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Valeton Phytoconstituents from *Curcuma Phaeocaulis* as Prion Protein Mutant V210I Inhibitors: A Computational Docking and Virtual Screening Study

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Abstract

Objective: *Creutzfeldt-Jakob disease (CJD), a neurological disorder that is sporadic, fatal, communicable, and worsens rapidly, is caused by abnormal folding of prion proteins. Identifying and assessing the potential of phytocompounds from the Curcuma phaeocaulis Valeton plant as a new therapeutic candidate aimed at the treatment of CJD is the objective of this research article.* ***Methods:*** *In this experiment, we assessed outcomes following an in-silico assessment to design a new oral treatment for Creutzfeldt-Jakob disease. (CJD). Phytocompounds from Curcuma phaeocaulis Valeton believed to have therapeutic effects were selected, put through a comprehensive virtual screening in this study, and screened for pharmacology and toxicology. Eventually, the screening was completed by docking the selected compounds with PyRx and Biovia against the prion protein target.* ***Results:*** *Germacrone, Cyclocurcumin, and demethoxycurcumin were found to be potentially effective therapeutic drugs against the target prion protein in docking analysis.* ***Conclusion:*** *According to this analysis, Curcuma phaeocaulis valeton can be a beneficial therapeutic herb for further research on the treatment of Creutzfeldt-Jakob disease.*

Keywords: Curcuma phaeocaulis valeton, creutzfeldt-jakob disease (CJD), prion disease, molecular docking, ADMET, toxicity prediction

INTRODUCTION

A kind of protein known to have been folded incorrectly and has the potential to propagate its abnormal shape to other, healthier versions of that same protein is called a prion. Prions are a major cause of several lethal and communicable neurological ailments affecting humans as well as animals [1]. Prion disorders are an untreatable group of neurological diseases that can affect both people and animals. The brain structure or other surrounding tissues are affected by all reported prion diseases in animals; they are invariably progressive, have no recognized effective treatments, and are all deadly [2]. The improper foldings and aggregation of these solvable cytoplasmic prion proteins (PrPC) to form scrapie variant prion proteins (PrPSc) within the cerebrospinal nervous contribute to cerebral harm and other neurodegenerative problems [3]. 90% of human prion disease cases instances of human prion disease are sporadic CJD [4].

The neurodegenerative disorder *Creutzfeldt-Jakob disease (CJD),* caused by prion proteins, is sporadic, fatal, contagious, and swiftly progressing [5]. The defining feature of this condition is "the misfolded prion proteins Scrapie (PrPSc) being aggregated in the brain" [4]. The symptoms were first reported by Hans Creutzfeldt in 1920 and later

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described by Alfons Jakob in 1921 and 1923. Clearance J. Gibbs later initiated to call the disease "Creutzfeldt-Jacob disease (CJD)", because that abbreviation sounds more similar to his name initials [5]. The aggregation of misfolded prion protein promotes the deterioration of brain tissue, which manifests in a variety of clinical symptoms such as advancing dementia, seizures, trembling, motor ataxia, and psychiatric indications [6]. Patients with CJD have a 6- to 12-month survival window due to these symptoms, since there is no known cure available for this disease [6]. The objective of treatment is to minimize symptoms and increase patient comfort. If there is pain, drugs can assist. Clonazepam and sodium valproate are two medications that may ease myoclonus, or jerky, uneven movements [7].

In East Asian countries, *Curcuma phaeocaulis Valeton (CpV)* belonging to the Zingiberaceae family (CpV) has been employed traditionally to treat blood stasis syndrome, which is characterized by symptoms including ataxia, menstrual irregularities, and joint pains [8]. Yunnan, a province in China, is its native land [9]. Recent studies on the plant's phytochemistry revealed sesquiterpenoid, one of its key components, to have anti-inflammatory, anti-tumor, as well as anti-platelet accumulation properties. Curcumin, which is rich in Curcuma longa, has been proven to be efficient in inhibiting prion formation in vitro [8]. It was also discovered along with the findings of several in vitro as well as in vivo studies, CpV extract efficiently decelerates prion proliferation, alters the progression of prion disorders by lowering PrPSc levels, and guards against neuropathological degeneration [8]. Therefore, in hopes of enhancing research into CJD treatment, Curcuma phaeocaulis Valeton is a potentially promising herb [8].

The mutant prion protein (2LV1) that induces CJD was molecularly docked against 20 phytochemical compounds from Curcuma phaeocaulis Valeton. An in-silico analysis was used. Twenty phytocompounds were evaluated pharmaceutically utilizing an ADMET study and toxicity screening. After 20 hits from phytocompounds, molecular docking was then used to identify the three most prominent protein-binding ligands, and their properties were investigated.

METHODS

Retrieval of Protein

The protein model chosen was the *solution-state NMR structure of the prion protein mutant V210I at neutral pH (2LV1*) with 147 macromolecule residues and just an A chain (Figure 1). This was taken using the PDB database (https://www.rcsb.org/) in the pdb format [10].

