# Journal of Integrated

## OMICS

a methodological journal

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## JIOMICS

#### Journal of Integrated OMICS

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Journal of Integrated OMICS, JIOMICS, provides a forum for the publication of original research papers, preliminary communications, technical notes and critical reviews in all branches of pure and applied "-omics", such as genomics, proteomics, lipidomics, metabolomics or metallomics. The manuscripts must address methodological development. Contributions are evaluated based on established guidelines, including the fundamental nature of the study, scientific novelty, and substantial improvement or advantage over existing technology or method. Original research papers on fundamental studies, and novel sensor and instrumentation development, are especially encouraged. It is expected that improvements will also be demonstrated within the context of (or with regard to) a specific biological question; ability to promote the analysis of molecular mechanisms is of particular interest. Novel or improved applications in areas such as clinical, medicinal and biological chemistry, environmental analysis, pharmacology and materials science and engineering are welcome.

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# Journal of integrated OMICS

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### EDITORIAL



JOURNAL OF INTEGRATED OMICS

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### The Advancement of Omics in Forensics Science: Unveiling Real and Current Cases

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In recent years, forensic science has undergone a profound transformation, driven by the integration of **omics sciences** - genomics, proteomics, metabolomics, transcriptomics, and other molecular approaches into the analysis of biological evidence. These advanced technologies are enabling unprecedented precision in identifying individuals, uncovering the circumstances surrounding deaths, and solving complex crimes that were once deemed unsolvable.

The growing application of omics analyses in forensic investigations is reshaping both the justice system and scientific understanding of biological traits. Real-world cases have demonstrated that omics can help to both incriminate the guilty and exonerate the innocent, illustrating its transformative potential.

#### What Are Omics Sciences?

Omics sciences involve the comprehensive study of biological systems at various molecular levels. **Genomics** focuses on the complete set of genes within an organism, while **proteomics** analyzes the full complement of proteins expressed in a biological sample.

**Metabolomics** and **transcriptomics** examine metabolites and RNA transcripts, respectively. By providing a multidimensional view of biological processes, these fields offer invaluable tools in forensic applications, where conventional methods may be limited or inconclusive.

Forensic omics can generate crucial data from tiny, degraded, or complex biological samples that are often present in crime scenes. For instance, where traditional DNA typing might fail due to sample degradation, newer omics approaches can reveal critical information through **mitochondrial DNA** (mtDNA) or **proteomic profiles**, even from bones or hair that have been damaged or aged [1].

### Omics in Action: Real Cases of Justice and Revelation

Several high-profile cases around the world have demonstrated the power of omics in forensic science, both in bringing criminals to justice and in protecting the innocent.

### 1. The Golden State Killer: Solving a Cold Case with Genomic Genealogy

Perhaps one of the most famous cases showcasing the impact of omics in forensics is that of the **Golden State Killer**, a serial rapist and murderer active in California in the 1970s and 1980s. For decades, investigators were stymied by the lack of leads, even though DNA evidence had been collected from multiple crime scenes. The breakthrough came in 2018, when forensic scientists used **genomic genealogy**, an omics-based technique that compares crime scene DNA with publicly available genetic profiles from ancestry databases. By tracing familial matches through distant relatives, authorities were able to narrow down the search to **Joseph James DeAngelo**, who was subsequently arrested and convicted of multiple murders and assaults [2].

This case exemplifies the intersection of genomics and forensic genealogy, where millions of DNA profiles in

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ancestry databases can serve as powerful tools for solving crimes. However, it also raises ethical concerns about privacy and consent, given that relatives of suspects often do not consent to their data being used in criminal investigations.

### 2. Exoneration through DNA: The Case of Kirk Bloodsworth

On the other side of justice, omics technologies have also been instrumental in **exonerating the wrongfully convicted**. One of the first people to be freed from death row through DNA evidence was **Kirk Bloodsworth**, a former U.S. Marine who had been convicted of the 1984 rape and murder of a young girl. After serving nearly nine years in prison, including two on death row, Bloodsworth was exonerated when DNA testing - performed using samples that had been preserved - proved that he was not the perpetrator [3,4,5].

This case highlights the vital role of omics, specifically forensic DNA testing, in ensuring justice for the wrongly accused. It underscores the need for constant review and application of advanced omics methods in cases where evidence is preserved but initial forensic techniques were insufficient.

### 3. Identifying the Unknown: Proteomics in Historical Cases

In some historical cases, omics approaches like **proteomics** and **metabolomics** are becoming essential tools in identifying human remains. A recent case in the United Kingdom involved the use of **forensic proteomics** to analyze proteins from ancient skeletal remains. By studying the unique protein profiles in the bones, forensic scientists were able to provide key information about the deceased's identity, including age, sex, and even dietary habits, helping to resolve historical mysteries that had baffled investigators for decades [6].

This type of analysis is proving especially valuable in archaeological forensics and cold cases involving skeletonized remains, where traditional DNA extraction may no longer be possible. Proteomics can offer critical insights into post-mortem changes and degradation, supporting both modern and historical forensics.

### 4. The Death of Alexander Litvinenko: Metabolomics in Action

The high-profile case of **Alexander Litvinenko**, a former Russian spy who was poisoned in London in 2006, demonstrated the power of metabolomics in forensic toxicology. Litvinenko was poisoned with **polonium-210**, a radioactive isotope that is extremely difficult to detect using standard toxicological methods. However, forensic scientists were able to use a combination of metabolomics and radiological analyses to trace the rare isotope in Litvinenko's body and link the assassination to Russian operatives [7].

