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Original Research

Targeting the Conserved Binding Pocket of Cadmium Transporters: Essential Oil-Derived Small Molecules Show Promise in Mitigating Cadmium Accumulation in Peppers

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Abstract

This research focused on the theoretical reduction of cadmium (Cd) in the above-ground parts of pepper plants. Utilising bioinformatics and transcriptome data, we identified 259 members of the heavy metal transport protein family within pepper. We then selected *CaHMA19*, *CaCAX11*, and *CaNRAMP8* as candidate genes that may influence Cd levels in pepper fruits, as indicated by RPKM/FPKM analysis and genotype clustering. Following this, we employed molecular simulation techniques to screen candidate small molecules with potential anti-heavy-metal activity. By conducting molecular docking with AutoDock software and evaluating energy scores and affinities using Discovery Studio, we identified three leading compounds, A, B, and C, and thoroughly examined their interactions with the proteins CaHMA19, CaCAX11, and CaNRAMP8. The findings indicated that these compounds were predicted to form interactions with the active sites of the target proteins, with compound A predicted to have broad-spectrum binding potential and compound C predicted to preferentially bind to CaHMA19. All findings in this study are derived from computational virtual screening and molecular docking simulations and require further experimental validation.

Keywords: discovery studio, essential oil-associated small molecules, molecular docking, inhibition effects

Introduction

Chilli peppers (*Capsicum annuum* L.) are a popular vegetable widely grown in Guizhou Province [1]. However, the rapid pace of urban development and industrial growth, along with the distinctive soil characteristics of Guizhou's karst landscape, have resulted in cadmium (Cd) pollution in the region's soil [2]. Cd, one of the most common heavy metals, can be found in soil, water, and air, and is easily taken up and stored by chilli plants [3]. Reports show that the Cd concentration in chilli fruits from certain areas in Guizhou has surpassed the national safety threshold of 0.05 mg/kg [4]. This situation poses a serious risk to both the production and consumption of local chillies, as well as to public health. Therefore, addressing

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the issue of Cd levels in chillies and improving the safety and quality of agricultural products are vital concerns for farmers in Guizhou Province and nationwide.

Pepper is notable for its ability to accumulate Cd, demonstrating a strong capacity for enrichment [5]. This research centers on Zunla No. 1, exploring its genomic and transcriptomic data while thoroughly examining the heavy metal transporters in pepper. The identified transporters include Ca^{2+}/H^{+} exchanger antiporters (CAXs) [6], heavy metal transporting ATPases (HMAs) [7], natural resistance-associated macrophage proteins (NRAMPs) [8], ATP-binding cassette C subfamily transporters (ABCCs) [9], oligopeptide transporter proteins (OPTs) [10], zinc-regulated transporter proteins (ZIPs) [11], Metal tolerance proteins (MTPs) [12], ATP-binding cassette G subfamily transporters (ABCGs) [9], Plant Cd resistance proteins (PCRs) [13], and multidrug and toxic compound extrusion proteins (MATEs) [14]. These proteins are essential for managing the absorption, transport, and distribution of Cd within the plant's tissues.

Studies show that various metal transporters play distinct roles in Cd transport. Specifically, CAXs are mainly responsible for the entry and exit of Cd in both roots and leaves [15], while HMAs are involved in the absorption and buildup of Cd in root systems [16]. NRAMPs play a role in maintaining the balance of iron and manganese ions, which can affect Cd levels, particularly with a noted tendency for higher accumulation in fruits [17]. ABCCs and OPTs are chiefly involved in the transport and storage of Cd [18, 19], whereas ZIPs and MTPs manage the distribution of Cd across different plant tissues, including roots, leaves, and fruits [11, 12, 20]. Additionally, PCRs and MATEs, among other transporters, influence Cd transport and accumulation through mechanisms related to microbial activity in the rhizosphere, drug efflux, and metabolic processes [21, 22].

Compounds derived from essential oils, particularly small molecules, are known for several key traits and benefits. Reduced toxicity [23, 24]: Compared with traditional pesticides, these compounds generally exhibit lower toxicity, thereby reducing their environmental impact and protecting ecosystems. Simple synthesis [25]: many of these compounds are sourced from natural plants and spices, characterized by straightforward chemical structures that make their synthesis easier. This simplicity not only lowers production expenses but also broadens their usability. Convenient storage and transport [26]: Essential oil compounds are often either insoluble or only slightly soluble in water, which contributes to their longer shelf life and ease of handling during storage and transport. Broad usage [27, 28]: Small molecules from essential oils have found extensive applications in areas such as drug development, biopesticides, and more, showcasing their considerable potential [29].

In agriculture, various essential-oil-derived medications can also act as insect deterrents. For example, tea tree oil is rich in insecticidal agents that can effectively manage a range of agricultural pests [30, 31], including aphids, thrips, whiteflies, and mealybugs [32]. Additionally, the wide-ranging antimicrobial effects of these essential oil-based treatments make them a key area of interest in the creation of biological pesticides [33]. Recently, there has been a growing application of essential oil compounds in the cultivation of vegetables [34], fruits [35, 36], tea leaves [37], and other crops [38, 39], contributing positively to the sustainable advancement of contemporary agriculture.

This research aims to identify candidate proteins associated with Cd accumulation in peppers by focusing on heavy metal transporters uniquely found in these plants. By employing computational docking methods, we seek to screen essential oil-associated small molecules that may potentially modulate Cd transport in pepper fruits. This study may provide theoretical and practical references for exploring feasible strategies to reduce Cd content in pepper and facilitate the development of sustainable agricultural practices. It also provides a preliminary theoretical framework for selecting candidate molecules for follow-up experimental research.

Materials and Methods

Identification of Heavy Metal Transporter Families in *Capsicum Annuum* L.

This research examined the genomic data of Zunla No. 1 and discovered ten families of heavy metal transport proteins in chilli peppers through genomic annotation, BLAST, and HMM analysis [40]. The protein families identified consist of CAXs, HMAs, NRAMPs, ABCCs, OPTs, ZIPs, MTPs, ABCGs, PCRs, and MATEs. Furthermore, functional and structural evaluations of these protein families were conducted using the NCBI-CDD database [41-43], establishing a foundation for future studies.