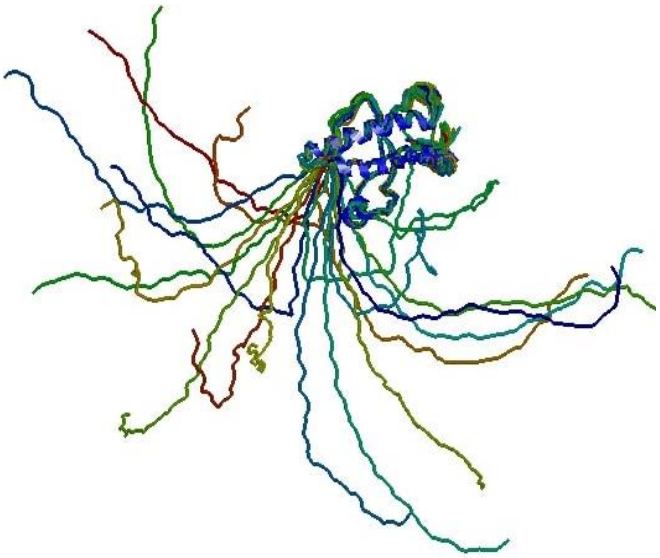

Figure 1. Unpurified target prion protein.

Retrieval of Ligands

Certain phytochemicals from *Curcuma phaeocaulis Valeton (CpV)* were acquired from the IMPPAT database, a compendium designed to document the phytochemicals in remedial herbs found in India, depending on their medicinal benefits [11]. After that, 20 phytocompounds' 3D structural data format structure (SDF) were gathered via PubChem (https://pubchem.ncbi.nlm.nih.gov/). Canonical SMILES were gathered to evaluate the physiochemical properties of phytochemicals [12].

Target Preparation

With the assistance of Biovia Discovery Studio Visualizer, its molecular graphics were altered (Free Download: BIOVIA Discovery Studio Visualizer - Dassault Systèmes (3ds.com)). The protein structure was purified using the methodologies mentioned here; the crystal structure and water molecule's free energy are incompatible (Figure 2). Molecules of water were entirely detached beforehand the process of molecular docking since those might disturb the docking outcomes. These formerly attached ligand compounds were taken out of the crystalline form to accelerate the bonding against the ligands preselected for the study. Then, to improve the degree of purity of purified structures, polarized atoms of hydrogen were added. The prediction concerning the protein characteristics was applied to the purified structure.

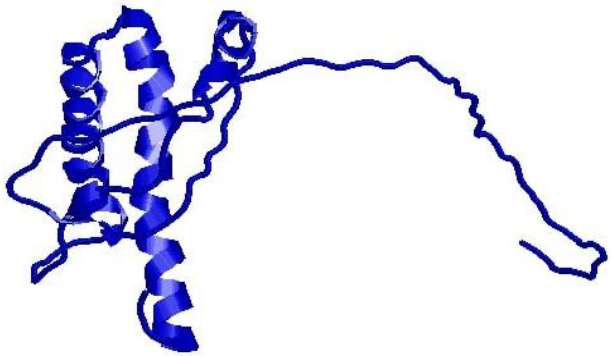

Figure 2. Purified target prion protein.

Target Structure Validation (Ramachandran Plot)

The hydropathy graph and properties associated with the emulated polypeptide were then generated with Pepstats online software (https://www.bioinformatics.nl/cgi-bin/emboss/pepstats) together with Pepwindow online software (https://www.bioinformatics.nl/cgi-bin/emboss/pepwindow). In windows of a specific size, Pepwindow evaluates hydropathy across the input sequence. When the generated protein model was uploaded to PDBsum generate (https://bio.tools/pdbsum_generate) to construct a Ramachandran plot as well as its information, the protein's motifs, and secondary framework had been determined.

Docking

Molecular docking is an eminent computational structure-based methodology that is frequently used in the discovery and development of drugs. Docking allows for the discovery of new drugs, the prediction of ligand-target interactions between ligand and target at the molecular level, or the identification of structure and activity relationships (SAR) without prior knowledge of the biochemical composition of target modulators [13]. The target protein structure was cleaned using the Discovery Studio, Biovia as a part of target preparation. The polypeptide structure after purification was loaded in the PyRx tool (https://pyrx.sourceforge.io/downloads), which has integrated editions of *Vina Wizard* (to see if there is a Vina output file already present (out. pdbqt) for each ligand), *Auto Dock Wizard* (the docking software**)**, and *Open Babel* (for energy minimization and ligands format conversion) [14]. The compounds were then sent for docking with protein target within Vina Wizard through energy reduction and augmenting grid dimensions (x:104.1255 y:63.5854 z:25.0000), ultimately acquiring binding energies for the ligands in a Comma- Separated Values file (CSV). The phytocompounds having the

lowest binding rapport scores were elected which were then sent to discovery studio Biovia. In Biovia hydrophobic and 2D structures as well as anticipated ligand-protein interactions were analyzed.

ADME

The acronym ADMET stands for Absorption, Distribution, Metabolism, Excretion, and Toxicity and it should be assessed at earliest as feasible since poor pharmacokinetic characteristics and toxicity of drug candidates are the primary factors of drug development failure [15]. Swiss ADME (http://www.swissadme.ch/) had been utilized to examine the ADME attributes of our acquired ligands. It provides variables for analyzing drugs, including BBB (stands for Blood Brain Barrier), Gastrointestinal (GI) absorption, PGP substrate, bioavailability score, lipophilicity, hydrogen atom acceptors, and hydrogen atom donors. Physiochemical properties, Pharmacokinetics, and Drug likeliness are the main variables that were focused on. The Swiss ADME was also used to retrieve the BOILED-Egg model. Surface area, water solubility, and rotatable bonds were all determined. Lipinski's 5 rules were applied to select and generate these significant factors. If there are 3 or more Lipinski violations, the ligand is required to be discarded from consideration for docking. But with these 20 ligands, there was no such case, so all twenty phytocompounds were taken further for docking.