This case showcases how metabolomics can be employed to detect poisons, drugs, and other toxic substances that are either present in minuscule quantities or metabolized rapidly, eluding conventional testing. By analyzing metabolic changes and breakdown products, metabolomics offers a new dimension of forensic investigation, crucial for cases of poisoning or overdose.

### Beyond DNA: Expanding the Forensic Toolkit with Proteomics and Metabolomics

While **DNA analysis** has been the gold standard in forensic science for the last few decades, **proteomics** and **metabolomics** are rapidly emerging as complementary techniques that expand the capabilities of forensic investigators. **Proteomics**, which studies the structure and function of proteins, is especially useful in identifying individuals from bone, hair, or other tissues that may no longer contain viable DNA. For instance, the analysis of keratin proteins from hair samples can provide clues about an individual's identity or history, such as exposure to certain chemicals or environmental factors [8].

Similarly, **metabolomics** - the study of small molecules, or metabolites, within a biological system - has found a niche in forensic toxicology and drug testing. In cases involving complex drug overdoses or poisonings, where traditional toxicology might fail to identify all substances, metabolomics can detect the subtle metabolic changes that result from drug or toxin exposure, helping to pinpoint the cause of death with higher accuracy [9,10].

### Ethical Challenges and the Future of Omics in Forensics

Despite the immense potential of omics technologies in forensics, several challenges remain. One of the primary issues is the **complexity of data interpretation**. Omics analyses generate vast amounts of data, and interpreting this data in a legally sound manner can be challenging. Forensic omics results must not only be scientifically accurate but also clear enough to be understood in courtrooms by judges and juries who may lack a technical background.

Moreover, the **ethical implications** of using large genetic databases in criminal investigations cannot be ignored. The use of public genealogical data, as seen in the Golden State Killer case, raises concerns about **privacy** and **consent**. While

the utility of these databases in solving crimes is undeniable, regulators and society at large must balance this with individuals' rights to privacy and protection from misuse of their genetic information [2].

#### Conclusion

#### **Omics at the Forefront of Forensic Science**

The integration of omics sciences - ranging from genomics to proteomics and metabolomics - into forensic science is revolutionizing the way we investigate crimes and deliver justice. From identifying individuals and uncovering hidden toxic substances to solving decades - old cold cases and exonerating the wrongfully accused, these technologies are pushing the boundaries of what is possible. However, as forensic omics continues to evolve, ethical and interpretative challenges must be carefully navigated to ensure that these powerful tools are used responsibly and effectively.

As the field advances, omics will likely become an indispensable component of forensic science, offering new insights and greater accuracy in the pursuit of truth. With ongoing technological innovations and interdisciplinary collaborations, forensic omics has the potential to solve some of the most challenging questions facing the justice system today.

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### **ORIGINAL ARTICLES**



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# Computational Screening of Natural Compounds as Antiviral Candidates Targeting the SARS-CoV-2 Main Protease

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ABSTRACT

The onset of the global pandemic caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in Wuhan in December 2019 has led to an urgent need for effective preventive and therapeutic solutions. Among the various approaches explored, natural products have shown potential in the fight against COVID-19. This study employed computational techniques to screen and evaluate six natural antiviral compounds for their effectiveness against the SARS-CoV-2 Main Protease (M<sup>pro</sup>). By using molecular docking simulations, the interactions between these natural compounds and the target proteins were predicted and analyzed, focusing on factors such as binding affinity, interaction patterns, and structural compatibility within the active sites. The analysis indicated that Cladosin C and Rhodatin formed the most stable interactions with M<sup>pro</sup>, engaging with several critical residues. Cannabidiol, Capsaicin, and Kappa-Carrageenan also demonstrated promising interactions, though with some variability. On the other hand, Astaxanthin exhibited the least stable binding, suggesting limited antiviral potential. This research provides insights into the possible roles of these natural compounds as antagonists of the SARS-CoV-2 M<sup>pro</sup> enzyme. Further in vitro and in vivo studies are necessary to confirm the antiviral properties of these compounds, and future research should investigate their broader antiviral applications.

Keywords: SARS-CoV-2, COVID-19, Main protease, Biomolecules, Molecular dynamics.

#### Introduction

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS -CoV-2), virus's ability to infect both humans and various mammalian species by exploiting different angiotensinconverting enzyme 2 (ACE2) membrane proteins has underscored its significant impact [1-3]. In response, substantial resources have been dedicated to developing vaccines and therapeutic treatments. This has resulted in the rapid development of several effective vaccines and the advancement of numerous potential treatments through clinical trials. These therapies primarily target viral proteins, including key viral enzymes, or host proteins crucial for viral entry, reflecting a dual approach to combating the virus [4].

Promising strategies including natural products have been involved in the fight against COVID-19 due to their diverse bioactive compounds derived from medicinal plants, animals, and marine sources. This study employs computational methods to screen and evaluate natural antiviral compounds for their effectiveness against the SARS-CoV-2 Main Protease (M<sup>pro</sup>) [5, 6]. By performing molecular docking simulations, the study aims to predict and analyze the interactions between these compounds and the viral target proteins. Additionally, an ADEMET (absorption, distribution, metabolism, excretion, toxicity) report will be used to assess the viability of these compounds. The findings from this research are expected to offer valuable insights and potential leads for future experimental validation and drug design efforts, contributing to the ongoing battle against COVID-19.

