**HMG-CoA reductase inhibition medicated hypocholesterolemic and antiatherosclerotic potential of phytoconstituents of an aqueous pod extract of *Prosopis cineraria* (L.) Druce: In silico, in vitro, and in vivo studies**

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**Abstract**

Hydrophilic bioactive compounds are copiously exhibited in aqueous extracts owed to solubility. The study was assigned to assess the ability of phytoconstituents of aqueous pod extract of *Prosopis cineraria* to inhibit 3-hydroxy-3-methylglutary-coenzyme A (HMG-CoA) reductase activity and regression in atherosclerotic plaque through in vitro, in vivo, and in silico assessments along with phytochemistry of extract. The test extract exhibited 17 leading compounds as examined by Liquid Chromatograph Triple Quadrupole Mass Spectroscope. In vitro assay of test extract showed 78.1% inhibition of HMG-CoA inhibition (IC50 was 0.03 μg/ml). In vivo assessments, hypercholesterolemia was induced by supplementing cholesterol powder and a high-fat diet. The treatment of test extract caused significant (*p* ≤ 0.001) improvements in the lipid profile and antioxidant levels. Subsequently, the reductions in the atherosclerotic plaque and improved lumen volume were pointedly observed. In silico analyses of molecular docking revealed potent interaction capabilities of cloprostenol with the target protein of HMGR. The interactions were validated through structural simulations of the molecular dynamics such as root mean square fluctuation, the radius of gyration, and solvent accessible surface area. The druggability of potent compounds was also examined. The results revealed that phytoconstituents of the test extract could inhibit HMGR and regress atherosclerotic plaque.

**1 INTRODUCTION**

The enzyme 3-hydroxy-3-methylglutary-coenzyme A (HMG-CoA) reductase is crucial in regulating the cholesterol biosynthetic pathway (Baskaran et al., [2015](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0008); Haber et al., [2013](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0021)). Inhibition of HMG-CoA reductase activity will reduce cholesterol synthesis and thus increase hepatic uptake of low-density lipids (LDL) by modulation of LDL receptors (Marahatha et al., [2021](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0040)). Using statins in humans as an HMG-CoA reductase inhibitor is associated with several adverse effects, such as hepatoxicity, myopathy, gastrointestinal upset, cataracts, rhabdomyolysis, and an increased risk of diabetes (Ramkumar et al., [2016](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0056)). Therefore, the present study evaluated dietary constituents that represent vital components utilized in folk medicine in India that may have capacities to inhibit several enzymes and scavenge free radicals (Nguyen et al., [2019](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0045); Tanwar et al., [2018](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0061)). Additionally, herbal formulations based on complementary medicine are receiving greater attention and acceptance in India and many other countries due to evidence-based studies of their safety and effectiveness. Several herbal formulations have been reported in the literature for the treatment of hypercholesterolemia and diabetes that were used by local people based on traditional knowledge passed down from generation to generation (Moss & Ramji, [2016](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0044); Patil et al., [2009](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0046); Tanwar et al., [2018](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0061); Yin et al., [2008](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0067)). Dietary supplements of antioxidant formulations are also potent in ameliorating risk factors associated with hypercholesterolemia (Mahdavi et al., [2020](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0038); Modak et al., [2007](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0043)). Accordingly, numerous studies have shown that using plant-derived antioxidant formulations is more preferred than synthetic drugs to reduce oxidative injury (Alamu et al., [2021](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0002); Marahatha et al., [2021](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0040)). Several phytoconstituents have also been reported to possess significant antioxidant properties (Forni et al., [2019](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0017); Zhang et al., [2015](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0068)). Phytoconstituents have been said to be “a revived medicine” and “a foundation of dietetic antioxidants” (Brahmachari et al., [2017](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0011); Mahmoud et al., [2019](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0039)). Along with this, local people in rural areas use plant-based products based on traditional knowledge to cure various diseases. Given this background, the present study evaluated the medicinal properties of *Prosopis cineraria*, a desert plant found in western Rajasthan, India. It can survive the adverse stress brought on by insufficient water availability. *P. cineraria* is a member of the Leguminosae and is used by local people to treat various ailments and as a nutritional supplement (Asati et al., [2022](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0005); Janbaz et al., [2012](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0026); Kumar et al., [2018](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0034); Ram et al., [2020](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0053)). Unripe pods of *P. cineraria*, locally known as “Sangari,” are stored after drying and used as a food supplement. The local people consume dried pods of *P. cineraria* in different food recipes and preparations. A plethora of myriad valued soluble bioactive phytocompounds has different degrees, as reported by our previous studies (Khokar & Menghani, [2015](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0031); Priyanka et al., [2022](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0051); Ram et al., [2020](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0053)). The current research is assigned to evaluate the antiatherosclerotic, anti-hypercholesterolemic, and antioxidant properties of an aqueous extract of *P. cineraria* pods through sequential in vitro, in silico, and in vivo assessments along with the phytochemical screening of the text extract.