RESULTS

Retrieval of Target Protein

The molecular docking procedure necessitates the purification of a target protein structure because this separation of protein and non-protein components allows for the characterization of a target protein's structure, function, and relationships. The protein is purified by removing any extra chains that are not needed and water molecules, then polar charges are added using Biovia Discovery Studio. Removing them enhances computations and frees up binding pockets since additional chains and water molecules that are not engaged in binding might interfere with essential docking interactions between the target and the ligand.

Pepstats and Pepwindow Study

The modeled protein comprised 147 residues, a charge of 3.0, and a molecular weight (MW) of 16633.53 according to the Pepstats analysis findings. The protein's isoelectric point was 7.4272, and the typical weight of residue was discovered 113.153. "Isoelectric Point" is a term that refers to the specific pH value where a certain molecule possesses no electrical charge at all (pI). In Table 1, the purified prion protein structure's amino acid composition is presented.

Table 1. Amino Acid Profile of amino acids present in protein 2LV1.

Properties	Residue	Numbers	Mole%age
Tiny	A+C+G+S+T	46	31.293
Small	A+B+ C+D+G +N+P +S+T+V	75	51.02
Aliphatic	A+I+L+V	25	17.007
Aromatic	F+H+W+Y	21	14.286
Non-polar	A+C+F+ G+I+L+M+P+ V+W+Y	73	49.66
Polar	D +E+H+ K+N+ Q+R+S+T+Z	74	50.34
Charged	B+D+E+H+K+R+Z	36	24.49
Basic	H+K+R	21	14.286
Acidic	B+D+E+Z	15	10.204

The hydrophobicity of a protein sequence may be seen using hydropathy graphs. The aquaphobic, as well as phillic features of amino acids, are utilized to create a hydropathy scale. The cumulative hydropathy at every point within the sequence is determined by a changing "window" (Y coordinate). Then, these amounts are plotted against the associated coordinates (X coordinate). These plots might be used to classify transmembrane proteins connected to membranes as well as the hydrophobic internal shares of globular polypeptides. With this computation, the window size is adjustable, making it possible

to alter the algorithm's sensitivity. Plots are "noisier" with smaller windows compared to bigger windows [16]. The Kyte Doolittle and Hopp Woods scales are two of the several methods used to evaluate hydropathy plots. Positive findings in Figure 3 imply that the amino acids appear to be hydrophobic, and thus might generate an alpha-helix that crosses the bilayer of lipids. Negative values, in contrast, indicating the amino acids seem to be in touch with solvents or water, which indicates that the amino acids are hydrophilic and most likely would be located at the external surface of protein.

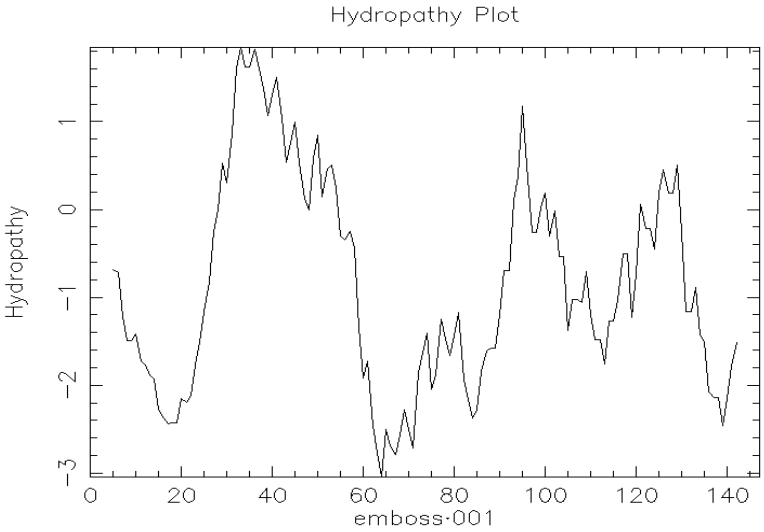

Figure 3. Hydropathy Plot generated for target protein (2LV1).

Macromolecular Structure Analysis

A Ramachandran plot is used to show the overall arrangement of the phi (φ) and psi (ψ) angles in statistical terms for every amino acid within the given structure. The Ramachandran plot's preferred regions are shown by the red region in Figure 4. The Ramachandran plot statistics showed that the regions with the most favorable regions had 108 residues and encased 86.4% amino acids (aa), whereas regions with added allowed regions possessed 16 residues and composed 12.8% aa, the regions generous allowed had only a single residue and conjured up to 0.8%. Lastly, the regions that are disallowed contained zero residue and composed 0.0%. Glycine (G) and proline (P) residues were detected at 15 and 6, respectively.