Identification of Heavy Metal Transporter Proteins with Specific Expression Patterns at the Transcriptional Level in *Capsicum Annuum* L. During Growth Stages

By conducting transcriptomic analysis on various tissue types throughout the growth of chilli peppers, we identified candidate proteins from ten families of heavy metal transporters that are specifically expressed during the fruit enlargement phase and will serve as our protein targets. We gathered samples from peppers at various developmental stages and tissue types. The transcriptome data for the growth stages included: 1. Post-pollination (0-1 cm); 2. Fruit (1-3 cm); 3. Fruit (3-4 cm); 4. Fruit (4-5 cm); 5. Fruit greening phase; 6. Fruit coloring phase; 7. Coloring phase (3 days); 8. Color change phase (5 days); 9. Color change phase

(7 days). We utilized differential expression analysis to explore the expression trends of ten heavy metal transporters throughout these stages and tissues. As a result, we focused on transporters that showed significant upregulation during the fruit enlargement phase and downregulation in other phases as primary protein targets for future molecular docking studies involving small molecules derived from essential oils.

Screening and Identification of Essential Oil-Derived Small Molecule Compounds Targeting Key Proteins

This research aimed to screen small molecules associated with essential oils that may potentially interact with important protein targets. To achieve this, molecular docking simulations were performed using Discovery Studio software. The compound library was constructed using a two-step strategy: first, the collection of well-documented natural essential oil constituents with reported antioxidant and metal-chelating activities; second, the expansion of chemical space by including structural analogues and derivatives that share core functional skeletons with the above active ingredients. This strategy is widely adopted in virtual screening for lead compound discovery. Initially, we performed a preliminary screening of the compound library to pinpoint those with potential binding capabilities to the target proteins. The compounds that showed promise were then refined further to identify the best candidates. These selected compounds underwent additional assessments, including calculations of charge distribution, analysis of intermolecular interactions, and evaluations of binding strength. Ultimately, our computational screening revealed essential oil-associated small molecules with predicted binding affinity for the target proteins, providing candidate molecules for follow-up experimental research.

Identification of the Pepper Heavy Metal Transporter Family

Genome Annotation

The genome files for our chilli peppers were derived from the wild Mexican variety known as Chiltepin and the Chinese inbred line Zunla-1 (also referred to as Zunla No. 1). The sequences for these chilli genomes were made public in January 2014 in the Proceedings of the National Academy of Sciences (PNAS). By comparing the annotation data of the pepper genome with established bioinformatics databases, we were able to pinpoint genes and their related characteristics, including specific sequence domains, exons, and introns. Throughout the genome annotation process, we utilized a range of software tools and functional modules to achieve the highest level of accuracy in predicting and annotating the functions of pepper genes.

Family Identification Analysis Using BLAST and HMM Software

BLAST and HMM are prominent algorithms utilized for classifying gene families. While BLAST focuses on local alignments, HMM uses matching techniques instead of alignment [40]. To identify the heavy metal transporter families in pepper, including *CaCAXs*, *CaHMAs*, *CaNRAMPs*, *CaABCCs*, *CaOPTs*, *CaZIPs*, *CaMTPs*, *CaABCGs*, *CaPCRs*, and *CaMATEs*, we applied these algorithms to gather and label genomic information. HMM models were developed using established sequences of heavy metal transporter families and were then compared against the pepper genome. Simultaneously, BLAST software was employed to align known protein sequences of heavy metal transporters with the pepper genome, thereby improving the accuracy and rate of family member identification. The integration of these two algorithms facilitates a more accurate identification of heavy metal transporter families in the pepper genome.

Functional and Structural Analysis Using the NCBI-CDD Database

The NCBI-CDD [41-43] serves as an extensive platform for conserved domains, integrating various databases of conserved domains, patterns based on positional features, tools for domain assembly, and multi-color alignment. This resource aids in examining domains, protein families, their functional traits, and interactions within protein or DNA sequences, which is essential for protein classification and functional predictions. By utilizing the NCBI-CDD database, we can perform in-depth functional and structural analyses of the sequences of the pepper heavy metal transporter protein family. Such investigations will allow us to uncover the biological roles, domains, and interactions among these proteins, providing valuable insights into the mechanisms governing heavy metal transport in chilli peppers. Furthermore, throughout our research, we can employ the NCBI-CDD database for additional analyses, trace the evolutionary lineage of the heavy metal transporter family, and investigate similarities and differences with transporter families found in other organisms.

Identification of Specific Expression Patterns for Key Protein Targets

Transcriptome Data

In accordance with our research objectives, we obtained transcriptomic data from the Chiltepin variety of Mexican wild chilli pepper and the Zunla-1 inbred line from China. This dataset was made publicly available in January 2014 through the Proceedings of the National Academy of Sciences (PNAS) and can be accessed at http://peppersequence.genomics.cn/. We chose

samples representing various developmental stages and tissue types for our analysis. The transcriptomic data encompassed: 1. Post-pollination (0-1 cm); 2. Fruit (1-3 cm); 3. Fruit (3-4 cm); 4. Fruit (4-5 cm); 5. Greening stage of fruit; 6. Color change stage of fruit; 7. Color change (3 days); 8. Color change (5 days); 9. Color change (7 days). This selection of samples provided a thorough overview of gene expression variations throughout the growth and maturation of the pepper, offering valuable data for further investigations.

Gene Expression Level Analysis across Developmental Stages

Using transcriptome data, we determined gene expression levels (RPKM/FPKM) and genotype values at each developmental stage with various software applications. We performed clustering analyses on gene families such as *CaCAXs, CaHMAs, CaNRAMPs, CaABCCs, CaOPTs, CaZIPs, CaMTPs, CaABCGs, CaPCRs, and CaMATEs* using TBtools. These analyses revealed changes in the expression of heavy metal transport genes during different reproductive phases and lay the groundwork for future differential gene expression analysis.

Differential Gene Screening

Through an analysis of gene expression at various developmental phases, we identified a set of significantly upregulated genes among 10 different families of heavy metal transporters, which include over 200 genes. These genes, which exhibit differential expression, are likely vital for the transport and accumulation of heavy metals under stress. Our focus was on the expression of genes related to Cd transport during the fruit enlargement phase, and we found notable upregulation at this specific stage, contrasting with significant downregulation observed in other phases. The comparison of expression levels across different stages indicates that these genes are essential for Cd accumulation and concentration in the fruit. Therefore, we propose these genes as potential key targets for protein studies.