**2 MATERIALS AND METHODS**

2.1 Pod procurement, authentication, and extraction

Dried pods of *P. cineraria* (L.) Druce were obtained from a local provisional store, and a botanical expert confirmed their taxonomic identity as per the issued herbarium voucher (Shetty 3487-BSJO). The dried pods were ground in a mixer, macerated after boiling in hot water, and then vacuum dried. The resulting sticky extract was stored under desiccated conditions by following the standard extraction methods (Poojary et al., [2015](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0048)). Atorvastatin, a type of statin, was purchased from a local medical store and used as a positive control. All chemicals and reagents were purchased from local suppliers in Jodhpur (Rajasthan), India.

2.2 Phytochemical analysis of the extract

LCMS and GCMS analysis performed a phytochemical analysis and identification of the small molecule compounds of the extract. The screened phytoconstituents were identified using METLIN software by the masses of the obtained peaks and based on the monoisotopic mass of standard compounds using M and M + H ions in QTOF mass hunter software. The default series for mass identification used a value greater than the 100 m/z ratio (Kind et al., [2018](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0032)).

2.3 LC/MS chemical analysis

A metabolomic analysis based on LC-MS was conducted to characterize the chemical fingerprint of the plant extract. Several mobile phase sequences were analyzed in the study to obtain a comprehensive characterization of chromatographic peaks (Zhu et al., [2013](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0069)).

2.4 Gas chromatography with tandem mass spectrometry (GC-MS/MS) analysis

GC-MS analysis of an ethanol pod extract of *P. cineraria* was conducted using a standard protocol. The sample was injected into a gas chromatograph interfaced with a mass spectrometer (GC-MS) (Baskaran et al., [2015](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0008)). The Liquid Chromatograph Triple Quadrupole Mass Spectroscope (LC-MS/MS and GC-MS/MS) were used to identify the phytoconstituents in the extract based on the separation and isolation of small molecular weight (MW) compounds based on their MW, retention time, and other chromatographic techniques (Keskes et al., [2017](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0030)).

2.5 In vitro assays

**2.5.1 HMG-CoA reductase activity**

In vitro inhibition of HMG-CoA reductase activity by the plant extract was performed using an HMG-CoA reductase assay kit (Sigma-Aldrich) based on the absorbance measurement in a spectrophotometer. The assays utilized increasing concentrations of the pod extract (1.56, 3.13, 6.25, 12.50, and 25 μg/ml) and pravastatin as a positive control. The concentration of the HMG-CoA reductase enzyme solutions ranged between 0.50 and 0.70 mg/ml. The different concentrations of the aqueous extract were mixed with a reaction mixture containing NADPH, HMG-CoA substrate, and HMGR. Pravastatin (Sigma Aldrich Co.) was used as a positive control, and distilled water served as a negative control (Baskaran et al., [2015](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0008); Ergin et al., [2013](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0015)).

**2.5.2 Antioxidants assays**

The in vitro total antioxidant potential of the pod extract was evaluated by following the suppression of ABTS (2,2'-azino-bis[3-ethylbenzthiazoline-6-sulfonic acid]) radical cations formation by antioxidant (Gupta et al., [2009](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0020)). The ferric reducing ability of plasma (FRAP) reagent was prepared at a ratio of 10:1:1 by mixing 300 mM acetate buffer (pH 3.6), 10 mM of 2,4,6-tripyridyl-s-triazine (TPTZ), and 20 mM of FeCl3⋅6H2O. The assays were carried out in triplicate in a 96-well plate by mixing 20 μl of plant extract at different concentrations with 180 μl of FRAP reagent. The reaction was incubated at 37°C in the dark for 30 min, and absorbance was measured at 593 nm. The antioxidant capacity was calculated as ferrous equivalents using a standard curve generated with FeSO4.

The ABTS reagent was prepared by mixing a 7 mM ABTS solution with 2.4 mM potassium persulfate. The reagent was stored in the dark for 16−24 h to stabilize it before use. A working solution was prepared by diluting the reagent with ethanol to obtain an absorbance of 0.70 at 734 nm at 37°C. Subsequently, 10 μl of the plant extract was mixed with 190 μl of diluted ABTS reagent in a 96-well plate. Trolox was used as a positive standard to measure the relative percentage scavenging activity of the aqueous pod extract of *P. cineraria*.

2.6 In vivo study

**2.6.1 Induction of hypercholesterolemia and experimental groups**

A rabbit (New Zealand white male) animal model was selected for use in the study, having an approximate weight of 1.5 ± 0.5 kg, 6−9 months in age and was deemed healthy after an inspection by a veterinarian. The animals were procured from Disease Free Small Animal House (DFSAH), Lala Lajpat Rai University of Veterinary and Animal Sciences, (Cash Memo-DFSAH/2019/441 dated 18.10.2019). The animals were kept under standard environmental conditions with a 12 h light/dark cycle. The rabbits were used for experimentation after 10 days of acclimatization to the laboratory conditions.