#### **Materials and methods**

In this study, we conducted an analytical *in silico* study to investigate the binding interactions between six natural molecules derived from different natural organisms against the target protein of the SARS-CoV-2 virus. The study employed computational docking techniques to predict and analyze the binding modes and affinities of the ligands within the target protein active sites, aiming to identify potential ligand-receptor interactions and gain insights into the binding mechanisms.

ADMET characteristics (absorption, distribution, metabolism, excretion, Toxicity) profiling validation, were utilized using the pkCSM package to identify molecules with favorable properties for oral bioavailability and potential as medications within the body, to predict the toxic effects of compounds, while also assessing their drug-like physical and chemical properties.

**Preparation of Ligand Structures:** Six natural molecules were chosen according to their antiviral characteristics from the literature, where we included two plant molecules [7, 8], Cannabidiol (from *Cannabis sativa*) and Capsaicin (from *Capsicum annuum*), two fungi molecules [9], Cladosin C (from microfungi *Cladosporium sphaerospermum*) and Rhodatin (from macrofungi *Rhodotus palmatus*), also, two algae molecules [10], Astaxanthin (from green microalgae *Haematococcus pluvialis*) and Kappa-Carrageenan (from red marine macroalgae *Kappaphycus alvarezii*) were selected as ligands. The structures of these six molecules were retrieved from the PubChem database, and further processed and optimized using ChemDraw and Chimera software.

ChemDraw was employed for drawing and editing the initial ligand structures, which facilitated the accurate representation of the ligands based on the available information from PubChem.

UCSF Chimera [11] was utilized to optimize the ligand structures, including energy minimization and geometry optimization, leading to more reliable and physiochemically reasonable conformations.

**Preparation of Receptor Structures:** The structure of the Main protease was retrieved from the PDB database and saved as PDB files.

The catalytic domains were selected for inhibitor docking of inhibitors against the SARS-CoV-2 targets using PyMol (The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC), as well as according to literature [12].

The 3C-like protease (M<sup>pro</sup>) catalytic sites: THR190, HIS41 CYS145, GLU166, GLY143, PHE140.

**Grid Generation:** To define the binding sites for docking, grids were generated around the active sites of the SARS-CoV-2 proteins, namely the Main Protease. The grid parameters, including size, spacing, and center coordinates, were set using the AutoGrid module of AutoDock [13].

**Docking Protocol:** All the retrieved compounds were docked using the selected catalytic site of the threedimensional structure. The ligands were prepared by adding polar hydrogens, merging nonpolar hydrogens, and assigning partial charges using AutoDock Tools [14]. The prepared ligands were then docked into the binding sites of the SARS-CoV-2 proteins using AutoDock Vina [15]. Multiple docking runs were conducted to explore different ligand conformations and orientations within the binding sites.

**Post-Docking Analysis:** After the docking simulations, the resulting poses were analyzed to identify potential ligand-receptor interactions and select the most favorable binding conformations. The docking results were visually inspected and analyzed using molecular visualization software AutoDock Tools. The binding poses were assessed based on their binding energies, ligand-receptor interactions, and conformational fit within the active site.

**Molecular dynamics simulations:** Schrödinger LLC Desmond software [16, 17] was employed for performing molecular dynamics (MD) simulations for a duration of 100 nanoseconds [18]. Prior to the MD simulation, a crucial step involved the protein-ligand docking, which predicted the static binding position of the ligand molecule at the active site of the protein as mentioned before [19]. MD simulations, incorporating Newton's classical equation of motion, simulate the movement of atoms over time and provide insights into the dynamic behavior and ligand-binding status in a physiological environment [20].

The ligand-receptor complex was preprocessed using Maestro's Protein Preparation Wizard, which performed optimization, minimization, and filling of any missing residues as necessary. The system was constructed using the System Builder tool. The MD simulation employed the TIP3P (Intermolecular Interaction Potential 3 Points Transferable) solvent model, utilizing an orthorhombic box with a temperature of 300 K, pressure of 1 atm, and the OPLS\_2005 force field [21]. To maintain physiological conditions, counter ions were introduced, and the system was neutralized using 0.15 M sodium chloride. Before the simulation, the models were equilibrated, and trajectories were recorded at regular intervals of 100 ps for subsequent analysis and inspection.

#### **Results and Discussion**

Six natural products; Astaxanthin, Cannabidiol, Capsaicin, Cladosin C, Rhodatin, and Kappa-Carrageenan were tested against SARS-CoV-2 target M<sup>pro</sup> (PDB ID. 7CAM). To test the relative stability of the 6 ligands, a 100 ns MD simulation was performed for the six complexes using Desmond in the Schrödinger package [16, 17].