Screening and Identification of Essential Oil-Derived Small Molecule Compounds

Acquisition and Computational Modeling of Three-Dimensional Structures of Small Molecules and Target Proteins

Gather structural data for essential oil small molecules from appropriate databases or scientific literature. For compounds that are already identified, retrieve the relevant SDF files from platforms like PubChem or ChemSpider. For newly discovered compounds, utilize molecular modelling tools such as Discovery Studio or Avogadro to predict and refine their structures. At the same time, acquire the three-dimensional structure file (in PDB format) for the target protein. Pre-existing three-dimensional structure files can be sourced from repositories like RCSB PDB or PDBj. If the protein's structure has not been resolved, bioinformatics techniques like Rosetta or SWISS-MODEL can be employed for modelling. Among these, RoseTTAFold stands out as a widely used, effective, and precise method for predicting protein structures [44, 45].

Ligand Optimization and Protein Preparation

For downloaded small molecules derived from essential oils, molecular simulation tools (such as Discovery Studio, AutoDock, and Glide) can be utilized for optimization and preprocessing. Within Discovery Studio, the Ligand Preparation feature allows for processes like deionization, hydrogenation, and modifications to increase the hydrophilicity of ligands, thereby improving their biological efficacy and reliability. At the same time, the Protein Preparation feature can be used to prepare proteins by executing tasks such as eliminating water molecules, incorporating hydrogen atoms, and rectifying structural issues.

Evaluation and Validation of Molecular Docking between Essential Oil Small Molecules and Target Proteins

A molecular docking assessment was conducted using software for drug-molecule interactions, focusing on refined small molecules from essential oils and their target proteins. The target protein was repaired and assigned charges via Prepare Protein, and Cavity Search was used to predict the binding pocket. The probe radii for C and H were set to 2.5 Å and 2.0 Å, respectively, with a grid spacing of 0.5 Å, and the docking sphere radius was set to 9 Å. Essential oil small molecules were preprocessed using Prepare Ligands. For LibDock docking, the hotspot count was 100, FAST search mode was adopted, a maximum of 10 conformations were output, the clustering radius was 0.5 Å, and the receptor was set as rigid. Re-docking could not be performed due to the absence of co-crystallized ligands. Reliability was comprehensively evaluated through CDOCKER refined docking, MM-GBSA free energy, and non-covalent interactions. These results represent only theoretical screening and require further experimental validation.

Distribution of Charge Potential and Evaluation of Intermolecular Interactions and Strengths

For the essential oil compounds that have undergone initial screening, assessments and comparisons are carried out through techniques such as calculations of charge distribution and evaluations of intermolecular interaction strengths. Analyzing these factors uncovers the molecular structural characteristics, stability, and interaction patterns with biological targets, which are vital for guiding future drug development.

Following this, pharmacological, toxicological, and pharmacokinetic analyses are conducted to assess their efficacy and safety in both in vitro and in vivo settings. The findings from these analyses help identify the most promising small molecules derived from essential oils, laying the groundwork for future validation studies. Molecular simulation software, including Discovery Studio, GROMACS, and AMBER, can be utilized to determine molecular mechanical properties (such as charge distribution and bond energy) and intermolecular interactions and to conduct molecular dynamics simulations. Additionally, bioinformatics resources like BLAST, GeneMANIA, and STRING can be used to forecast molecular biological functions and pathways.

Results

Identification of Pepper Heavy Metal Transporter Families

By utilizing genomic data from pepper, we conducted an analysis employing BLAST and HMM algorithms, which led to the successful identification of 259 members across various heavy metal transporter families. Specifically, the *CaOPTs* family revealed 20 OPT proteins known for their efficient ion transport and selectivity; the *CaCAXs* family uncovered 14 CAX proteins that are integral to the transport and storage of heavy metal ions; the *CaPCRs* family identified 12 PCR proteins essential for ion and proton transport; the *CaNRAMPs* family found 8 NRAMP membrane proteins that facilitate ion transport through cell membranes; the *CaZIPs* family included 12 members adept at transporting ions and substances such as glucose from the extracellular environment into the cell; the *CaMATEs* family identified 70 MATE proteins, which are vital for the transport and metabolism of amphetamine-like substances; the *CaHMAs* family revealed 51 HMA membrane proteins that play a role in the efflux and distribution of heavy metal ions; the *CaABCs* family included 23 ABCC and 44 ABCG members, which are significant for ion transport, metabolism, and drug excretion; finally, the *CaMTPs* family identified 5 MTP proteins involved in the transport and storage of ions in peppers. We also organized these proteins according to their chromosomal arrangement.

Potential Protein Targets Affecting Cd Accumulation in Pepper Fruits

This research focused on examining gene expression in chilli peppers throughout the stages from pollination to maturity by utilizing transcriptomic data. We specifically looked for heavy metal transporter genes that were significantly upregulated. To validate our findings, we employed RPKM/FPKM metrics and conducted clustering analysis based on genotype values.

During the stage of fruit growth, we observed that the expression levels of *CaHMA19*, *CaCAX11*, and *CaNRAMP8* in peppers initially increased before declining (Fig. 1), a pattern specifically triggered during this growth phase. These genes are likely involved in the transport and accumulation of heavy metals in the fruit, making them potential protein targets that could affect Cd levels in pepper fruits. CaHMA19 is a member of the HMA membrane protein family, which has been shown to facilitate the transport and efflux of copper (Cu) and Cd ions in various plant species. Meanwhile, CaCAX11 and CaNRAMP8 are associated with the CAX and NRAMP membrane protein families, respectively, and previous research has indicated their roles in the transport and efflux of heavy metal ions in plants. Additionally, we confirmed the similarities and differences in the expression patterns of *CaHMA19*, *CaCAX11*, and *CaNRAMP8* across different pepper genotypes using genotype-value clustering analysis.

Screening and Identification of Essential Oil-Related Small Molecules

Utilizing bioinformatics techniques, this research acquired the amino acid sequences for the CaHMA19, CaCAX11, and CaNRAMP8 proteins found in chilli peppers. The three-dimensional structures were modeled with RoseTTAFold (Fig. 2a). We employed PyMOL software to visualize and examine the 3D configurations of these proteins, ensuring their structural correctness and integrity. Ultimately, we exported the PDB files for each protein to support future molecular docking studies.

To determine similarities and differences among these proteins, we performed topological superposition analysis of the chilli pepper CaHMA19, CaCAX11, and CaNRAMP8 proteins using PyMOL software (Fig. 2b). The analysis showed distinct structural differences among the three proteins, while their active site amino acid sequences and spatial arrangements were notably similar. This similarity provides a structural basis for the identification of a common binding pocket.

