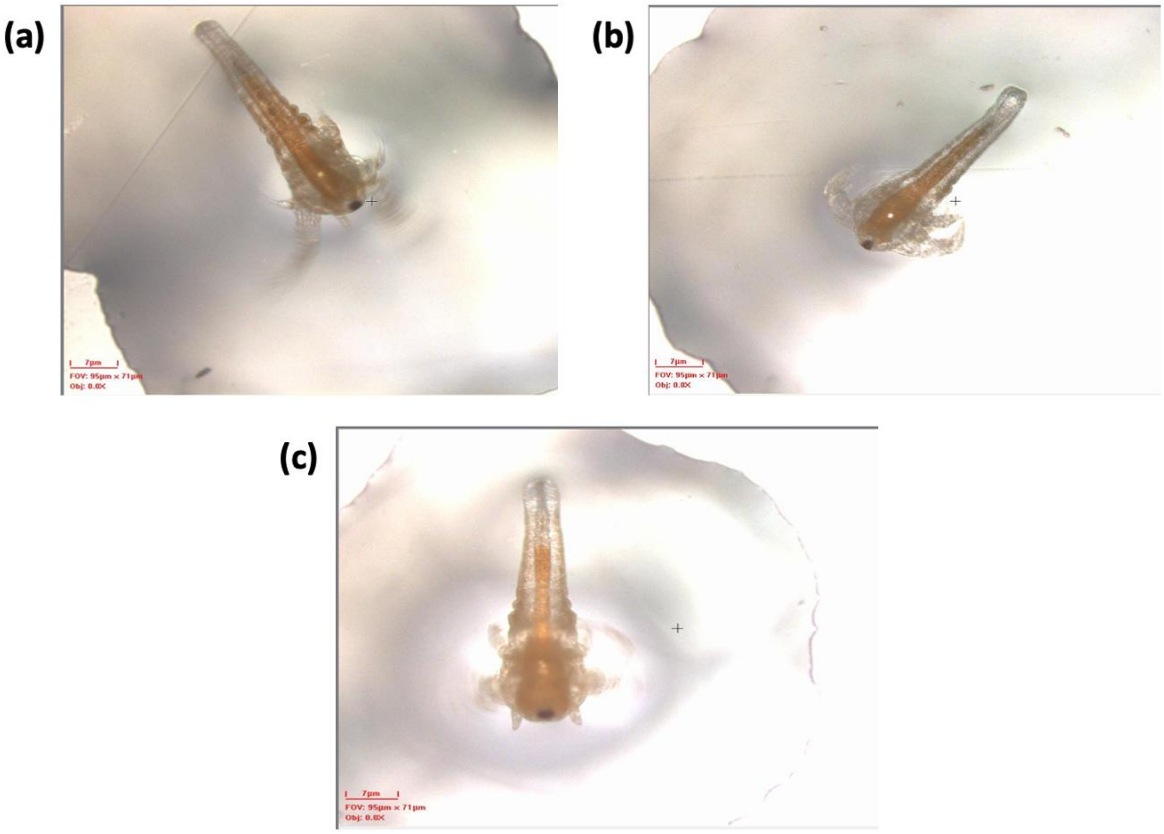

Figure 5. (a), (b), (c) Three-dimensional images of *Artemia salina* after 24 h exposure to three different concentrations of the extract: 100 μg/mL, 500 μg/mL, and 1500 μg/mL, respectively