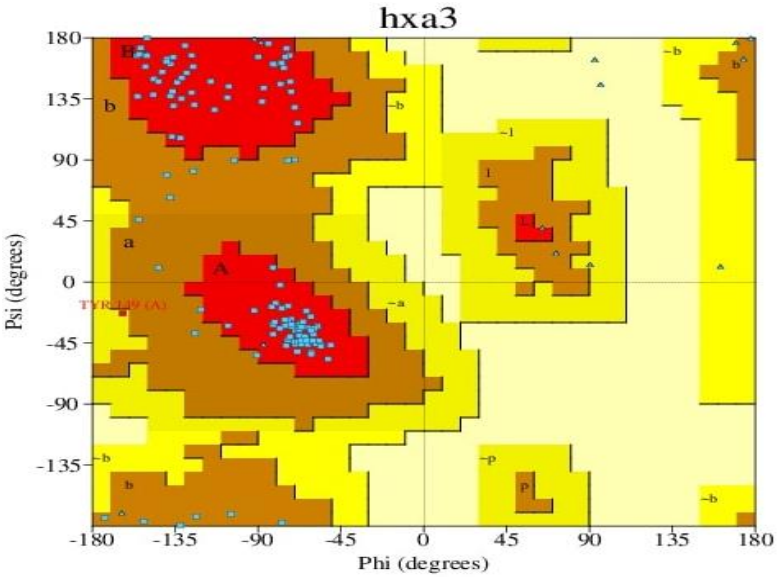

Figure 4. Ramachandran Plot generated for the target protein (2LV1).

PDBsum is an online platform that provides structural details on Protein Data Bank entries (PDB). PDBsum generate is used for assessing the target protein's secondary structure and also to generate its Ramachandran Plot through Procheck [17]. According to PDBsum generate results, there are 4 helices, 1 beta sheet, 2 beta strands, 1 beta hairpin, 4 helix-helix interactions, 2 gamma turns, and one disulphide in the target's 2° structure (Figure 5).

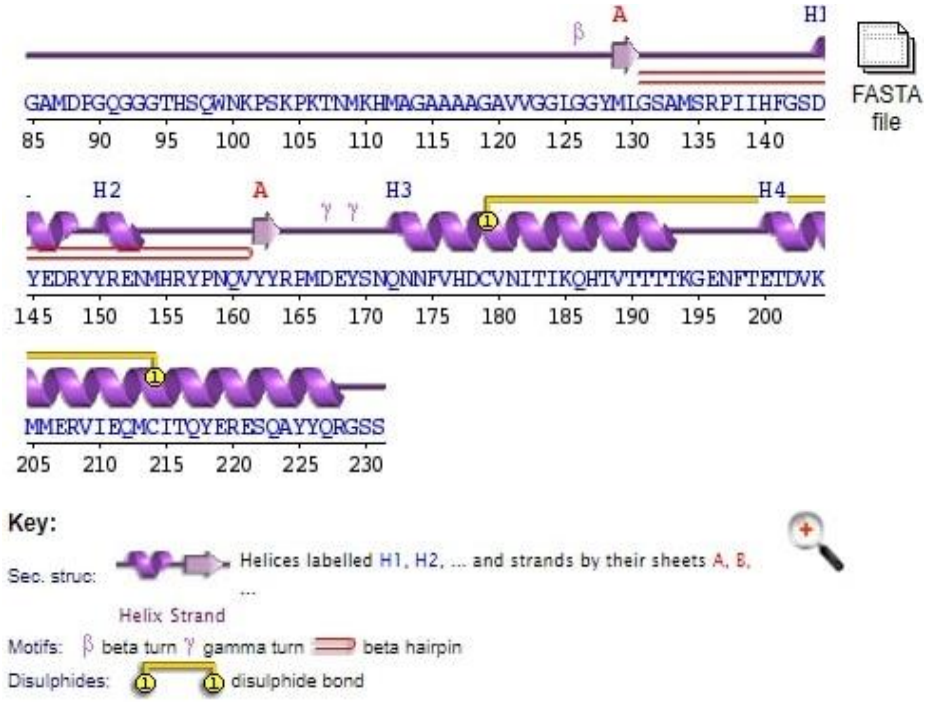

Figure 5. 2LV1 protein's 2° structure determined via PDBsum generate.

Ligands Retrieval

Based on the Lipinski rule of 5, drug-likeness assesses the ability of a given molecule to become a pill-based pharmaceutical. The phytocompounds' drug-likeness is assessed using ADMET analysis to evaluate whether they possess structural or physicochemical characteristics that are sufficiently developed to be considered oral drug candidates (Tables 2–5).

At the very least, the Csp3 fraction containing Sp3 hybridized atoms of carbon ought to be 0.25 and ideally. The MW should be at intervals 150 and 500 g/mol by mass. The polarity should have a topological polar surface space amidst 20 and 130.

According to rules by Lipinski, the optimal range of molar refractivity (MR) is between 40 and 130, the molecular weight (MW) of the phytocompound should always be less than 500 Daltons, MlogP value not more than 4.15, the hydrogen atom donors should be less than 5 and hydrogen atom acceptors must be 5 and 10, respectively. Considering Table 3, it is apparent that the ligands Beta-elemene, Zingiberene, alpha-curcumene, Campesterol, beta-Pinene, Camphene, and beta-Farnesene do not adhere to the Lipinski Rule of 5 because these ligands have MlogP values more than 4.15. The Lipinski criteria are satisfied by the other ligands though. The other four factors i.e., the molecular weight, H donors, H acceptors, and MR are all within the appropriate range according to Lipinski's rules.

Together with solubility, lipophilicity also affects permeability, potency, selectivity, characteristics of ADME, and toxicity. Minimal solubility, poor oral absorption, and fast metabolic cycle are frequently caused by high lipophilicity (logP>5). Moreover, highly lipophilic substances have a propensity to attach to hydrophobic receptors besides the chosen target, raising the possibility of promiscuity and toxicity [18].

Table 2. Physiochemical Properties of the selected compounds.