The active regions of CaHMA19, CaCAX11, and CaNRAMP8 were identified by examining pertinent studies and analyzing protein structures and sequences. Various elements were taken into account, including the positioning of amino acid residues, their chemical characteristics, and their interactions. This analysis resulted in the identification of the second pocket (site 2) as the candidate active site for the proteins being compared (Fig. 3).

A total of 35,263 candidate small-molecule essential oil-associated compounds and their structural analogs with potential anti-heavy-metal activity were retrieved from the PubChem database. The library was constructed using a two-step strategy as described in Materials and Methods, including both natural essential oil constituents and their structural analogs sharing core active skeletons. Utilizing their PubChem CID numbers,

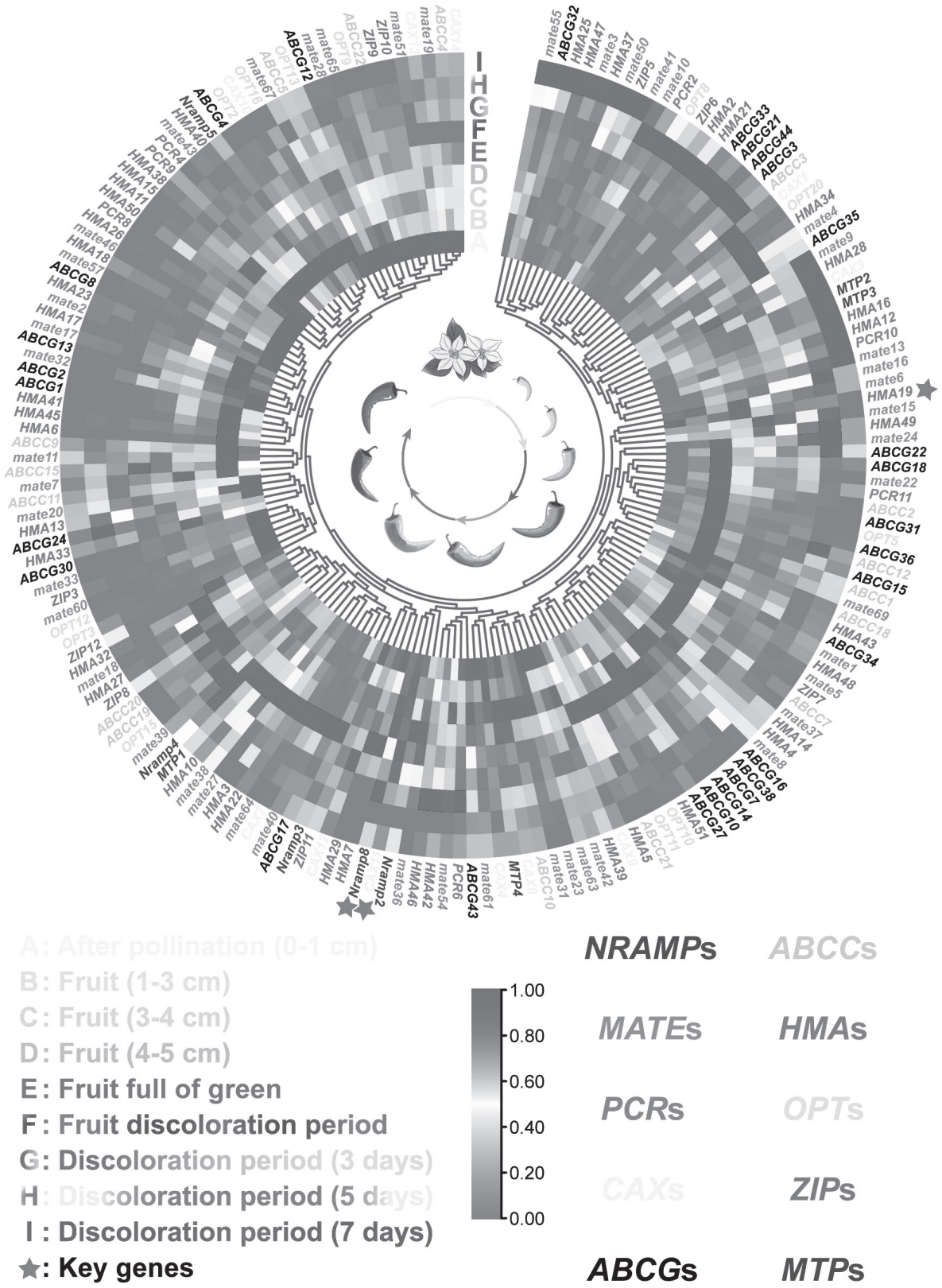


Fig. 1. Clustering of expression levels of heavy metal transport genes at 9 fruit developmental stages after pollination.
Note: *HMAs* (Heavy Metal ATPases): Heavy metal ATPase family; *CAXs* (Cation/H^+ Exchangers): Cation/hydrogen exchanger family; *NRAMPs* (Natural Resistance-Associated Macrophage Proteins): Natural resistance-associated macrophage protein family; *ZIPs* (Zrt/Irt-like Proteins): Zinc-regulated transporter/iron-regulated transporter-like protein family; *ABCCs* (ATP-Binding Cassette subfamily C): ATP-binding cassette transporter subfamily C; *ABCGs* (ATP-Binding Cassette subfamily G): ATP-binding cassette transporter subfamily G; *MATEs* (Multidrug and Toxic Compound Extrusion proteins): Multidrug and toxic compound extrusion protein family; *PCRs* (Plant Cadmium Resistance proteins): Plant Cd resistance protein family; *OPTs* (Oligopeptide Transporters): Oligopeptide transporter family; *MTPs* (Metal Tolerance Proteins): Metal tolerance protein family.