**ADMET characteristics:** The ADMET properties of the selected six molecules are represented in Table 1. These properties fulfil the Lipinski's rule criteria and the extra condition on the number of rotatable bonds, suggesting their good pharmacokinetic permeability and their oral bioavailability [22, 23].

| 398.455<br>3.4701<br>1<br>6<br>3 | 250.298<br>1.56907<br>4  | 305.418<br>3.7896  | 314.469  | 598.868   | MOL_WEIGHT  |
|----------------------------------|--|--|--|---|---|
| 1                                |  | 3.7896   |  |   |   |
| 6                                | 4  |  | 5.8465   | 9.1294  | LOGP  |
|                                  |  | 9  | 6  | 11  | ROTATABLE_BONDS   |
| 3                                | 4  | 3  | 2  | 4   | ACCEPTORS   |
| 3                                | 4  | 2  | 2  | 2   | DONORS  |
| 169.004                          | 106.029  | 132.74   | 140.109  | 265.976   | SURFACE_AREA  |
| -3.635                           | -2.147   | -3.842   | -3.396   | -6.127  | Water solub ility   |
| 562                              | -256   | 1.338  | 956  | 827   | Caco2 penneability  |
| 91.746                           | 72.937   | 92.836   | 91.534   | 93.641  | Intestinal absorption (human)   |
| -2.827                           | -2.755   | -3.133   | -2.764   | -2.659  | Skin Penneability   |
| Yes                              | No   | Yes  | Yes  | Yes   | P-glycoprotein substrate  |
| Yes                              | No   | No   | Yes  | No  | P-glycoprotein I inhibitor  |
| Yes                              | No   | No   | No   | Yes   | P-glycoprotein II inhibitor   |
| 42                               | -0.37  | 142  | 618  | -784  | VDss (human)  |
| 0.04                             | 474  | 115  | 0.04   | 0   | Fraction unbound (human)  |
| -802                             | -939   | -411   | 7  | 395   | BBB permeability  |
| -2.327                           | -3.421   | -2.328   | -1.293   | -1.383  | CNS permeability  |
| No                               | No   | No   | No   | No  | CYP2D6 substrate  |
| Yes                              | No   | Yes  | No   | Yes   | CYP3A4 substrate  |
| No                               | No   | Yes  | Yes  | No  | CYP1A2 inhibitior   |
| No                               | No   | No   | No   | No  | CYP2C19 inhibitior  |
| No                               | No   | Yes  | No   | No  | CYP2C9 inhibitior   |
| No                               | No   | No   | No   | No  | CYP2D6 inhibitior   |
| No                               | No   | Yes  | No   | No  | CYP3A4 inhibitior   |
| 319                              | 332  | 1.294  | 1.165  | 849   | Total Clearance   |
| No                               | No   | No   | No   | No  | Renal OCT2 substrate  |
| No                               | No   | No   | No   | No  | AMES toxicity   |
| -759                             | 257  | 302  | -202   | -424  | Max. tolerated dose (human)   |
| No                               | No   | No   | No   | No  | hERG I inhibitor  |
| No                               | No   | Yes  | Yes  | No  | hERG II inhibitor   |
| 2.61                             | 2.726  | 2.566  | 2.35   | 4.061   | Onal Rat Acute Toxicity (LD50   |
| 1.909                            | 1.31   | 1.85   | 2.517  | 2.28  | Otal Rat Chuonic Toxicity   |
| No                               | No   | No   | No   | No  | Hepatotoxicity  |
| No                               | No   | No   | No   | No  | Skin Sensitisation  |
|                                  | -3.635<br>562<br>91.746<br>-2.827<br>Yes<br>Yes<br>Yes<br>42<br>0.04<br>-802<br>-2.327<br>No<br>Yes<br>No<br>Yes<br>No<br>No<br>No<br>No<br>No<br>No<br>No<br>No<br>No<br>No<br>No<br>No<br>No | -3.635         -2.147           562         -256           91.746         72.937           -2.827         -2.755           Yes         No           Yes         No           Yes         No           42         -0.37           0.04         474           -802         -939           -2.327         -3.421           No         No           Yes         No           No         No | -3.635         -2.147         -3.842           562         -236         1.338           91.746         72.937         92.836           -2.827         -2.755         -3.133           Yes         No         Yes           No         No         No           Yes         No         No           42         -0.37         142           0.04         474         115           -802         -939         -411           -2.327         -3.421         -2.328           No         No         No           Yes         No         No           No         No         Yes           No         No         No           Yes         No         No           No         No         Yes           No         No         Yes           No         No         No           No         No         No | -3.635         -2.147         -3.842         -3.396           562         -256         1.338         956           91.746         72.937         92.836         91.534           -2.827         -2.755         -3.133         -2.764           Yes         No         Yes         Yes           Yes         No         No         Yes           Yes         No         No         Yes           0.4         No         No         No           42         -0.37         142         618           0.04         474         115         0.04           -802         -939         -411         7           -2327         -3.421         -2.328         -1293           No         No         No         No           No         No         No | -3635-2.147-3.842-3.396-6.127562-2561.3389568279174672.93792.83691.53493.641-2.827-2.755-3.133-2.764-2.659YesNoYesYesYesYesNoNoYesYes12827-0.37142618-7840.044741150.040-802-939-4117395-2.327-3.421-2.328-1.293-1.383NoNoNoNoNoYesNoNoNoNo91-3.421-2.328-1.293-1.383NoNoYesNoNoNoNoNoNoNoNoNoYesNoNoNoNoYesNoNoNoNoYesNoNoNoNoYesNoNoNoNoYesNoNoNoNoYesNo< |