Hypercholesterolemia was induced by oral administration of 500 g of cholesterol powder mixed with 5 ml of coconut oil for 15 days, along with a high-fat diet (21% fat) (Ram et al., [2014](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0055)).

Animals were divided into four groups, with each group containing five rabbits. The experiment ran for 60 days. The experimental groups were as follows:

* Group 1: Vehicle Control, treated with only distilled water for 60 days.
* Group 2: Hypegrcholesterolemic control, diseased animal model.
* Group 3: Treatment with aqueous pod extract of *P. cineraria* (400 mg/kg for 45 days, oral).
* Group 4: Treatment with atorvastatin (25 mg/kg) (Wang et al., [2013](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0066)).

After completing the assigned duration of the experiments, the overnight fasted animals were killed under the recommended anesthesia of ketamine by following the standard norms (Close et al., [1997](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0013); Iung et al., [2020](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0025)).

**2.6.2 Biochemical analyses**

Blood was collected from animals in each treatment group, and serum was separated from blood by centrifugation using a standard protocol and stored at −20°C. After thawing, assessments were made of total cholesterol (Abell et al., [1952](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0001)), triglyceride (Klotzsch & McNamara, [1990](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0033)), HDL-cholesterol (Hirano et al., [2008](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0022)), glucose (Trinder, [1969](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0063)), and total protein (Lowry et al., [1951](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0037)). All measurements were conducted by following the standard methods. The lipid profile and atherogenic indices were calculated using Friedewald's formula (Dobiášová, [2004](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0014); Vujovic et al., [2010](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0065)).

**2.6.3 Serum antioxidant assay**

The antioxidant capacity of the serum was evaluated by measuring catalase, SOD (superoxide dismutase) (Beauchamp & Fridovich, [1971](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0009)), GSH (Rahman et al., [2007](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0052)), lipid peroxidation (LPO) (Buege & Aust, [1978](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0012)), and total antioxidant activity (Benzie, [1996](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0010)). Thiobarbituric acid reactive substances were examined as an index of LPO.

**2.6.4 Histopathology and planimetric analysis**

The aortas of the four different experimental groups were obtained from autopsied animals and processed for histopathological examination. The aortas were fixed in 10% formalin and processed for embedding and sectioning. Tissue sections were mounted on glass slides, stained, and then covered with a cover slip for evaluation through microphotography at a magnification of ×200 of H&E stained objects (Micklem & Sanderson, [2001](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0042)). Planimetric studies of the aorta wall, lumen volume, and atherosclerotic plaque were conducted using a Camera Lucida (Ram et al., [2014](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0055)).

2.7 Molecular docking

**2.7.1 Ligand preparation**

Sixteen molecules (Table [1](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-tbl-0001)) were docked onto human HMG-COA reductase receptors (PDB ID:1HWK). The molecules in structure data format were downloaded from Pubchem using their structure CIDs. The geometry of the molecules was optimized using the OPLS2005 force field and low energy conformers generated in the LigPrep module of Schrodinger (Peasari et al., [2018](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0047)). The generated conformers were then used in molecular docking studies.

**Table 1.**Molecular docking study of top three potent phytoconstituents of aqueous pod extract of *Prosopis cineraria* (L.) Druce

| **S. No.** | **Pubchem ID** | **Name** | **Glide *g* score** | **Interaction** |
| --- | --- | --- | --- | --- |
| Positive control |
| 1. | 60823 | Atorvastatin | −7.9 | ARG590, VAL683, SER661, GLY860 |
| 2. | 54687 | Pravastatin | −7.1 | ASP690, LYS691, LYS692, SER684, CYS688 |
| Identified compounds |
| 3. | 5311053 | Cloprostenol | −5.923 | ASP767, CYS526 |
| 4. | 6443798 | Cinecromen | −4.625 | ASN658, GLU665, ARG590, SER684, ASN686 |
| 5. | 6473883 | Dirithromycin | −4.289 | ASN658, GLU528, LYS691, ARG590, SER684 |

**2.7.2 Receptor preparation**

The three-dimensional crystal structure of human HMG-COA reductase was obtained from a protein data bank and resolved using the X-ray diffraction method (PDB ID: 1HWK). The protein structure was prepared in the “PrepWiz” module of Schrodinger (Barua et al., [2019](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0007)). Hydrogens were added, bond orders were assigned, and proper ionization states were assigned during preprocessing. The structure was optimized using restrained minimization by the OPLS2005 force field. The prepared structure was used in the docking studies.

**2.7.3 Grid generation and docking**

The cocrystallized ligand was used as a reference to define the receptor binding sites, and a receptor grid was generated around the centroid of the cocrystallized ligand (atorvastatin). The binding pocket residues at MET657, SER661, VAL683, ARG590, SER684, CYS688, ASN686, ASP690, LYS691, and LYS692 were used for grid generation. The docking was done using the Glide module of Schrodinger in a standard precision mode. The molecules were ranked after docking based on their glide *g*-score, which utilizes different parameters such as lipophilic terms, hydrogen bond terms, metal−ligand interaction, Vander Waals interaction, solvation, π−π, and cation−π interactions to calculate the glide score (Jasmine & Vanaja, [2013](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0027)).