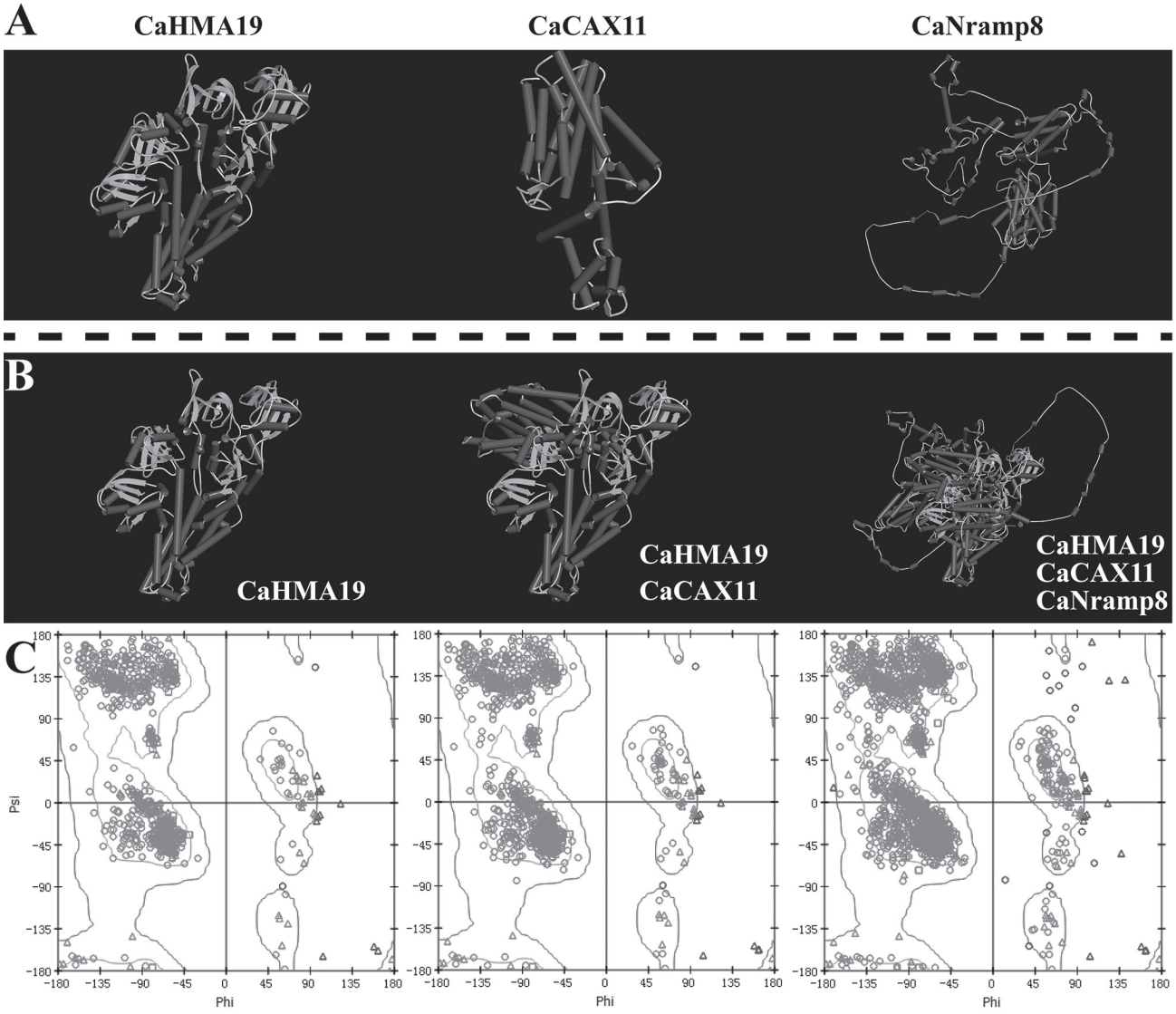


Fig. 2. 3D structure and superposition analysis of CaHMA19, CaCAX11, and CaNRAMP8 proteins in chilli pepper. a) Schematic diagram of the three-dimensional structure of the three proteins. The red cylinder represents the α-helix, the cyan band represents the β-fold, and the white line represents the random curl and ring region. b) Protein structure topological superposition analysis. The left panel shows the reference structure of CaHMA19, the middle panel shows the superimposed structure of CaHMA19 and CaCAX11, and the right panel shows the common superimposed structure of the three proteins. c) Ramachandran plots for three protein structure models. The horizontal axis is the Phi dihedral angle, and the vertical axis is the Psi dihedral angle. The blue and purple contours represent the most favorable and permissible conformational regions, respectively. Green circles are residues in reasonable regions and red triangles are residues in unreasonable regions.

we gathered further pertinent details, which included 17,064 ketones, 4,747 carboxylic acids, 12,824 alcohols, 57 terpenoids, and 571 esters. This classification is based on the main functional groups annotated by PubChem, not on natural source attribution. We then processed these compounds with Discovery Studio software, performing tasks such as energy minimization, charge balancing, and constrained conformation analysis. Following computational modeling via LibDock, we discovered 6,282 small molecules (including isomers) that were predicted to interact with the target proteins. The leading 10 compounds were chosen based on their docking scores (Table 1; Fig. 4).

Molecular docking simulations were conducted with AutoDock software to assess interactions between small molecules derived from essential oils and the proteins CaHMA19, CaCAX11, and CaNRAMP8, focusing on the energy scores and binding affinities of each complex. Subsequently, we utilized PyMOL and Discovery Studio to visualize the three best-performing complexes after docking (Fig. 5) for a detailed examination of the interactions between the compounds and proteins.

According to structural evaluations, the three identified compounds are: (1) [(2R,3R,4R,5R,6R)-3-hydroxy-4,5-bis(phenylmethoxy)-6-(phenylmethoxymethyl)oxan-2-yl]-phenylmethanone

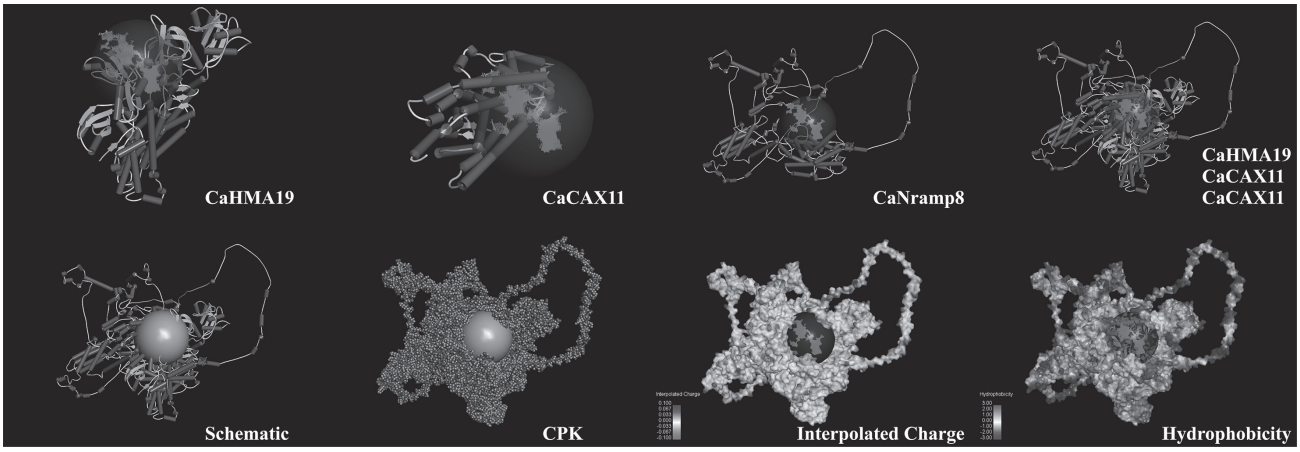


Fig. 3. Combined pocket (active site) definitions (CaHMA19, CaCAX11, and CaNRAMP8 proteins separately and in combination).

