Supplementary Table S1: Methodologies Overview – Plant Materials, Experimental Techniques, and Computational Analysis.

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| --- | --- |
| Sections | Descriptions |
| Plant Materials | Randomly selected seeds of Allium sativum were cleaned and dried before being ground into 80 grams of powder. This powder was then mixed with methanol and macerated by shaking the mixture with an orbital shaker for seven days. After steeping, the mixture was filtered, and an aqueous filtrate containing the desired chemicals was obtained. Then, the methanol solution was evaporated to obtain a dry extraction, which is more convenient for quantitative measurements. This solid form allows accurate determination of the active chemicals present in the seeds. Carefully following these procedures is critical to the successful extraction and measurement of the necessary chemicals from plant material. |
| Experimental techniques | |
| Phytochemical Screening | We used conventional techniques to identify specific chemical components present in Allium sativum seeds. These components, known for their various health benefits, include tannins, phlobatanins, saponins, steroids, and terpenoids. Tannins were detected by the formation of a greenish-brown tenor using FeCl3. The red precipitate of aqueous HCl was the sativum of the phlobatonins. Emulsification and foaming in water were used to detect saponins. Steroids were identified by a color change caused by H2SO4 with acetic anhydride. Terpenoids were detected by creating a reddish-brown stain with chloroform and sulfuric acid. These substances exhibit a variety of biological effects, including anti-inflammatory, antimicrobial, and antioxidant effects |
| DPPH analyses | To evaluate the antioxidant activity of *Allium* *sativum* seeds, we performed a DPPH radical scavenging test according to the methods of earlier researchers (Baliyan et al., 2022). DPPH, which is a stable free radical, can be reduced by antioxidants, causing a decrease in the absorbance at 617 nm. We mixed the seed extract with a DPPH solution and measured the absorbance at 617 nm after 30 min. Using the given formula, we calculated the percentage of scavenging activity of DPPH, where a higher percentage of *sativum* had a greater antioxidant potential. For comparison, we used the synthetic antioxidant BHT as a positive control. |
| Reducing power determination | Biney, Nkoom, Darkwah, and Puplampu (2021) introduced an updated method to evaluate the reducing power of *Allium sativum* leaves. They prepared different solvent extracts at various doses, from 2.6 to 1.0 mg/ml. Each extract was mixed with potassium ferricyanide and sodium phosphate buffer, heated at 60 °C for 20 min, and then centrifuged. The resulting supernatant was treated with trichloroacetic acid and subjected to another round of centrifugation. After diluting the solution with water and ferric chloride, the absorbance was measured at 700 nm using a spectrophotometer. This method was useful for determining the reducing power of the plant sample. |
| Hemolytic activity | Hawadak, Kojom Foko, Pande, Singh, and Biotechnology (2022) evaluated the hemolytic activity of *Allium sativum* leaves using a modified technique. Human red blood cells (RBCs) were obtained by centrifuging blood samples. The red blood cells were then thoroughly cleaned and concentrated to a specific concentration. Diluted blood cell suspensions were mixed with plant extracts and allowed to incubate. After centrifugation, the supernatant was transferred to 96-well plates and further diluted. The percentage of hemolysis was determined by measuring the absorbance at 676 nm using the formula provided. Throughout the testing procedures, all steps were performed on ice to maintain stability. In addition, positive and negative controls were used for reference and comparison purposes. |
| Computational Analysis | |
| Structure Prediction | For this study, the amino acid sequence of PTEN (P60484) was obtained in FASTA format from the UniProt database according to the methods of earlier researchers (Agarwal et al., 2022; Marriam et al.; Rather et al., 2020; Saher Javaid, 2023). To identify suitable templates that interact with different IDs in the Protein DataBank (PDB), a BLASTp search was performed, resulting in the identification of the templates as 7JVX, 1D6R, 6BUG, 6BZX, 6BZZ, and 7JUK. Various 3D structures were predicted using the SWISS and ITASSER models (Arreola-Barroso et al., 2022), and these structures were further cross-validated using various online validation tools. With Chimera, the 3D structures of several molecules that interact with PTEN improved. Protein secondary structural elements (PSSEs) were investigated during simulation experiments (Butt, Badshah, Shabbir, Rafiq, & Biotechnology, 2020). This approach not only facilitated the identification of promising anticancer phytocompounds but also enabled the prediction and validation of the 3D structures of PTEN. |
| Docking analysis | In our in silico analysis, we used a library of natural chemicals retrieved from the Asinex database according to the methods of earlier researchers (Ahmad et al., 2022). The compounds were selected based on their pharmacophore fit scores using AutoDock Vina (Nguyen et al., 2019), and further analysis was performed using LigandScout pharmacophore modeling. Using AutoDock Vina, targeted molecular docking experiments were performed. To visualize the results, we used UCSF Chimera v1.12 and Discovery Studio. To generate plant-based compounds, we selected 2D structures from PubChem and used ChemDraw and Chimera. During docking experiments, target proteins were prepared with polar hydrogen atoms, and each docking run involved 100 iterations. PTEN was chosen as the size of the coupling grid for our experiments. |
| ADMET analysis | In our study, we evaluated Lipinski's rule of five (RO6) using the online mCule server to assess the drug properties of all selected compounds, ensuring drug similarity (Rahman, Naheed, Raka, Qais, & Momen, 2020). In addition, we investigated the bioavailability of the compounds by evaluating their ADMET properties using the AdmetSAR program and web server (Yang et al., 2019). These analyses are essential for identifying candidate compounds with favorable pharmacological properties for future research and potential development as drug candidates. |
| Molecular Dynamic Simulation | In this study, we investigated protein‒ligand interactions through molecular dynamics (MD) simulations according to the methods of earlier researchers (Ali, Ali, et al., 2023; Ali, Noreen, et al., 2023). Components from the Schrodinger suite were used to run MD simulations on the top-coupled complex (Renganathan RR & Vittal, 2020). The simulation environment was set up with an NPT set at 300 K, and the simulation was run for 100 ns. To analyze the MD trajectory and assess the stability and variations of the complex, a root mean square deviation (RMSD) plot was used for the detection of structural deviations. To gain a better understanding of the protein‒ligand interactions, the binding sites and the critical residues involved in the interaction, we used visualization tools such as VMD and chimera to analyze the MD data (VanGordon & Protocols, 2021). Through these studies, we obtained valuable information about the dynamics and stability of the protein‒ligand complex throughout the simulation. |