2.8 Absorption, distribution, metabolism, excretion, and toxicity (ADMET) and blood-brain barrier (BBB) studies

Analysis of the identified compounds' ADMET properties utilizing Drulito online software. The violation of ideal drug properties such as MW, partition coefficient (log P), octanol-water partition coefficient (AlogP), H-bond donor, H-bond acceptor, total polar surface area, nHB (number of hydrogen bonds), and the number of acidic groups present was evaluated for violation of the Lipinski rule of five and for the ability of the identified compounds to pass through BBB filters.

2.9 Molecular dynamics

The top two docked complexes of 1HWK; cloprostenol and cinecromen, showing the highest binding affinity, were subjected to molecular dynamics simulations using GROMACS 2020.2 package to study the structural deviations in a dynamic environment for a time scale of 10 ns (Jose et al., [2022](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0029)). The molecular dynamic simulations were examined based on interaction energy, free energy of solvation (DGsolv), RMSF, solvent accessible surface area (SASA), radius of gyration (Rg), and root mean square deviation (RMSD) values as a function of time.

2.10 Statistical analysis

Results of the biochemical assessments, organ weights, and planimetric studies are expressed as a mean ± standard error of the mean. Significant statistical differences were determined by one-way ANOVA and the student “*t*” test (Assaad et al., [2014](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0006)).

**3 RESULTS**

3.1 In vitro investigations

**3.1.1 HMG-CoA reductase inhibition**

Increasing concentrations of the pod extract and a standard drug (pravastatin) were evaluated for their ability to inhibit HMG-CoA reductase. The IC50 of the pod extract was IC50 = 0.03 μg/ml and performed 78.1% inhibition of HMG-CoA reductase (Figure [1B](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0001)), while the standard drug inhibited 93.1% of HMG-CoA reductase activity at IC50 = 0.02 μM (Figure [1B](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0001)).

**Figure 1**

[**Open in figure viewer**](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42)[**PowerPoint**](https://onlinelibrary.wiley.com/action/downloadFigures?id=efd242-fig-0001&doi=10.1002%2Fefd2.42)

(A) Concentration dependent HMG-CoA reductase inhibition by pravastatin (equation: *y* = 11.335ln(x) + 96.013, *R*² = 0.9517; IC50 = 0.02 μM. (B) Concentration dependent HMG-CoA reductase inhibition by aqueous pod extract of *Prosopis cineraria* (L.) Druce (Equation: *y* = 8.0719, *n*(x) + 76.947; IC50 = 0.03 μg/ml). HMG-CoA, 3-hydroxy-3-methylglutary-coenzyme A.

**3.1.2 Antioxidant assays of extract**

A standard curve was generated using ferrous sulfate to determine Fe2+ equivalents for the different concentrations of pod extract as a measure of antioxidant potential. The absorbance value of the different concentrations of pod extract was converted into FRAP equivalents using the slope of the standard curve. Results revealed that the antioxidant activity of aqueous pod extract was concentration dependent over the range of 12.5−200 μg/ml. The maximum value of Fe2+ equivalent, 217.71 ± 0.042 mol μFe2+E/g was obtained at 200 μg/ml of aqueous pod extract (Figure [2A](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0002)).

**Figure 2**

[**Open in figure viewer**](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42)[**PowerPoint**](https://onlinelibrary.wiley.com/action/downloadFigures?id=efd242-fig-0002&doi=10.1002%2Fefd2.42)

(A) ABST assessment of antioxidant potential of aqueous pod extract of *Prosopis cineraria* (L.) Druce. (B) FRAP assay of total antioxidant potential of aqueous pod extract of *P. cineraria* (L.) Druce.

The TEAC antioxidant assay using ABTS also indicated that the antioxidant scavenging activity of the pod extract as measured by Trolox equivalents was also concentration dependent. The absorbance values obtained with different concentrations of pod extract were used to calculate percentage scavenging utilizing the equation: 1.0 − sample absorbance/control absorbance) multiplied by 100. Results indicated that the maximum percentage of scavenging activity (34%) was obtained at *t* 200 µg/ml pod extract (Figure [2B](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0002)).

3.2 In vivo studies

**3.2.1 Lipid profile and atherogenic indices**

Lipid profile parameters (total cholesterol, triglyceride, LDL-cholesterol) and atherogenic indices (LDL/HDL and triglyceride/HDL) were significantly (*p* ≤ 0.001) altered in the hypercholesterolemic groups where LDL cholesterol and total cholesterol increased up to sevenfold in comparison to the untreated control. Notably, treatment of hypercholesterolemic rabbits with either the pod extract or a standard statin drug resulted in a significant reduction in the lipid profile and atherogenic indices (Figure [3](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0003)).