(PubChem CID: 10962558); (2) bis[1-(1H-indol-2-ylmethyl)-2-piperazin-1-ylpiperidin-4-yl]methanone (PubChem CID: 129870149); and (3) (3S)-3-(4-nitrophenyl)-1-phenyl-3-(phenylmethoxyamino)propan-1-one (PubChem CID: 101110084). Comprehensive details can be found in Fig. 6.

In order to thoroughly assess and contrast the predicted binding affinity of the various complexes, we employed Discovery Studio software to analyze and compare factors such as charge distribution, intermolecular interactions, and their respective strengths. This analysis included hydrogen bonds, hydrophobic interactions, van der Waals forces, and ionic bonds, which indicate the interaction potential between compounds and proteins, as well as the stability of the complexes.

After completing the aforementioned screening and assessment, we identified the three leading compounds, designated as A, B, and C, based on their evaluation scores. A thorough investigation was carried out to examine how these compounds interact with the proteins CaHMA19, CaCAX11, and CaNRAMP8. The findings reveal that these compounds are capable of establishing stable complexes with the active sites of the target proteins, although differences were noted among the various proteins.

Compound A (ID: 10962558) established hydrogen bonds and hydrophobic interactions with the active sites of the CaHMA19, CaCAX11, and CaNRAMP8 proteins, suggesting its potential broad-spectrum binding activity. In contrast, Compound B (ID: 129870149) was predicted to form hydrogen bonds and van der Waals interactions

Table 1. Basic information on the top 10 candidate small molecules.

PubChem CID	IUPAC Name	Chemical Formula	Molecular Weight (g/mol)	Match Ranking
10962558	[(2R,3R,4R,5R,6R)-3-hydroxy-4,5-bis(phenylmethoxy)-6-(phenylmethoxymethyl)oxan-2-yl]-phenylmethanone	$C_{34}H_{34}O_6$	538.6	1
129870149	bis[1-(1H-indol-2-ylmethyl)-2-piperazin-1-ylpiperidin-4-yl] methanone	$C_{37}H_{50}N_8O$	622.8	2
101110084	(3S)-3-(4-nitrophenyl)-1-phenyl-3-(phenylmethoxyamino)propan-1-one	$C_{22}H_{20}N_2O_4$	376.4	3
101540215	4,5-dimethylidene-1-naphthalen-2-yl-8-phenyl-3-(2-phenylethyl) octan-1-one	$C_{34}H_{34}O$	458.6	4
101189086	(3S)-3-(benzhydryloxyamino)-3-(2-chlorophenyl)-1-phenylpropan-1-one	$C_{28}H_{24}ClNO_2$	441.9	5
101540217	1-(furan-2-yl)-4,5-dimethylidene-8-phenyl-3-(2-phenylethyl)octan-1-one	$C_{28}H_{30}O_2$	398.5	6
70139661	1-(1-benzofuran-2-yl)-3-(1-benzylpiperidin-4-yl)prop-2-en-1-one	$C_{23}H_{23}NO_2$	345.4	7
10472370	1-[(2S,3S)-2,3-bis(phenylmethoxymethyl)aziridin-1-yl]propan-1-one	$C_{21}H_{25}NO_3$	339.4	8
69951057	1-(1,3-benzothiazol-2-yl)-3-(1-benzylpiperidin-4-yl)prop-2-en-1-one	$C_{22}H_{22}N_2OS$	362.5	9
101108326	[(3S,4R)-1,4-diphenylpyrrolidin-3-yl]-pyrimidin-2-ylmethanone	$C_{21}H_{19}N_3O$	329.4	10

Notes: Compounds are ranked by LibDock docking score. The screening library includes natural essential oil constituents and their structural analogs.