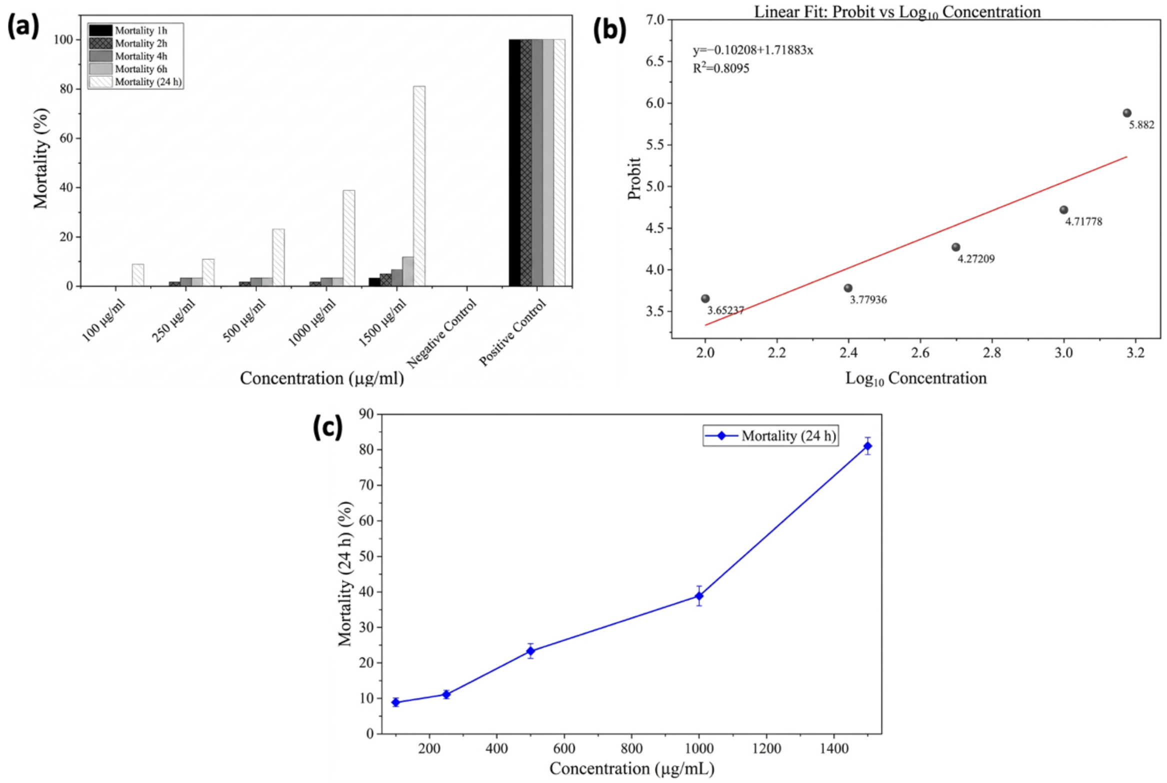

Figure 6. (a) Represents the mortality differences observed at different exposure periods, **(b)** Represents the LC_{50} value by the probit analysis and **(c)** Dose-Mortality representation of 24 hr percentage mortality.

4. Conclusion

The findings of the present study confirm the phytochemical composition and biological potential of *Prosopis cineraria*. The characterization of the extract through UV–VS, FTIR and GC–MS confirmed the presence of various functional groups and identified the major classes of secondary metabolites. The antioxidant activity by ABTS and hydroxyl radical scavenging activity observed in the extract could be linked to the presence of phenolic compounds, flavonoids and other bioactive constituents identified *P. cineraria* seeds. The brine shrimp lethality assay indicates that extract with LC_{50} values below this threshold are regarded as biologically active and weakly toxic. In conclusion, the results support the traditional medicinal relevance of *Prosopis cineraria* seeds and highlight their potential as a source of biologically active compounds with antioxidant and moderate cytotoxic properties, suggesting possible applications in the management of oxidative stress-related conditions.

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Sivasankar Murugesh: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, visualization, Validation, Writing - Original Draft. Mrudhulla Sivakumar: Data Curation, Formal Analysis, Visualization. S. Monisha: Data Curation, Formal Analysis, Visualization. Santhoshkumar Muthu: Conceptualization, methodology, Supervision, validation, Writing - Review & Editing, Project administration. All the authors read and approved the final version of the manuscript.

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Competing Interests

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Data Availability

The data supporting the findings of this study can be obtained from the corresponding author upon reasonable request.

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