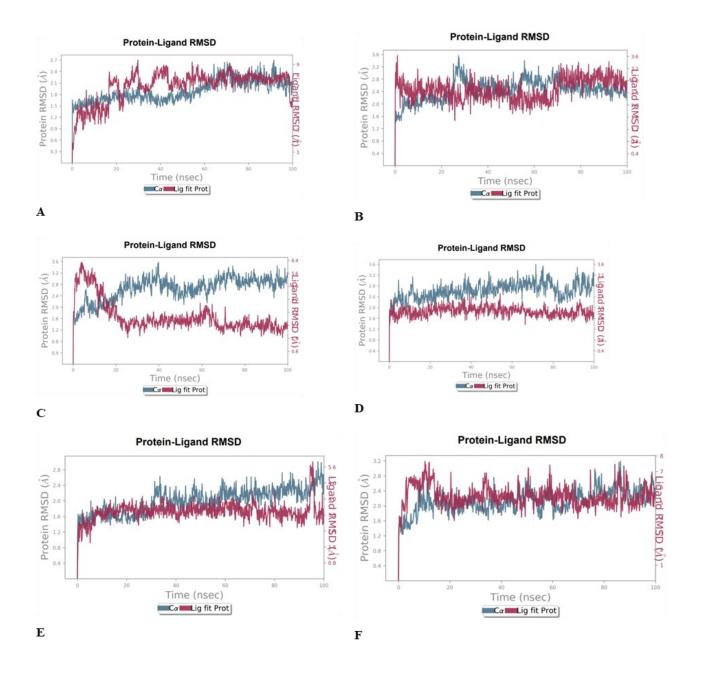
**Table 1** - ADMET characteristics of the six biomolecules predicted by pkSCM package.

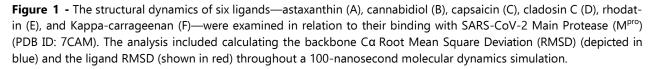
Figure 1 gives insight towards the binding stability of the 6 target ligands against SARS-CoV-2  $M^{pro}$  using Root Mean Square deviation (RMSD) plots for both protein backbone and the ligand. Protein Ca RMSD represents the stability of the protein when the ligand binds to it. The less fluctuation the more stability it possesses. Similarly, ligand RMSD represents the ligand stability upon binding to the target protein. As shown in figure 1, all the 6 ligands showed very good binding stability with low oscillations where cladosin C and rhodatin seems to have the best RMSD with fluctuation around 1.6 Ao (figure 1.D and E). Also, capsaicin (figure 1.C) showed a stable binding conformation after 20 ns from the

beginning of the simulation where in the last 80 ns RMSD of both the protein backbone and capsaicin were oscillating between 1 and 1.4 Ao which is a good indication for their stable binding. Both cannabidiol and Kappa-carrageenan (figure 1.B and 1.F, respectively) possess a higher but still acceptable RMSD fluctuation range; between 1.6 and 3.2 Ao, for both complexes. On the other hand, astaxanthin bound to M<sup>pro</sup> was found to be the least stable complex where in the first 20 ns upon astaxanthin binding to M<sup>pro</sup> shows a high fluctuation between 0.9 and 1.8 Ao and the second 40 ns show also a relatively non-stable binding with a reported RMSD ranging between 1.3 and 2.7 Ao while only in the last 40 ns this binding starts to be relatively stable; oscillating through plotting the Root Mean Square fluctuation (RMSF) of around 2.1 Ao (figure 1.A). each complex where green-colored vertical bars represent the

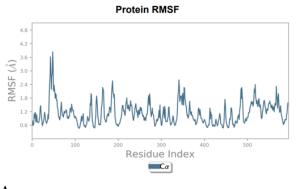
A deeper insight for the contact of each ligand towards M<sup>pro</sup> target was evaluated via studying the protein amino acids involved in binding interactions formed with each ligand

through plotting the Root Mean Square fluctuation (RMSF) of each complex where green-colored vertical bars represent the residues involved in the interaction. Secondly, the ligandprotein interactions with each residue are categorized into four types of interactions: Hydrogen bonds, hydrophobic interactions, ionic interactions and water bridges. Also, the



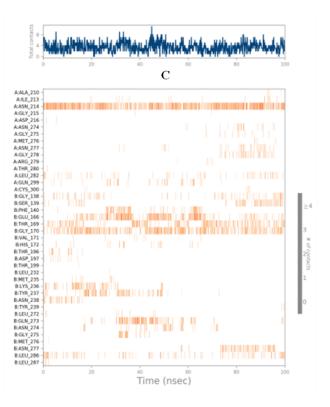


total number of contacts formed between the ligand and its was able to form a good contact with astaxanthin being able to target protein was plotted throughout the 100 ns trajectories form H-bond interaction with a fraction of about 0.3 and this and a schematic diagram for ligand 2D atomic interactions with was confirmed in figure 2.D where only Asn 214 was able to each protein residue in each trajectory frame (0 through 100 maintain a good interaction with astaxanthin throughout the ns) was presented for each complex. RMSF of M<sup>pro</sup> in complex whole simulation. Thr 169 and Gly 170 also were able to form H with astaxanthin show high fluctuation with no reported -bonds with astaxanthin but they were observed to be very interaction (no green bars) throughout the whole 100 ns week (with interaction fraction less than 0.1) figure 2.B and also meaning that no stable binding (figure 2.A) and this is cannot be maintained during the simulation figure 2.D. confirmed through 2D ligand-protein contact shown in figure Accordingly, no significant interaction is reported for 2.E that also shows no interaction with the pocket residues. astaxanthin-M<sup>pro</sup> complex which complies with the complex Figure 2.B shows the interaction fraction the ligand can form RMSD plot (figure 1.A). with the pockets' residues. It is noticeable that only Asn 214



