**Algal-Derived Phytochemicals as Potential Bioproducts: Computational and Experimental Strategies for Drug Discovery and Therapeutics**

Sudipta Sardar\*, Srija Hazarika 2, Somenath Dutta1\*

1. Biomolecular Engineering Laboratory, Pusan National University, Busan, South Korea
2. CSIR – Northeast Institute of Science and Technology (NEIST), Jorhat, Assam, India

**Supplementary Table 1: Comparison of Docking Tools and Their Features**

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Docking Tool** | **Search Algorithms** | **Scoring Functions** | **Auxiliary Tools** | **Partial Charges** | **Evaluation Methods** | **Type of Docking** | **Supported Platforms** | **License** | **References (PMID)** |
| **AutoDock** | Genetic Algorithm, Local Search | Free Energy of Binding, Empirical Force Field | AutoGrid, AutoDockTools | Gasteiger | RMSD, Binding Affinity | Rigid and flexible ligand | Windows, macOS, Linux | Open-source (GPL) | [27077332](https://pubmed.ncbi.nlm.nih.gov/27077332) |
| **AutoDock Vina** | Iterated Local Search Global Optimizer | Empirical Scoring Function | AutoDockTools, OpenBabel | Gasteiger, AutoDock Charges | RMSD, Binding Affinity | Rigid and flexible ligand | Windows, macOS, Linux | Open-source (Apache) | [19499576](https://pubmed.ncbi.nlm.nih.gov/19499576) |
| **DOCK** | Anchor-and-Grow, Hierarchical Search | Grid-based Energy Scoring | Chimera, UCSF DOCK Suite | AMBER | RMSD, Energy Scores | Flexible ligand, rigid protein | Windows, macOS, Linux | Open-source (GPL) | [25914306](https://pubmed.ncbi.nlm.nih.gov/25914306) |
| **FlexX** | Fragment-based Incremental Construction | Empirical, Forcefield-based | SYBYL, BioSolveIT | Gasteiger | Energy Scores, Visual Inspection | Rigid and flexible ligand | Windows, Linux | Commercial | [17886339](http://www.ncbi.nlm.nih.gov/pubmed/17886339) |
| **RosettaDock** | Monte Carlo, Low-Resolution Search | Rosetta Energy Function | PyMOL, RosettaScripts | AMBER, CHARMM | Interface RMSD (iRMSD), Energy Scores | Protein-protein docking | Linux | Open-source | [18442991](https://pubmed.ncbi.nlm.nih.gov/18442991) |
| **ICM** | Biased Probability Monte Carlo (BPMC) | Empirical, Docking Score, Free Energy | ICM Browser, PyMOL | Gasteiger | RMSD, Binding Affinity | Flexible ligand, receptor flexibility | Windows, Linux | Commercial | <https://doi.org/10.1002/jcc.540150503> |
| **GOLD** | Genetic Algorithm | ChemScore, Piecewise Linear Potential (PLP) | Hermes Visualizer, PyMOL | Gasteiger, MMFF94 | RMSD, Binding Affinity | Flexible ligand, protein flexibility | Windows, Linux | Commercial | 12910460 |
| **Prescience In-Silico** | Custom Algorithms | Proprietary Scoring Function | Prescience Suite | AMBER, MMFF94 | Binding Affinity, Energy Scores | Flexible ligand, custom protocols | Windows, macOS, Linux | Commercial | <https://www.prescience.in/prins> |
| **MOE** | Simulated Annealing, Genetic Algorithm | Forcefield-based, Free Energy, Empirical | PyMOL, MOE Visualizer | AMBER | RMSD, Binding Affinity | Rigid and flexible ligand | Windows, macOS, Linux | Commercial | 19075767 |
| **Schrödinger Glide** | Systematic Search, Monte Carlo | GlideScore (empirical), ChemScore, Emodel | Maestro, PyMOL | OPLS, MMFF94 | RMSD, Energy Scores | Flexible ligand | Windows, Linux | Commercial | <https://www.schrodinger.com/> |
| **LeDock** | Systematic Search | Empirical Scoring | PyMOL, LePro | AMBER | Binding Affinity, Visual Inspection | Rigid and flexible ligand | Windows, macOS, Linux | Free for academic use | 10.1088/1755-1315/218/1/012143 |

**Supplementary Table 2: Comparison of Docking Tools and Their Features**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Tool Name** | **Supported Platform** | **Search Algorithms** | **Availability** | **References (PMID)** |
| **CASTp 3.0** | Any | Alpha Shape, Delaunay triangulation, Discrete Flow | Web: <http://sts.bioe.uic.edu/castp/> | 29860391 |
| **fpocket** | Linux, MacOS, Docker, Conda | Voronoi tessellation, Alpha Spheres | GitHub: <https://github.com/Discngine/fpocket>  Web: <https://bioserv.rpbs.univ-paris-diderot.fr/services/fpocket/>  Standalone: <https://sourceforge.net/projects/fpocket/> | 20478829 |
| **3D-LigandSite** |  | HHSearch, TMAlign | Web: Under Maintenance | 35412635 |
| **CAPLA** | Any | CNN | GitHub: <https://github.com/lennylv/CAPLA> | 36688724 |
| **BindWeb** | Any | GNN,  Bi-LSTM,  CNN | Web:  <http://www.csbio.sjtu.edu.cn/bioinf/BindWeb/> | 36190332 |