**Figure 3**

[**Open in figure viewer**](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42)[**PowerPoint**](https://onlinelibrary.wiley.com/action/downloadFigures?id=efd242-fig-0003&doi=10.1002%2Fefd2.42)

Lipid profile and atherogenic indices of various treated groups. Data are means ± SEM (*n* = 5); c*p* ≤ 0.001 - significant; and d was nonsignificant as compared to the respective control values. g*p* ≤ 0.001 - significant; and h was nonsignificant as compared to the respective values of the hypercholesterolemic control group. HDL, high density lipids; LDL, low-density lipids.

3.3 Serum antioxidant capacity

Treatment of rabbits with either atorvastatin or the pod extract resulted in significant (*p* ≤ 0.001) changes in the level of catalase, SOD, GSH, LPO, and total antioxidants (Figure [4](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0004)). SOD, GSH, and catalase levels were significantly decreased in the diabetic control group, while the level of LPO increased. Total antioxidant levels were also considerably lower in the hypercholesterolemic control rabbits relative to the untreated control rabbits. Treatment of the hypercholesterolemic rabbits with a standard statin drug or pod extract significantly alleviated catalase, SOD, GSH, and total antioxidant levels relative to the levels in hypercholesterolemic rabbits. The level of LPO was also considerably decreased in treatment groups (Figure [4](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0004)).

**Figure 4**

[**Open in figure viewer**](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42)[**PowerPoint**](https://onlinelibrary.wiley.com/action/downloadFigures?id=efd242-fig-0004&doi=10.1002%2Fefd2.42)

Serum antioxidants status of various treated groups. Data are means ± SEM (*n* = 5); c*p* ≤ 0.001 was significant as compared to the respective control values. g*p* ≤ 0.001 was significant as compared to the respective values of the hypercholesterolemic control group. SOD, superoxide dismutase.

**3.3.1 Histopathology and planimetric study of aorta**

The histoarchitecture of the aorta in untreated control rabbits exhibited a typical layered wall. In contrast, the aorta of hypercholesterolemic rabbits revealed increased thickness in their intima and media, bulging depositions of fatty substances and the presence of foam cells and a fatty band of atherosclerotic plaque. The normal layering of the aorta cell wall was absent, and the lumen volume was decreased (Figure [5B](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0005)). Treatment of hypercholesterolemic rabbits with atorvastatin or pod extract resulted in a significant reduction in plaque area, intima, and total volume. Consequently, the lumen volume of the aorta increased significantly in the treated rabbits (Figure [5C,D](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0005); Figure [6](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0006)).

**Figure 5**

[**Open in figure viewer**](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42)[**PowerPoint**](https://onlinelibrary.wiley.com/action/downloadFigures?id=efd242-fig-0005&doi=10.1002%2Fefd2.42)

(A) Histology of aorta of intact vehicle control (x200 H&E): Aortal wall exhibiting regular arrangement of intima, media, and adventitia. (B) Histology of hypercholesterolemic aorta exhibiting with atherosclerotic plaque (x200 H&E): Arrow indicating the atherosclerotic plaque with remarking of foam cells and fatty strick. (C) Histology of aqueous pod extract of *Prosopis cineraria* (L.) Druce treated aorta (x200 H&E): Arrow indicating regression in atherosclerotic plaque area. (D) Histology of atorvastatin treated aorta (x200 H&E): Arrow indicating regression in area of atherosclerotic plaque.

**Figure 6**

[**Open in figure viewer**](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42)[**PowerPoint**](https://onlinelibrary.wiley.com/action/downloadFigures?id=efd242-fig-0006&doi=10.1002%2Fefd2.42)

Aortal wall and plaque regression status in various treated groups. Data are means ± SEM (*n* = 5); c*p* ≤ 0.001 was significant as compared to the respective control values. g*p* ≤ 0.001 was significant as compared to the respective values of the hypercholesterolemic control group.

3.4 In silico studies

**3.4.1 Molecular docking**

The docking was accomplished, and the best conformation of each ligand was determined based on its binding to HMG-COA reductase. More negative ΔG values indicate stronger binding to the receptor. Based on the glide score, the top three binding molecules were cloprostenol, cinecromen, and dirithromycin, with glide scores of −5.923, −4.625, and −4.289, respectively. The score of the hydrogen bond interactions of the compounds with amino acids is listed in Table [1](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-tbl-0001).

The docked pose, indicating the hydrogen bonds and the H-bond distances of cloprostenol, cinecromen, and dirithromycin, are shown in Figures [7A−C](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0007), as well as in Table [1](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-tbl-0001). Atorvastatin and pravastatin were used as positive controls in in vivo and in vitro assessments, respectively (Table [1](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-tbl-0001)). Besides this, the chemical structures of top-docked phytocompounds (cinecromen and cloprostenol) show the existence of potent functional groups, which resulted in variations in gscores (Figure [7D](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0007)).