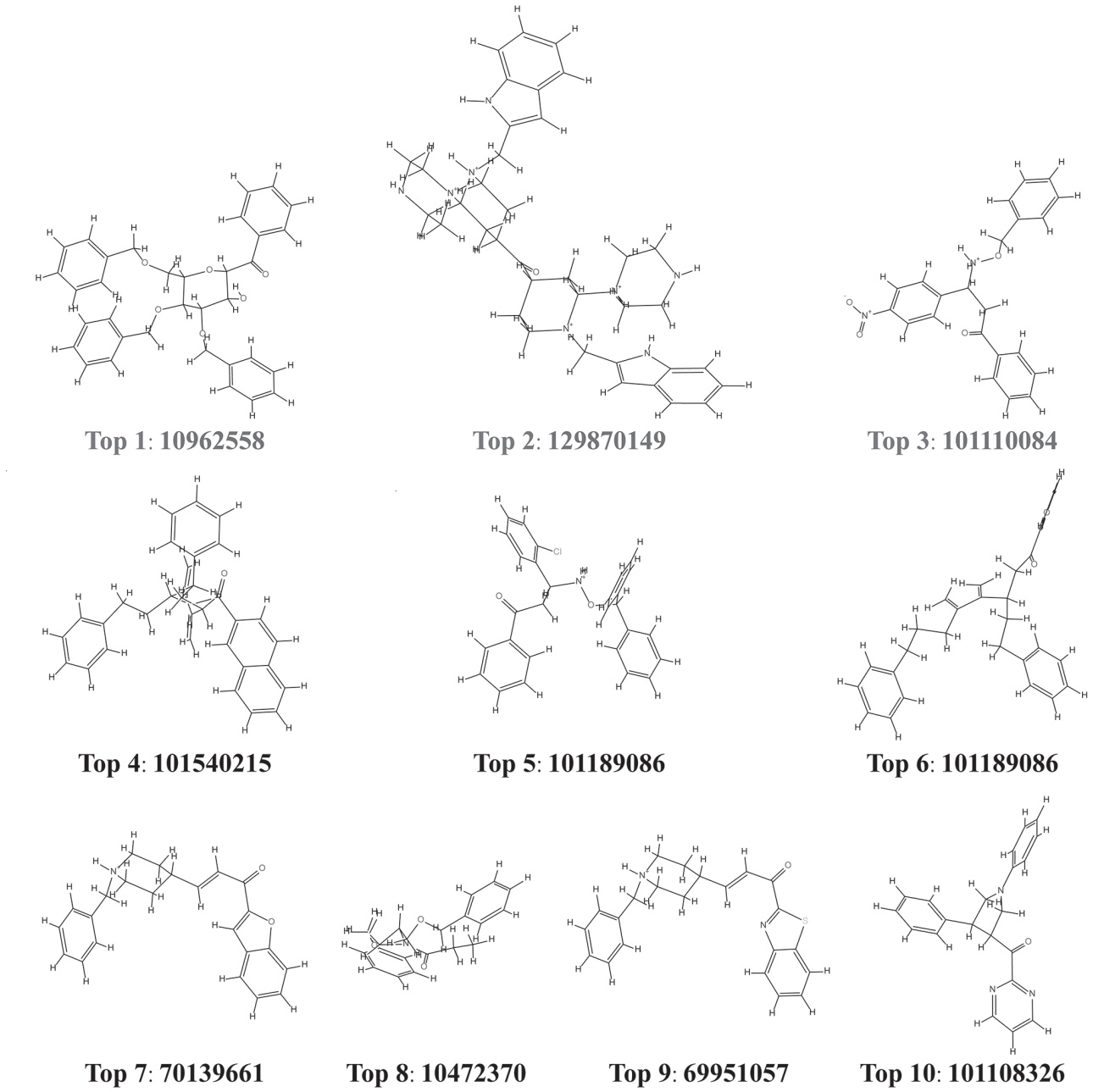


Fig. 4. Structural characteristics of the top 10 small-molecule compounds of optimal essential oils.

with the active sites of CaHMA19 and CaCAX11, while its interactions with CaNRAMP8 were comparatively weaker. Meanwhile, Compound C (ID: 101110084) was predicted to form hydrogen bonds and van der Waals interactions with all three target proteins, with the most significant interaction occurring with CaHMA19, suggesting potential preferential binding to HMA19.

Our findings from molecular docking, computational evaluations, and interaction assessments suggest that compounds A, B, and C may contribute to the tolerance of chilli peppers against heavy metal toxicity. These compounds show certain application potential in practical scenarios and may serve as candidate additives for pesticides or fertilizers, with preliminary prospects for agricultural application.

In conclusion, this study investigated potential protein targets in chilli peppers and performed molecular docking and interaction assessment of essential oil-derived small molecules, enabling the preliminary identification of several candidate compounds with heavy metal toxicity alleviation activity. We also provided insights into their interaction patterns and potential mechanisms with target proteins. These results may offer a preliminary theoretical basis and experimental reference for advancing research on chilli metabolic processes, as well as the development and application of resources against heavy metal toxicity.

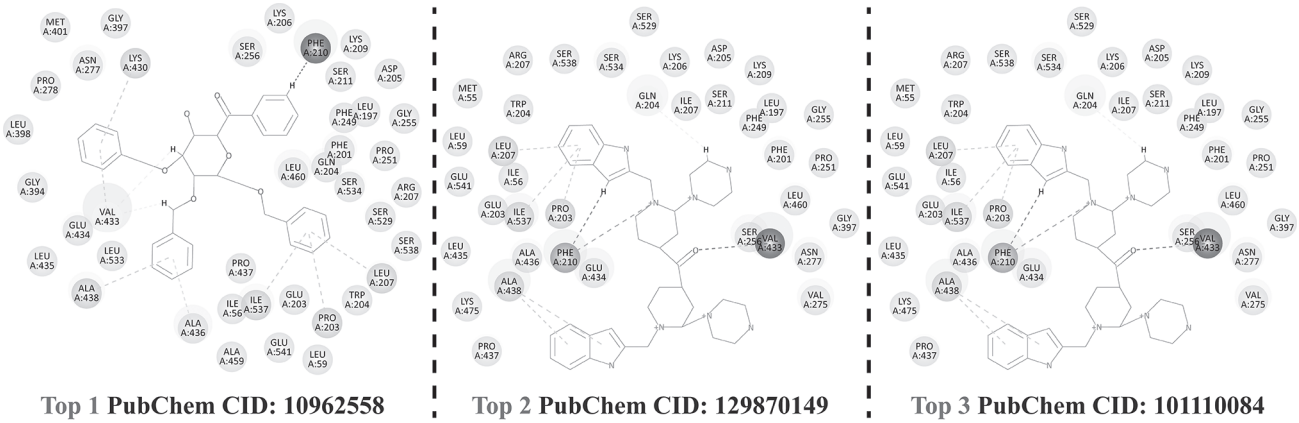


Fig. 5. Visual analysis of molecular docking of small-molecule compounds of optimized essential oils.
Notes: Green dashed lines indicate conventional hydrogen bonds; red dashed lines indicate strong hydrogen bonds; yellow dashed lines indicate π-alkyl interactions; pink-shaded residues indicate hydrophobic interactions. All three compounds bind to the same conserved pocket, with PHE210/213, ALA438, SER255, and VAL433 serving as common key interacting residues.