Ligands Name	Molecular Weight	Csp3 Fraction	Rotatable Bond	Topological PSA	Lipophilicity
Curcumin	368.38	0.14	8	93.06	3.27
Curdione	236.35	0.73	1	34.14	2.8
Germacrone	218.33	0.53	0	17.07	2.96
Curzerene	216.32	0.47	2	13.14	3.17
Beta - elemene	204.35	0.6	3	0	3.37
Demethoxycurcumin	338.35	0.1	7	83.83	2.78
Bisdemethoxycurcumin	308.33	0.05	6	74.6	1.75
Curlone	218.33	0.53	4	17.07	3.14
Alpha-Tumerone	218.33	0.53	4	17.07	3.11
Zingiberene	204.35	0.6	4	0	3.65
alpha-Curcumene	202.34	0.47	4	0	3.5
Cyclocurcumin	368.38	0.19	5	85.22	3.12
Nerolidol	222.37	0.6	7	20.23	3.64
Campesterol	400.68	0.93	5	20.23	4.92
beta-Pinene	136.23	0.8	0	0	2.59
Camphene	136.23	0.8	0	0	2.58
Limonene	136.23	0.6	1	0	2.72
Kaempferol	286.24	0	1	111.13	1.7
Myrcene	136.23	0.4	4	0	2.89
beta-Farnesene	204.35	0.47	7	0	3.86

PSA: Polar Surface Area.

Table 3. Lipinski filter analysis of selected phytocompounds.

Ligand	MW	MLogP	H bond donors	H bond acceptors	MR
Curcumin	368.38	1.47	2	6	102.8
Curdione	236.35	2.54	0	2	72.03
Germacrone	218.33	3.37	0	1	70.88
Curzerene	216.32	3.33	0	1	68.74
Beta - elemene	204.35	4.53	0	0	70.42
Demethoxycurcumin	338.35	1.8	2	5	96.31
Bisdemethoxycurcumin	308.33	2.13	2	4	89.82
Curlone	218.33	3.37	0	1	70.88
Alpha-Tumerone	218.33	3.37	0	1	70.88
Zingiberene	204.35	4.53	0	0	70.68
alpha-Curcumene	202.34	5.75	0	0	69.55
Cyclocurcumin	368.38	1.16	2	6	100.78
Nerolidol	222.37	3.86	1	1	74
Campesterol	400.68	6.54	1	1	128.42
beta-Pinene	136.23	4.29	0	0	45.22
Camphene	136.23	4.29	0	0	45.22
Limonene	136.23	3.27	0	0	47.12
Kaempferol	286.24	-0.03	4	6	76.01
Myrcene	136.23	3.56	0	0	48.76
beta-Farnesene	204.35	4.84	0	0	72.32

MW: *Molar Weight, MR: Molar Refractivity.*

The blood arteries' ability to accurately regulate the movement of chemicals, and ions, in addition to the travel of cells amongst both blood and brain is made possible by BBB. Crucial elements like GI absorption must be taken into account during drug development. Substratum for permeability

glycoproteins (PGP) helps the efflux system pump things out of the cell. The fact that it is there means that chemicals can now be pushed away from the cell. The ligand's solubility has to be 6 in order to satisfy the drug-likeliness. The compounds are easier to develop if the SA score is less than six. The amount of a chemical that may enter the bloodstream and reach its intended location is measured by its bioavailability, which is dependent on how well it is secreted and absorbed. With a 0.55 bioavailability score, the substance complies with Lipinski's rule. PAINS (Pan Assay Interference) are those compounds that provide false-positive findings of interaction. Non-specific interactions between PAIN molecules and the target protein. According to Table 4, no ligands present here display PAIN characteristics.

There are several toxicity classifications based on LD50 estimates and toxicity levels. The 6 main groups are as follows: Class I (LD50≤5) and Class II (5 known toxicity, Class I lethal, Class II lethal, Class III (50 toxic, Class IV (300) dangerous, class V hazardous (2000 class VI (LD50>5000) non-toxic.

Protox (https://tox-new.charite.de/protox_II/) tool was used to determine the toxicity predictions of selected phytocompounds. According to Table 5, all the phytocompounds fall under Class IV and V, which means that they may or may not be dangerous if ingested. None of the phytocompounds selected are lethal in nature.

Boiled Egg Interpretation

Boiled Egg is an acronym for Brain or Intestinal Estimated Permeation Predictive Model and it predicts properties like GI absorption capacities of a beneficial compound, which is a significant aspect in the process of designing and developing pharmaceuticals. In contrast to those falling within the yolk and yellow areas of the BOILED-Egg, which are predicted to have elevated levels of GI absorption, ligand molecules falling in the white part are predicted to have a higher possibility of bridging the BBB. The white part of the boiled egg has greater GI absorption rates for Curcumin, Demethoxycurcumin, Cyclocurcumin, and Kaempferol however the yellow area exhibits advanced rates of BBB permeability for other ligands, as seen in Figure 6. A red circle point denotes the lack of PGP, whereas blue circle represents inclusion of PGP within ligands. There are no blue points found in the diagram of boiled egg.

Table 4. ADME results and Structure-Activity relationships analysis.