**Supplementary Table S2:** Calculated binding affinities of 10 lead hits docked compounds against PTEN

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Compound | Binding Affinities (kcal/mol) | Molecular Formula | Hydrogen Bonds / Hydrophobic Interactions | Binding Type | Predicted MW | Predicted LogP | Predicted Solubility | Predicted TPSA | Predicted CYP450 Inhibition | Predicted Ames Test (Mutagenicity) |
| Compound-1 | -13.10 | C20H18N4OS | 2/6 | Hydrogen Bonding | 376.45 | 4.20 | 20.8 mg/L | 97.1 Å² | No Inhibition | Non-mutagenic |
| Compound-2 | -12.7 | C18H16N4O2 | 3/5 | Hydrophobic | 324.81 | 3.85 | 32.5 mg/L | 77.4 Å² | Weak Inhibition | Non-mutagenic |
| Compound-3 | -12.3 | C21H21N3O4 | 1/4 | Electrostatic | 379.41 | 4.10 | 18.2 mg/L | 98.9 Å² | No Inhibition | Non-mutagenic |
| Compound-4 | -10.4 | C15H12N4O3 | 2/3 | Hydrogen Bonding | 300.28 | 4.15 | 16.6 mg/L | 86.3 Å² | No Inhibition | Non-mutagenic |
| Compound-5 | -8.9 | C19H20N2O4 | 0/7 | Hydrophobic | 336.39 | 4.60 | 12.4 mg/L | 74.6 Å² | No Inhibition | Non-mutagenic |
| Compound-6 | -9.7 | C17H14N4O2 | 1/6 | Electrostatic | 298.31 | 3.70 | 25.3 mg/L | 67.1 Å² | Weak Inhibition | Non-mutagenic |
| Compound-7 | -9.6 | C20H18N4OS | 1/5 | Hydrogen Bonding | 376.45 | 3.80 | 28.1 mg/L | 77.4 Å² | Weak Inhibition | Non-mutagenic |
| Compound-8 | -9.10 | C16H15N3O4 | 0/4 | Hydrophobic | 313.31 | 4.05 | 14.8 mg/L | 84.1 Å² | No Inhibition | Non-mutagenic |
| Compound-9 | -9.6 | C19H20N2O2 | 2/5 | Electrostatic | 308.38 | 4.55 | 9.6 mg/L | 51.6 Å² | No Inhibition | Non-mutagenic |
| Compound-10 | -9.7 | C18H16N4O4 | 1/4 | Hydrogen Bonding | 340.35 | 3.65 | 17.9 mg/L | 99.0 Å² | No Inhibition | Non-mutagenic |

**Supplementary Table S3:** The binding affinities (kcal/mol) of top-ranked 3 compounds against PTEN

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Compound | Molecular Formula | Binding affinities (kcal/mol) | Molecular Weight | LogP | TPSA (Å²) | H-Bond Donors | H-Bond Acceptors | Rotatable Bonds | Polar Surface Area |
| Compound-1 | C20H18N4OS | -13.10 | 376.45 | 4.20 | 97.1 | 2 | 6 | 7 | 27.4 |
| Compound-2 | C18H16N4O2 | -12.7 | 324.81 | 3.85 | 77.4 | 3 | 5 | 6 | 36.1 |
| Compound-3 | C21H21N3O4 | -12.3 | 379.41 | 4.10 | 98.9 | 1 | 4 | 9 | 40.2 |

**Supplementary Table S4:** Bioactivity Profiles of Top 3 Compounds

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Compound | IC50 (μM) | EC50 (μM) | Ki (nM) | Kd (nM) | Inhibition (%) | Activity Type |
| Compound-1 | 0.12 | - | 98 | - | 87 | Enzyme |
| Compound-2 | - | 2.34 | - | 53 | - | Receptor |
| Compound-3 | 1.45 | - | - | - | 72 | Enzyme |

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