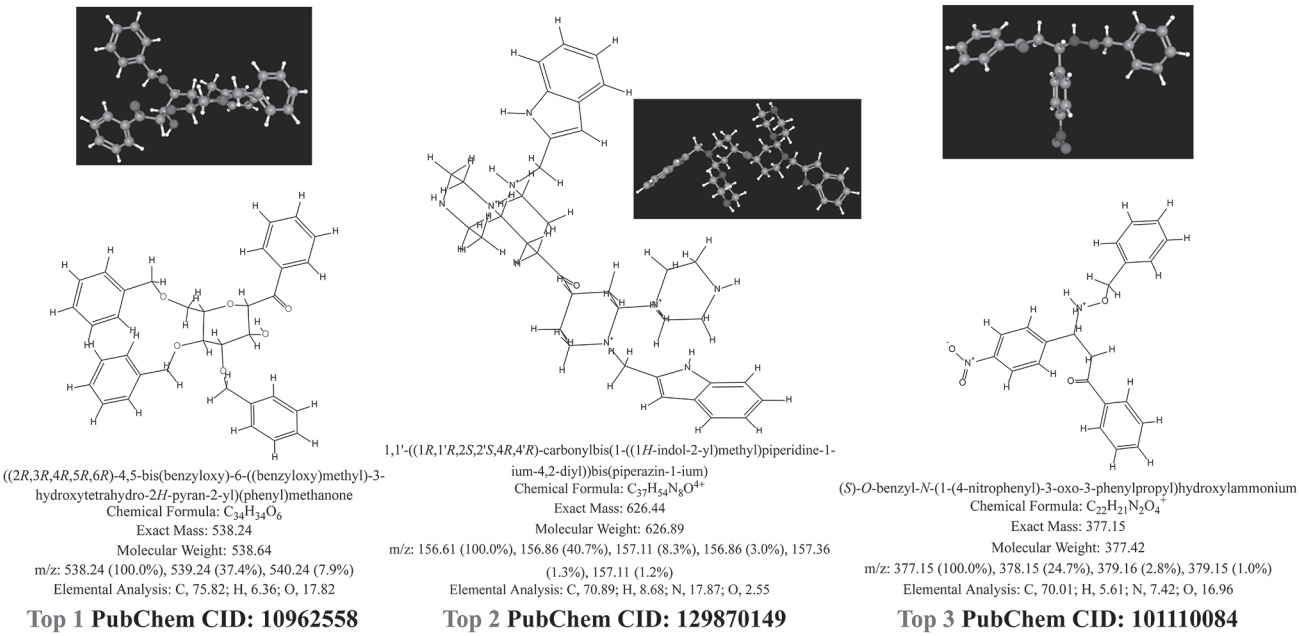


Fig. 6. Detailed information and 3D topology of optimized compounds

Discussion

Essential Oil Small Molecules Based on the Common Binding Pocket of Three Cd Transport Protein Complexes

This research utilizes a molecular screening strategy focused on the common binding pocket of candidate Cd-accumulating proteins CaHMA19, CaCAX11, and CaNRAMP8 in peppers, marking a shift away from traditional models that concentrate on individual protein targets.

Conventional studies aimed at creating regulatory molecules for plant heavy metal transporters have mainly concentrated on the roles and specific interactions of individual proteins [46, 47]. However, why do these investigations frequently produce limited practical outcomes? It is suggested that this limitation arises because the uptake, transport, and compartmentalization of Cd ions in plants depend on the collaborative function of various protein families, such as HMA [48], CAX [49], and NRAMP [50]. Focusing on the regulation of a single protein is inadequate for effectively hindering Cd accumulation pathways. This research utilized three-dimensional protein structure superposition analysis, revealing significant similarities in both amino acid sequences and spatial arrangements within the active sites of the three proteins. This finding provides a structural basis for the development of molecules targeting a shared binding pocket. Compound A, identified through this approach, was predicted to form hydrogen bonds and hydrophobic interactions

with the active sites of all three proteins simultaneously. This outcome suggests that multi-target regulation has certain research potential and may provide new research ideas for dealing with the challenges faced by single-target regulation.

Investigation of the Targeting Characteristics of Essential Oil-Derived Small Molecules and their Association with Protein Structures

The candidate compounds screened in this research exhibit predicted characteristics of both broad-spectrum binding and selective affinity. A thorough investigation into the mechanisms driving this occurrence is warranted.

Compound A was predicted to interact with three proteins, CaHMA19, CaCAX11, and CaNRAMP8, at the same time. In contrast, compound C showed a predicted stronger binding affinity for CaHMA19 than for the other two proteins. Could this phenomenon be linked to the structural variations among the proteins? Prior research indicates that during the evolution of plant heavy metal transport protein families, there has been a notable balance between the preservation of essential functional domains and structural variations driven by the need for species adaptability [51]. The pepper CaHMA19 protein features distinct structural characteristics in its transmembrane helices and metal-binding pockets, which may account for the preferential binding of compound C [52, 53]. This finding not only provides preliminary insights into the selective interactions of small-molecule compounds but may also serve as a foundational reference for future targeted molecular modifications based on protein structure.

Analysis of the Application Value of Essential Oil-Derived Small Molecules in the Prevention and Control of Agricultural Heavy Metal Pollution

Essential oil-derived small molecules are notable for their low toxicity, straightforward synthesis, and eco-friendliness, making them particularly advantageous for applications aimed at mitigating heavy metal pollution in agriculture [54].

Small molecules derived from plants have been reported to be associated with the regulation of Cd in crops by influencing the function of transport proteins [55]. This investigation screened candidate small molecules predicted to target multiple Cd transport proteins simultaneously. However, the actual effects of these compounds on Cd accumulation require verification through further experimental studies. Nonetheless, there is a lack of studies focusing on compounds that can target the three main transport proteins: HMA, CAX, and NRAMP simultaneously. This investigation identifies essential oil small molecules that can act on various stages, including the absorption, transport, and compartmentalization of Cd ions. Relative to single-target regulatory agents, these compounds may act through a broader range of functional pathways. Given the natural attributes of essential oils, their application in agricultural settings may help alleviate the ecological harm associated with chemical agents while supporting the reduction of Cd concentrations in chilli fruits, thus providing a potential strategy for safeguarding agricultural product safety.

Study Limitations

The conclusions of this study are constrained by its exclusively computational design: no experimental validation, transporter activity assays, physiological Cd measurements, or field trials were performed, and molecular dynamics simulations were not conducted to evaluate complex stability. The modeled protein structures may also differ from in vivo conformations. With these limitations understood, the screening results presented here may serve as a preliminary computational reference for future experimental work on Cd transporter-targeting small molecules.

Conclusions

This study targeted the common binding pocket of the complex of key proteins CaHMA19, CaCAX11, and CaNRAMP8 predicted to be involved in Cd accumulation in peppers. Computational analyses indicated that these compounds were predicted to form interactions with the three core transport proteins simultaneously. Among them, compound A was predicted to have broad-spectrum binding activity, and compound C showed predicted CaHMA19-targeted selectivity. Based on evolutionary analysis, the structural conservation of transport proteins and species-specific differentiation may jointly determine the binding characteristics of small-molecule compounds. This may offer a preliminary theoretical perspective for understanding the regulatory mechanisms of plant metalloid transport and the actual application effects of these compounds. The actual Cd-regulating effects of the candidate compounds need to be verified by further biological experiments, including transporter activity assays, physiological measurements, and greenhouse or field validation.

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AI Usage Disclosure Statement

This article only uses artificial intelligence to complete the text polishing, and the rest of the content is original, without AI-assisted creation.

Conflict of Interest

The authors declare no conflict of interest.

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