Ligand	BB	GI absorption	PGP substrate	Solubility	PAINS	Bioavailabilty	SA
Curcumin	No	High	No	Slightly soluble	0	0.55	2.97
Curdione	Yes	High	No	Soluble	0	0.55	4.44
Germacrone	Yes	High	No	Soluble	0	0.55	3.9
Curzerene	Yes	High	No	Slightly soluble	0	0.55	4.05
Beta - elemene	No	Low	No	Soluble	0	0.55	3.63
Demethoxycurcumin	No	High	No	Slightly soluble	0	0.55	2.82
Bisdemethoxycurcumin	Yes	High	No	Slightly soluble	0	0.55	2.59
Curlone	Yes	High	No	Soluble	0	0.55	4.17
Alpha-Tumerone	Yes	High	No	Soluble	0	0.55	4.53
Zingiberene	No	Low	No	Soluble	0	0.55	4.81
alpha-Curcumene	No	Low	No	Slightly soluble	0	0.55	2.31
Cyclocurcumin	No	High	No	Slightly soluble	0	0.56	4.21
Nerolidol	Yes	High	No	Soluble	0	0.55	3.53
Campesterol	No	Low	No	Slightly soluble	0	0.55	6.17
beta-Pinene	Yes	Low	No	Soluble	0	0.55	3.73
Camphene	Yes	Low	No	Soluble	0	0.55	3.5
Limonene	Yes	Low	No	Soluble	0	0.55	3.46
Kaempferol	No	High	No	Soluble	0	0.55	3.14
Myrcene	Yes	Low	No	Soluble	0	0.55	2.85
beta-Farnesene	No	Low	No	Soluble	0	0.55	3.42

PGP: *Permeability Glycoprotein, GI: Gastrointestinal, SA: Synthetic Availability, BBB: Blood Brain Barrier*

Table 5. Toxicity Study for selected phytocompounds.

Phytocompounds	Predicted LD 50	Toxicity class
Curcumin	2000 mg/kg	4
Curdione	5000 mg/kg	5
Germacrone	2950 mg/kg	5
Curzerene	590 mg/kg	4
Beta - elemene	5000 mg/kg	5
Demethoxycurcumin	2000 mg/kg	4
Bisdemethoxycurcumin	2560 mg/kg	5
Curlone	4600 mg/kg	5
Alpha-Tumerone	2500 mg/kg	5
Zingiberene	1680 mg/kg	4
alpha-Curcumene	2000 mg/kg	4
Cyclocurcumin	1500 mg/kg	4
Nerolidol	5000 mg/kg	5
Campesterol	890 mg/kg	4
beta-Pinene	4700 mg/kg	5
Camphene	5000 mg/kg	5
Limonene	4400 mg/kg	5
Kaempferol	3919 mg/kg	5
Myrcene	5000 mg/kg	5
beta-Farnesene	5000 mg/kg	5

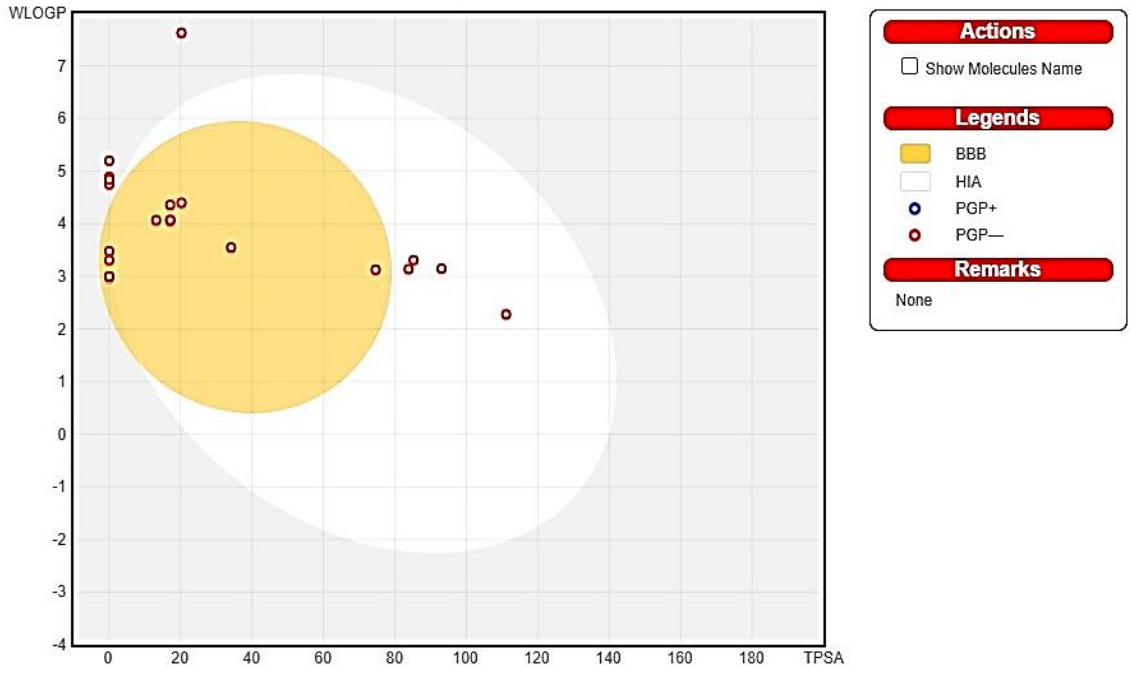

Figure 6. The boiled egg or selected phytocompounds.

Molecular Docking and Visualization

As none of the 20 phytochemicals had more than three Lipinski violations, they were all docked to the *prion mutant protein (2LV1),* which is the target protein.