**Figure 7**

[**Open in figure viewer**](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42)[**PowerPoint**](https://onlinelibrary.wiley.com/action/downloadFigures?id=efd242-fig-0007&doi=10.1002%2Fefd2.42)

(A−C) Interactions with target enzyme HMG-CoA and phytochemicals (A) cloprostenol, (B) cinecromen, and (C) dirithromycin. (D) Chemical structures of cinecromen and cloprostenol. HMG-CoA, 3-hydroxy-3-methylglutary-coenzyme A.

**3.4.2 Molecular dynamics**

The ligands' RMSD was recorded to observe ligand stability during simulations. The RMSD value for cloprostenol was recorded at 0.45 nm, and for cinecromen, it was marked with a value of 0.55. The RMSD value for cloprostenol remains lower than cinecromen during the simulation, indicating cloprostenol's stability over cinecromen (Figure [9A](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0009)). The RMSF values of the ligand−protein complexes were calculated to understand the local fluctuations taking place for assessing the flexibility of the atoms. The RMSF values for both complexes remain under 0.2 nm (Figure [9B](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0009)). The average Lennard−Jones short-range value for both complexes was found to be −175 KJ/mol. Both complexes' solvation-free energy remains static with an average value of −25 DGsolv (Figure [9C](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0009)). A plot of the Rg spanning over 10 ns is analyzed to display the compactness of the protein during MD simulations. Throughout simulations, the Rg for the 1HWK-cloprostenol complex remains lower than the 1HWK-cinecromen complex, indicating that the 1HWK-cloprostenol complex remains more stable during simulation studies (Figure [9D](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0009)). The average Coulomb's short-range value for complex 1HWK-cinecromen was found to be −25 KJ/mol, and for 1HWK-cloprostenol, the value was recorded at −150 KJ/mol, indicating that 1HWK-cloprostenol interaction is more favorable (Figure [9E](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0009)). Theoretically, the SASA gives an insight into how accessible a protein is to the solvent it resides. Throughout the simulations, SASA fluctuates around 195 nm2 for both complexes. SASA value for the 1HWK-cloprostenol complex remains low in the middle of simulations (Figure [9F](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0009)).

**3.4.3 ADMET analysis**

The pharmacokinetic analysis conducted to evaluate the ADMET properties of the identified compounds for their potential use as pharmaceutical drugs indicated that they conformed to the Lipinski rule of five, except for cinecromen, lupeol, and ophiobolene (Table [2](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-tbl-0002)).

**Table 2.**Pharmacokinetics ADMET prediction of phytoconstituents of aqueous pod extract of *Prosopis cineraria* (L.) Druce by Drulito against Lipinski rule of five and blood-brain-barrier filter

| **Compound** | **MW** | **logP** | **AlogP** | **HBA** | **HBD** | **TPSA** | **nHB** | **nAcidic group** | **Filter (L/B)** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Atorvastatin | 558.25 | 3.442 | 1.093 | 7 | 4 | 110.1 | 11 | 1 |  |
| Pravastatin | 424.25 | 2.275 | −1.89 | 7 | 4 | 124.29 | 11 | 1 | L |
| Prosolanapynone II | 304.17 | 4.875 | 1.033 | 4 | 1 | 55.76 | 5 | 0 | L/B |
| Ophiobolin B | 402.28 | 3.522 | 1.489 | 4 | 2 | 74.6 | 6 | 0 | L |
| Cinene | 136.13 | 3.729 | 2.142 | 0 | 0 | 0.0 | 0 | 0 | L/B |
| Lupeol | 426.39 | 11.901 | 3.231 | 1 | 1 | 20.23 | 2 | 0 |  |
| Cineole | 154.14 | 2.595 | 1.311 | 1 | 0 | 9.23 | 1 | 0 | L/B |
| Prosogan | 369.08 | 0.667 | 0.538 | 5 | 1 | 82.26 | 6 | 0 | L/B |
| Ophiobolene | 358.32 | 8.823 | 3.613 | 1 | 1 | 20.23 | 2 | 0 | B |
| Oxethazaine | 467.31 | 3.08 | 2.354 | 6 | 1 | 64.09 | 7 | 0 | L |
| Ophiobolin A | 400.26 | 3.143 | 1.49 | 4 | 1 | 63.6 | 5 | 0 | L |
| Cloprostenol | 424.17 | 2.138 | −0.49 | 6 | 4 | 107.22 | 10 | 1 | L |
| (2s,3s) -(-)-3-propyloxiranemethanol | 116.08 | 0.525 | −1.22 | 2 | 1 | 32.76 | 3 | 0 | L/B |
| β-d-mannofuranoside, methyl | 194.08 | −1.607 | −2.10 | 6 | 4 | 99.38 | 10 | 0 | L |
| Cinecromen | 651.28 | 1.117 | 0.117 | 13 | 1 | 134.33 | 14 | 0 |  |

* Abbreviations: ADMET, absorption, distribution, metabolism, excretion, and toxicity; AlogP, octanol–water partition coefficient; B, blood brain barrier; Filter L, Lipinski rule of five; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; logP, partition coefficient; MW, molecular weight; nAcidic group, number of acidic group; nHB, number of hydrogen bond; TPSA, total polar surface area.