**Protein-Ligand Contacts** 

H-bonds Hydrophobic lonic Water bridges

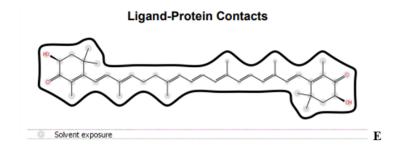


A

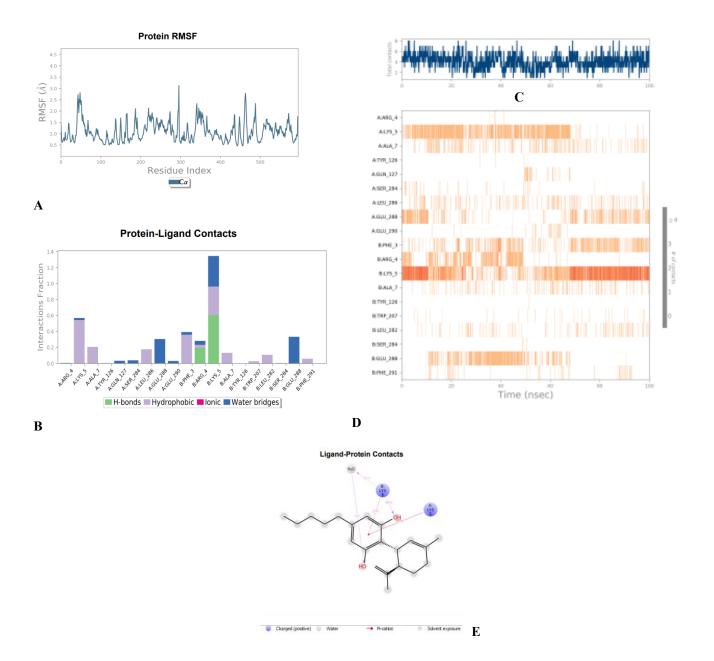


Interactions Fraction





**Figure 2** - Structural Dynamics of Astaxanthin with SARS-CoV-2 M<sup>pro</sup>: (A) Protein RMSF, (B) Interaction Fractions Shown in a Stacked Bar Chart, (C) Timeline of Specific Interactions, (D) Residue-Ligand Interactions per Trajectory Frame, (E) 2D Ligand-Protein Interaction Map.

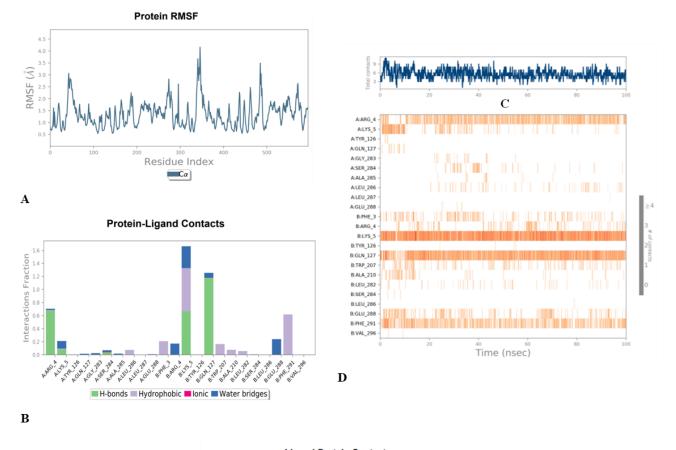


**Figure 3** - Structural Dynamics of Cannabidiol Bound to SARS-CoV-2 M<sup>pro</sup>: (A) Protein RMSF, (B) Stacked Bar Chart of Protein-Ligand Interaction Fractions, (C) Timeline of Total Specific Interactions, (D) Residue-Ligand Interactions per Trajectory Frame, (E) 2D Map of Ligand-Protein Interactions.

protein RMSF was observed indicating unreasonable binding highest reported interaction fraction (more than 1.6) (figure (figure 3.A). Only 2 residues were able to form H-bonds; Arg 4 4.B). Also, Lys 5 in chain A was able to form a Pi-cation (interaction fraction = 0.2) and Lys 5 (interaction fraction = 0.6) interaction and was noticed at the first 10 ns from the in chain B also Lys 5 in chain A was a Pi-cation reaction beginning of the simulation (figure 4.D). Another 2 important (interaction fraction = 0.55) (figure 3.B). Figure 3.E shows the H- residues were reported in this simulation; Arg 4 (chain A) and bond formed with Lys 5 in chain B and Pi-cation interaction Gln 127 (chain B) with interaction fractions = 0.7 and 1.3, with Lys 5 in chain A where only interactions that occur more respectively (fig 4. B). Both were able to form H-bonds with the than 30.0% of the simulation are reported. Lys 5 in both chains ligand and these bonds were stable during the simulation (a and B) seems to be a key residue responsible for cannabidiol (figure 4. D). Although good binding interactions are observed binding against M<sup>pro</sup> as they are noticed to be formed in most for this complex, the protein RMSF is reported to be highly trajectory frames during the 100 ns simulation (figure 3.D).

Similar interactions were observed upon capsaicin binding to M<sup>pro</sup> (figure 4.E), where also Lys 5 in chain B was able to form

Regarding, cannabidiol-SARS-CoV-2 M<sup>pro</sup> complex also high H-bond and Pi-cation interactions with capsaicin showing the fluctuating (figure 4.A). An explanation for this high residue RMS fluctuation can be concluded from the RMSD plot shown in figure 1.C, where the complex starts to stabilize its binding interaction 20 ns after the beginning of the simulation.