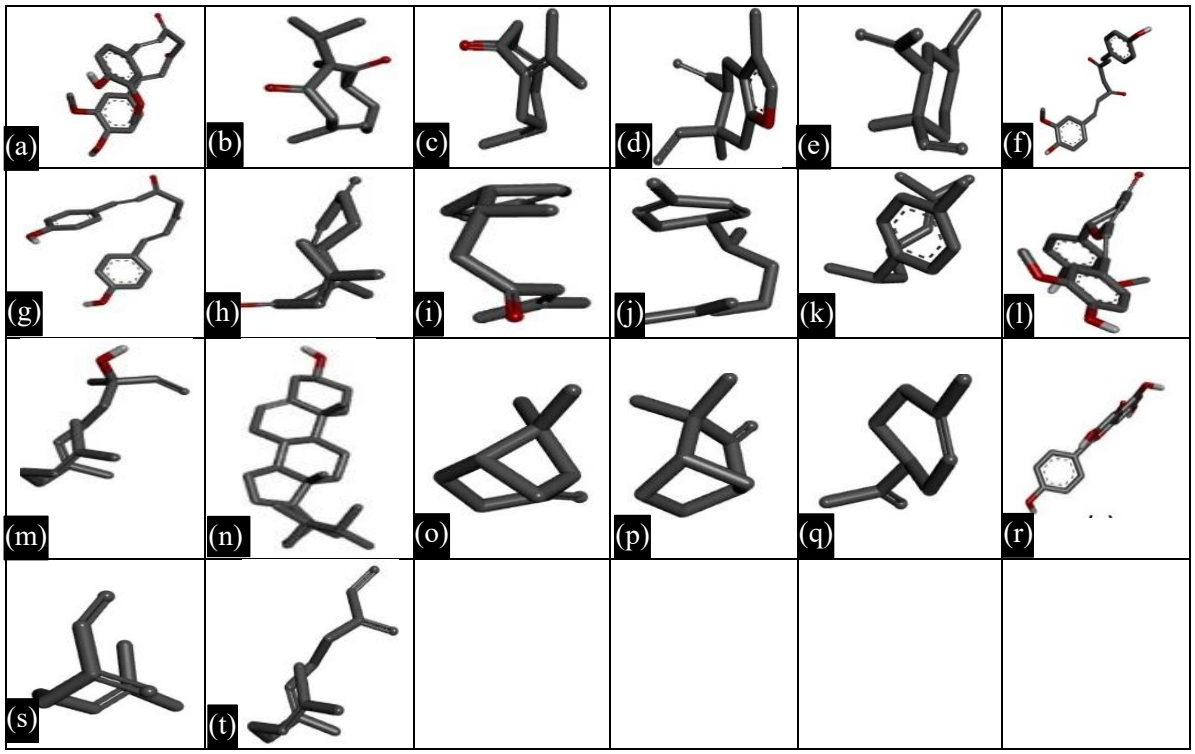

Figure 7. 3D structures of phytocompounds from Curcuma phaeocaulis Valeton. (a) Curcumin, (b) Curdione, (c) Germacrone, (d) Curzerene, (e) Beta elemene, (f) Demethoxycurcumin, (g) Bisdemethoxycurcumin, (h) Curlone, (i) Alpha-Tumerone, (j) zingiberene, (k) alpha-Curcumene, (l) Cyclocurcumin, (m) Nerolidol, (n) Campesterol, (o) beta-Pinene, (p) Camphene, (q)Limonene, (r)Kaempferol, (s) Myrcene, and (t) beta-Farnesene.

The ligands with the strongest negative binding were chosen for visualization (Table 6). Germacrone, Cyclocurcumin, and demethoxycurcumin were determined as the three ligands with binding affinities of -7.1, -6.7, and -6.5, respectively.

Visualization of Docked Target-Ligand Complexes

Docking of Germacrone with the Modeled Target Prion Protein

Germacrone-prion Protein Interaction

There were six amino acids isoleucine, Glutamine, Valine, Histidine, and Asparginine present in this 2d diagram. All these amino acids were attached by using van der Waals interactions. Valine also used the Alkyl groups for attachment (Figure 8).

Docking of Cyclocurcumin with the Modeled Target Prion Protein

Cyclocurcumin-Prion Protein Interaction

The 2d diagram here confined four amino acids that were Serine, Methionine, Phenylalanine, and Proline. Serine and Methionine were attached through carbon-hydrogen bonds. Proline was bound using Pi-alkyl groups and Phenylalanine was bound through Pi-Pi T-shaped groups (Figure 9).

Docking of Demethoxycurcumin with the Modeled Target Prion Protein

Demethoxycurcumin-Prion Protein Interaction

Tyrosine, Glutamic acid, and Phenylalanine are the three amino acids that are constrained in this 2d interaction structure. Carbon-hydrogen bonds were employed to bind Glutamic acid. Tyrosine was attached by using Unfavourable donor-donor and Pi-Pi Stacked groups. And phenylalanine was attached through Pi-Pi T-shaped groups (Figure 10).

Table 6. Binding affinity of ligands as per the results of molecular docking.