Cinecromen violated the rule as its MW is above 500 g/mol and possesses 13 potential hydrogen acceptor groups. Cloprostenol conformed to the Lipinski rules for an ideal drug compound but could not cross the BBB since it keeps an acidic group in its structure. Cinene, cineole, prosogan, and prosolanapynone II were all identified as suitable drug candidates based on their ADMET properties and because they conformed to the Lipinski rules and their ability to cross the BBB.

3.5 Phytochemistry of the pod extract

The METLINE mass hunter software screened out the presence of 17 bioactive compounds, with cloprostenol, cinecromen, and dirithromycin being the most dominant in test extract by results of LC-MS/MS and GC-MS/MS (Table [3](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-tbl-0003), Figure [8A](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0008),[B](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-fig-0008)).

**Table 3.**Identified masses by UPLC-QTOF mass spectroscopy and GC-MS/MS investigations from aqueous pod extract of *Prosopis cineraria* (L.) Druce

| **S. No.** | **Identified compound name** | **MIT mass** | **Retention time (tR) (min)** | **Pubchem ID** |
| --- | --- | --- | --- | --- |
| Identified compounds from UPLC-QTOF mass spectroscopy |  |
| 1. | Prosoanapynone II | 304.38 | 3.22 | 11822857 |
| 2. | Prosogan | 369.36 | 3.58 | 3883 |
| 3. | Lupeol | 426.386 | 4.35 | 259846 |
| 4. | Cineole | 154.253 | 11.1 | 2758 |
| 5. | Cinene | 136.238 | 11.9 | 22311 |
| 6. | Ophiobolene | 358.61 | 12.0 | 46173837 |
| 7. | Cinecromen | 651.713 | 12.4 | 6443798 |
| 8. | Ophiobolin A | 400.551 | 12.9 | 5281387 |
| 9. | Ophiobolin B | 402.575 | 13.2 | 12303081 |
| 10. | Clopnostenol | 424.918 | 13.6 | 5311053 |
| 11. | Oxethazaine | 467.654 | 14.1 | 4621 |
| 12. | Rifamximin | 785.891 | 14.8 | 6436173 |
| 13 | Dirithromycine | 836.086 | 15.43 | 6473883 |
| Identified compounds from GC-MS/MS analysis |  |
| 14. | β-d-mannofuranoside, methyl | 194 | 17.54 | 6420200 |
| 15. | Tetracosanoic acid, trimethylsilyl ester | 444 | 20.530 | 522540 |
| 16. | 11-eicosenoic acid, trimethylsilyl ester | 382 | 20.716 | 5366418 |
| 17. | (2s,3s) -(-)-3-propyloxiranemethanol | 116 | 24.612 | 10313120 |

* Abbreviation: GC-MS/MS, gas chromatography with tandem mass spectrometry.

**Figure 8**

[**Open in figure viewer**](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42)[**PowerPoint**](https://onlinelibrary.wiley.com/action/downloadFigures?id=efd242-fig-0008&doi=10.1002%2Fefd2.42)

(A) UPLC chromatograph of aqueous pod extract of *Prosopis cineraria* (L.) Druce. (B) UPLC chromatograph of aqueous pod extract of *P. cineraria* (L.) Druce. (C) GC-MS analysis of aqueous pod extract of *P. cineraria* (L.) Druce. GC-MS, gas chromatography with tandem mass spectrometry.

**Figure 9**

[**Open in figure viewer**](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42)[**PowerPoint**](https://onlinelibrary.wiley.com/action/downloadFigures?id=efd242-fig-0009&doi=10.1002%2Fefd2.42)

(A−B) Molecular dynamics assessments of RSMD (A) and RSMF (B). (C) Molecular dynamics assessments of free energy of solvation (C), radius of gyration (D), ligand interaction energy (E), and solvent accessible surface area (SASA) (F). RSMF, root mean square fluctuation.

**4 DISCUSSION**

Hypercholesterolemia and atherosclerosis are associated with dysregulation of the cholesterol biosynthetic pathway and further consequent events (Prasad & Lee, [2007](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0050)). The present study determined the effectiveness of an aqueous pod extract of *P. cineraria* (L.) Druce is an antiatherosclerotic plaque and anti-hypercholesterolemia agent, as evidenced by its ability to inhibit HMG-CoA reductase activity and enhance antioxidant capacity in hypercholesterolemic rabbits. The food preparations follow the different solvent-based recipes where direct consumption (fresh plant material) and water essence-based recipes exhibited hydrophilic bioactive compounds, leading from ancient civilizations (Altemimi et al., [2017](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0004); Iloki-Assanga et al., [2015](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0023); Stéphane et al., [2012](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0060)). Accordingly, the test extract's LC-MS/MS and GC-MS/MS showed occurrences of 17 leading phytocompounds. Leading compounds perform this potentiality through in vitro assay of test extract against the HMG-CoA reductase (HMGCR) activity. The intensity of inhibition of HMGCR is the representation of the interaction between the ligand and the target protein, as revealed by the molecular docking parameters of gscore. The main phytoconstituents identified in the pod extract were cloprostenol, cinecromen, and dirithromycin, all of which showed significant molecular interaction with HMG-CoA reductase in the docking studies. This kind of examination validated by structural simulations of molecular dynamics that the structural size of ligands showed a significant structural distance between coordinates, revealing how much the protein conformation has changed. Accordingly, the RSMF data indicating the displacement of a particular atom, or group of atoms, relative to the reference structure, averaged over the number of atoms as reported by earlier studies (Ram et al., [2022](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0054); Ishak et al., [2017](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0024); Sargsyan et al., [2017](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0058)). The effective interactions and compactness of cloprostenol and cinecromen with target protein are displayed by the Rg of total and axis, as reported by previous studies. It relates to how standard secondary structures could be competently crammed into a three-dimensional protein structure. The lowest Rg and, consequently, the strongest folding are characteristic of proteins (Lobanov et al., [2008](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0036)).