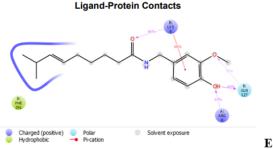
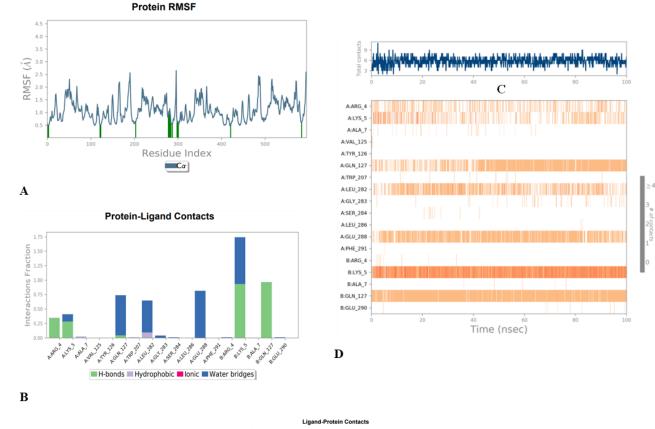
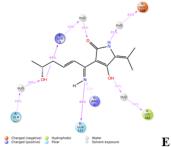


Figure 4 - Structural Dynamics of Capsaicin with SARS-CoV-2 M<sup>pro</sup>: (A) Protein RMSF, (B) Bar Chart of Protein-Ligand Interaction Fractions, (C) Timeline of Total Specific Interactions, (D) Residue-Ligand Interactions per Trajectory Frame, (E) 2D Ligand-Protein Interaction Map.

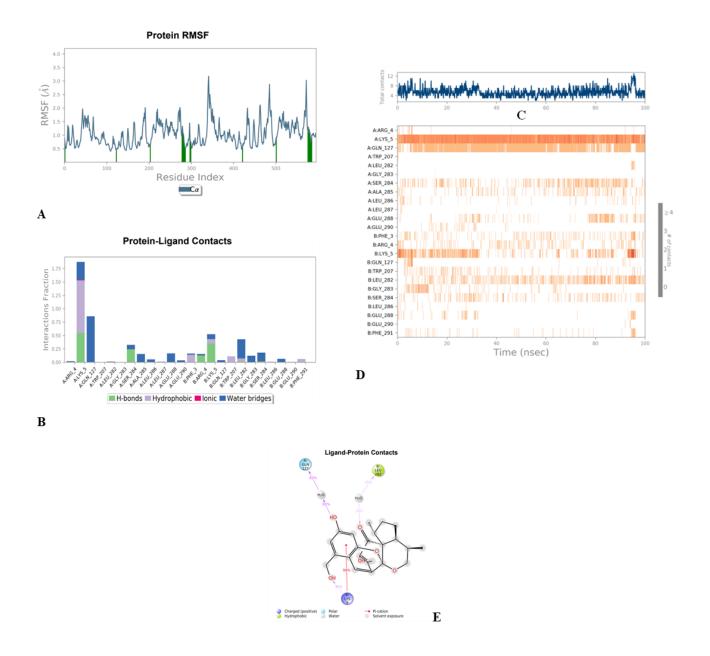
best and the most stable RMSD fluctuation (figure 1.D and E), observed with Lys 5 (chain B) which was able to form both Hinteractions with 6 and 3 residues, respectively, in M<sup>pro</sup> active simulation (figure 5.D). For rhodatin, Lys 5 (chain A) showed to pocket. Some of them are previously reported with cannabidiol be the main residue involved in the interaction with interaction with both cladosin C and rhodatin ligands was Leu 282 forming trajectory frame (figure 6.D). The protein RMSF for both another water bridge with Mpro Glu 288 active pocket (figure

As reported previously that cladosin C and rhodatin show the 5.E). Regarding cladosin C, the highest interaction fraction was the best binding interactions were reported for the 2 bond and water bridge interactions (figure 5.B). It also complexes. Cladosin C and rhodatin were able to form possesses the most permanent interaction along the 100 ns and capsaicin Lys 5. Also Gln 127 as a key residue was reported fraction of more than 1.75. The second important residue was when M<sup>pro</sup> was in complex with capsaicin, cladosin C and Gln 127 (interaction fraction > 0.75) (figure 6.B) where both rhodatin (figure 5.E and 6.E). A third pocket residue reported were able to interact with the rhodatin in almost each a water bridge with both pockets. Cladosin C was able to form cladosin C and rhodatin complexes with M<sup>pro</sup> show a low





**Figure 5** - Structural Dynamics of Capsaicin with SARS-CoV-2 M<sup>pro</sup>: (A) Protein RMSF, (B) Bar Chart of Protein-Ligand Interaction Fractions, (C) Timeline of Total Specific Interactions, (D) Residue-Ligand Interactions per Trajectory Frame, (E) 2D Ligand-Protein Interaction Map.