**Supplementary Table 3: Comparison of different algal extraction methods, highlighting their principles, benefits, and limitations.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Category** | **Extraction Methods** | | **Description** |  | | --- | --- | | **Benefits** | **Limitations** |
| **Conventional Methods** | **Soxhlet Extraction** | Continuous cyclic extraction with boiling solvents. | Easy automation, effective for non-volatile compounds. | Long procedure, high energy usage, restricted to specific compounds. |
| **Hydrodistillation** | In order to extract volatile compounds, algal biomass is boiled with water. | Easy to set up, good for volatile Compounds. | Heat-sensitive compounds should not be used with this energy-intensive method. |
| **Maceration** | Consists of soaking algal biomass in a solvent at room temperature or heating it in order to extract it. | Simple to execute, requiring little equipment. | Low efficiency, time-consuming, and requiring solvent evaporation. |
| **Advanced Methods** | **Supercritical Fluid Extraction (SFE)** | Makes effective, non-toxic extraction using supercritical CO₂ (or other fluids) as the solvent. | Selective extraction that preserves bioactivity and is environmentally friendly. | Expensive to operate and needs specialist equipment. |
| **Microwave-Assisted Extraction (MAE)** | Heats the solvent and algae biomass using microwave energy to facilitate quick extraction. | Quick and energy-efficient, ideal for compounds that are sensitive to heat | If expensive equipment isn't optimized properly, it could degrade. |
| **Enzyme-Assisted Extraction (EAE)** | Releases compounds through the hydrolysis of cell walls using certain enzymes. | Eco-friendly, mild conditions, yield-boosting conditions. | Enzymes are expensive, and each variety of algae requires optimization. |
| **Aqueous Extraction** | **Hot Water Extraction** | Extracts water-soluble substances from algae biomass using hot water. | Easy, economical, and environmentally beneficial. | Restriction to hydrophilic compounds and potential for thermal deterioration. |
| **Enzymatic Extraction** | Improves the release of bioactive compounds into water by using enzymes to hydrolyze algal cell walls. | High efficiency, eco-friendly, and mild conditions. | Eco-friendly, highly efficient, and mild environments. |
| **Green Extraction Methods** | **Ionic Liquid Extraction** | Makes effective use of ionic liquids (ILs) as environmentally friendly solvents to extract bioactive compounds. | Solvents that are highly selective, recyclable, and reduce harmful waste. | Expensive, few large-scale uses, and some ILs may be hazardous. |
| **Deep Eutectic Solvent (DES) Extraction** | Uses non-toxic, biodegradable DES as solvents for ecologically sustainable extraction. | Eco-friendly, extremely flexible, and suitable for both polar and non-polar compounds. | Scalability issues and complicated solvent preparation. |
| **Non-Solvent-Based Methods** | **Hydrothermal Liquefaction** | Utilizes water at high pressure and temperature to turn wet algal biomass into bio-oil. | Biofuel may be produced efficiently without the requirement to dry biomass. | High energy usage, restricted to particular kinds of compounds. |
| **Mechanical Extraction** | Compounds can be extracted physically using techniques like grinding or crushing. | Cost-effective, appropriate for large-scale operations, and doesn't require compounds. | Low efficiency: purification after extraction might be necessary. |
| **Integrated Extraction Methods** | **Extraction with Fractionation** | Separates substances according to their chemical composition by combining fractionation and extraction. | High efficiency simultaneous extraction and purification. | Requires knowledge and advanced equipment. |
| **Cascade Extraction** | Multiple compounds can be extracted from the same biomass using sequential extraction techniques. | Reduces waste and makes the most use of available resources. | Complex procedure, extended extraction periods, and exact condition control are all necessary. |

**Supplementary Table 4: An overview of the biological activity tests that are frequently used to assess algal extracts.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Category** | **Test** | **Procedure** | **Differences** |
| **Antioxidant Assay** | DPPH Assay | Measure the compound's absorbance at 517 nm after adding DPPH solution and letting it sit in the dark. | Free radical scavenging is measured by colour change; it's easy to use and popular. |
| ABTS Assay | Produce ABTS radicals, react with the substance, and then measure the absorbance at 734 nm. | Suitable for antioxidants that are lipophilic or hydrophilic. |
| Superoxide Anion Scavenging | Assesses the scavenging of superoxide anion (O2) | Evaluates defence against the oxidative damage that superoxide causes. |
| **Anti-inflammatory Assay** | Nitric Oxide Inhibition Assay | Apply the chemical to the cells, then use the Griess reagent at 540 nm to determine the NO levels. | Indicates anti-inflammatory effectiveness by evaluating the reduction of NO generation. |
| COX Inhibition Assay | Measure the amounts of the product (e.g., PGE2) and test the substance against COX enzymes. | Useful to research on inflammation; targets cyclooxygenase pathways. |
| **Cytotoxicity Assay** | MTT Assay | Add the MTT reagent, incubate the cells with the chemical, and measure the absorbance at 570 nm. | Evaluates mitochondrial activity as a measure of the health of cells. |
| LDH Release Assay | Measure the amount of LDH released in the medium after treating cells with a chemical. | Demonstrates membrane damage and enhances the MTT test for evaluating cytotoxicity. |
| **Enzymatic Assays** | GSH-Px Activity | The activity of glutathione peroxidase is measured. | Shows how well the antioxidant defense system can detoxify peroxides. |
| Catalase Activity | Measures the catalase activity | Assesses the capacity of hydrogen peroxide to break down into oxygen and water. |