Consequently, the free energy of solvation and surface SASA indicate the ligand-binding affinities and capabilities of interactions (Gonçalves & Stassen, [2002](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0018); Martins et al., [2014](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0041)). Accordingly, the assessments indicated cloprostenol showed significant values and SASA and free energy of solvation and protein-ligand interaction energy. Along with this, the ADMET analysis predicted the potential of different phytoconstituents present in the pod extract to have the ability to cross the BBB, which remarks the druggability (Tian et al., [2015](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0062)).

Besides this, the HMGCR is a crucial enzyme in the cholesterol biosynthesis pathway, which proceeds the first stable product; therefore, it is considered a rate-limiting step. Accordingly, the statins (HMGCR inhibitors) are served as leading therapeutic agents for hypercholesterolemia. The extract treatment caused a significant reduction in total cholesterol levels, as well as intermediate fractionates such as LDL, HDL, and triglyceride. The similar kinds of observation seen by some previous studies and our experimentation with different extracts of the *P. cineraria* may follow the mechanism of action like statins or mimic phenomena of HMGCR statins (Priyanka et al., [2022](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0051); Ram et al., [2020](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0053); Wang et al., [2013](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0066)). Consequently, the treatment of test extract caused significant ameliorations in antioxidant levels and regression in atherosclerotic plaques. These alterations may follow the scavenging capabilities of phytoconstituents and subside the sequential steps of the progression of atherosclerotic plaque, as reported by earlier studies (Almeida & Budoff, [2019](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0003); Feig, [2014](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0016)).

Oxidative stress results from the excessive generation of free radicals in cells and leads to several metabolic disorders and animal models that affect the quality of life. Plants possess various secondary metabolites that exhibit potent antioxidants (REF). Including these plants and their phytoconstituents in the human diet can prevent oxidative stress in cells and tissues and help maintain normal physiology (Lee et al., [2017](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0035); Tungmunnithum et al., [2018](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0064)). Different phytoconstituents have varying antioxidant capacity levels, and these secondary metabolites should be considered a valuable resource in human diets, as reported in several ancient medical systems (Grover et al., [2002](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0019); Johar et al., [2018](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0028)). Treatment of hypercholesterolemic rabbits with pod extract resulted in a significant increase in the level of catalase, SOD, and GSH, a decrease in LPO, and an increase in the total antioxidant capacity in blood serum. These changes boosted the free radical scavenging capacity in the body. As reported in previous studies, the phytoconstituents most likely induced them in the pod extract and atorvastatin (Prasad, [2008](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0049); Salvamani et al., [2016](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0057); Schneider, [1999](https://onlinelibrary.wiley.com/doi/full/10.1002/efd2.42#efd242-bib-0059)).

**5 CONCLUSION**

It can be concluded that the cloprostenol and potent phytocompounds of an aqueous pod extract of *P. cineraria* pods can potentially subside the hypercholesterolemia through inhibition of HMG-CoA reductase and scavenging the free radicals.

**AUTHOR CONTRIBUTIONS**

**Heera Ram and Bhim Pratap Singh**: designing experimental protocols, drafting, and review of the manuscript. **Noopur Jaipal and Heera Ram**: conducted in vivo studies. **Jaykaran Chara**: conducted the in vitro analysis of HMG-CoA reductase activity. **Pratap Singh and Anshuman Dixit**: conducted the in silico study. **Ashok Kumar and Anil Panwar**: molecular dynamics. **Pratap Singh and Garima Singh**: conducted the phytochemical analysis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

**ETHICS STATEMENT**

The current study has a vital part of animal experimentations for in vivo research, which was approved and provided ethical clearance by the Institutional Animal Ethics Committee (IAEC), Department of Zoology, Jai Narain Vyas University, Jodhpur (Rajasthan)−342001, India is registered under CPCSEA (Reg. No.1646/GO/a/12/CPCSEA valid up to 27.03.23). All authors reviewed the manuscript and agreed with the final version to proceed with publication.