**Figure 6** - Structural Dynamics of Rhodatin with SARS-CoV-2 M<sup>pro</sup>: (A) Protein RMSF, (B) Stacked Bar Chart of Protein-Ligand Interaction Fractions, (C) Timeline of Specific Interactions, (D) Residue-Ligand Interactions per Trajectory Frame, (E) 2D Ligand -Protein Interaction Map.

fluctuation with the same reported amino acids (shown in binding interaction fractions = 1 and 0.75, respectively. These 3 green lines in figure 5.A and 6.A).

with M<sup>pro</sup> pocket residues with a steady stable RMSD (figure is not reported in 2D interaction is Thr 154. Thr 154 was able to 1.F), it shows a different binding mode interacting with interact Kappa-carrageenan via water bridges and hydrophobic different pocket residues. As shown in figure 7.E, 4 residues interaction (figure 7.B) and maintained these interactions within were involved in this binding interaction; Glu 14, Gly71, Ser 121 most of the trajectory frames (figure 7.D). Also, figure 7.B and Pro 122. Glu 14 showed the highest interaction fraction showed that Ser 121 and Pro 122 maintained their interactions (~1.75) followed by both Ser 121 and Pro 122; exhibiting

residues were able to interact with the ligand via both H-bond While Kappa-carrageenan was able to form a stable interaction and water bridges (figure 7.B). A fifth important residue which in most of the simulation frames while Glu 14 is almost the main key interaction in through all the 100 ns simulation.

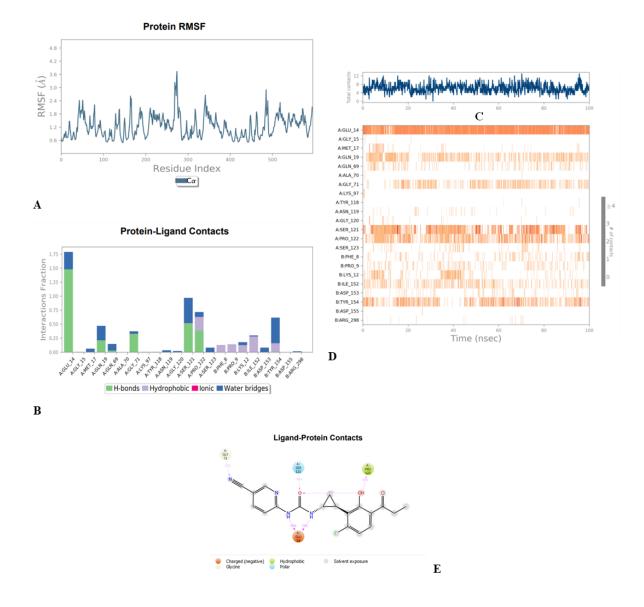


Figure 7 - Structural Dynamics of Kappa-Carrageenan with SARS-CoV-2 M<sup>pro</sup>: (A) Protein RMSF, (B) Stacked Bar Chart of Protein-Ligand Interaction Fractions, (C) Timeline of Specific Interactions, (D) Residue-Ligand Interactions per Trajectory Frame, (E) 2D Ligand-Protein Interaction Map.

#### Conclusion

In the quest to combat the devastating impact of SARS-CoV-2, [1] Carabelli, A.M., Peacock, T.P., Thorne, L.G. et al. SARS-CoV-2 this study undertook a comprehensive investigation of natural variant biology: immune escape, transmission and fitness. Nat antiviral compounds, focusing on their interactions with the Rev Microbiol, 2023, 21, 162-177. critical viral target, the Main Protease (Mpro). Six natural molecules, including Astaxanthin, Cannabidiol, Capsaicin, Cladosin [2] Zatla I, Boublenza L, Hassaine H. SARS-CoV-2 origin, classifi-C, Rhodatin, and Kappa-Carrageenan, were assessed using a cation and transmission: a mini-review. Curr Top Virol. 2021; 18: combination of molecular docking simulations and molecular 31-38. dynamics. This research has shed light on their potential as antagonists against the viral M<sup>pro</sup> enzyme. Among these com- [3] Zatla I, Boublenza L, Hassaine H. SARS-CoV-2 morphology, pounds, Cladosin C and Rhodatin emerged as the most prom- genome, life cycle and our bodies' immune response: a review. ising, demonstrating stable binding interactions with Mpro and Curr Top Virol. 2021; 18: 15-24 forming crucial associations with key residues. Cannabidiol, Capsaicin, and Kappa-Carrageenan also showed encouraging [4] Li, G., Hilgenfeld, R., Whitley, R. et al. Therapeutic strategies interactions, though with some variability in stability. On the for COVID-19: progress and lessons learned. Nat Rev Drug Disother hand, Astaxanthin exhibited the least stable binding, sug- cov, 2023, 22, 449-475. gesting limited potential as an antiviral agent against SARS-CoV-2. These findings contribute to our understanding of the [5] Zatla, I., & Boublenza, L. In Silico Evaluation of Natural Antistability and efficacy of these natural compounds as potential viral Compounds Targeting the RBM of SARSCoV-2 Spike Glyantiviral agents. The next step is to validate these computation- coprotein. Trop J Nat Prod Res, 2023, 7(10), 4273–4283. al results through in vitro and in vivo studies, offering a pathway for the development of novel antiviral therapies in the [6] Wang, Z., Wang, N., Yang, L., Song, X-q. Bioactive natural ongoing battle against COVID-19. This research also under- products in COVID-19 therapy. Front Pharmacol, 2022, scores the importance of further exploration of naturel sources 13:926507. containing these compounds, potentially uncovering new sources of antiviral agents.

#### Ethics approval and consent to participate

Not applicable.

#### **Consent for publication**

Not applicable.

#### Availability of data and materials

from the corresponding author upon request.

#### **Competing interests**

The authors declare no conflict of interest.

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#### **Authors contributions**

data analysis, manuscript writing, manuscript editing. Lamia (13):1605-12. Boublenza: Manuscript review, supervision of the project.

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