Ligand	Binding affinity
Curcumin	-5.9
Curdione	-6.3
Germacrone	-7.1
Curzerene	-6.1
Beta - elemene	-5.7
Demethoxycurcumin	-6.5
Bisdemethoxycurcumin	-5.8
Curlone	-5.8
Alpha-Tumerone	-5.8
Zingiberene	-5.7
alpha-Curcumene	-6
Cyclocurcumin	-6.7
Nerolidol	-5.8
Campesterol	-7
beta-Pinene	-5.7
Camphene	-5.4
Limonene	-5.2
Kaempferol	-6.4
Myrcene	-4.7
beta-Farnesene	-5.7

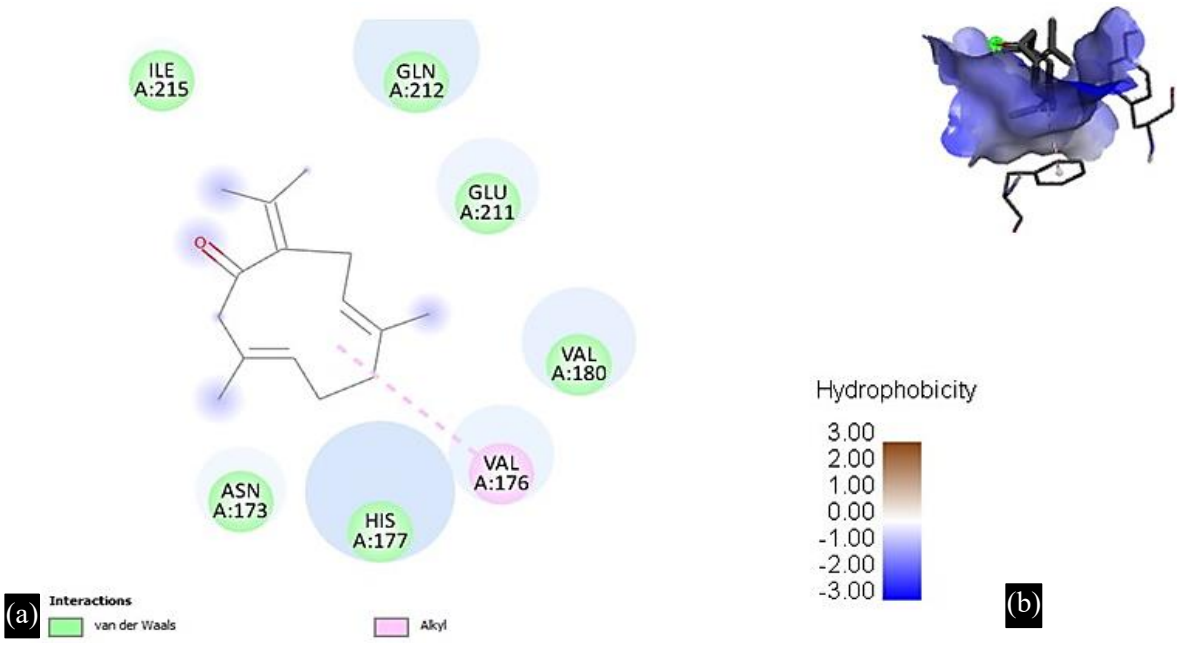

Figure 8. Visualization of Germacrone-prion protein interaction. (a) 2-dimensional, (b) 3-dimensional.

DISCUSSION

The descriptor "proteinaceous infectious particle" is the origin of the word "prion" [19]. Prions are zoonotic that, under particular conditions, are believed to be responsible for apparently transferring prion disorders among people as well as animals. The development of prions in the brain appears to occur

spontaneously in sporadic CJD, the utmost ubiquitous human prion ailment [20]. Decline of intelligence, memory loss, personality changes, diminished stability and coordination, difficulty speaking, visual impairments and blindness, irregular twitching motions, and a gradual decline in cognitive function and mobility are all hallmarks of CJD [21]. The majority of CJD patients pass away within twelve months of their symptoms manifesting, generally from infection for the reason that they are more susceptible to infection due to their immobilization [21]. Although very uncommon, they are lethal and have no cure [22]. The only kind of therapy available is palliation, and an early diagnosis is crucial for easy navigation to symptom management and final-stage care services [23].

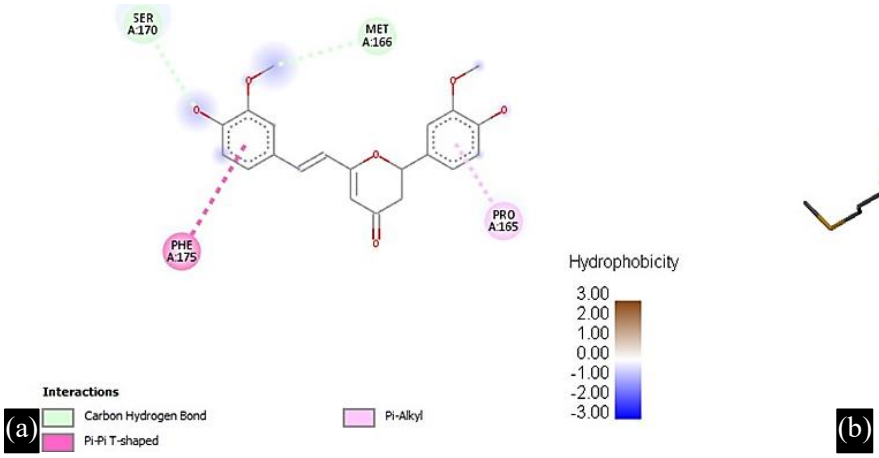

Figure 9. Visualization of Cyclocurcumin-prion protein interaction. (a) 2-dimensional, (b) 3-dimensional.

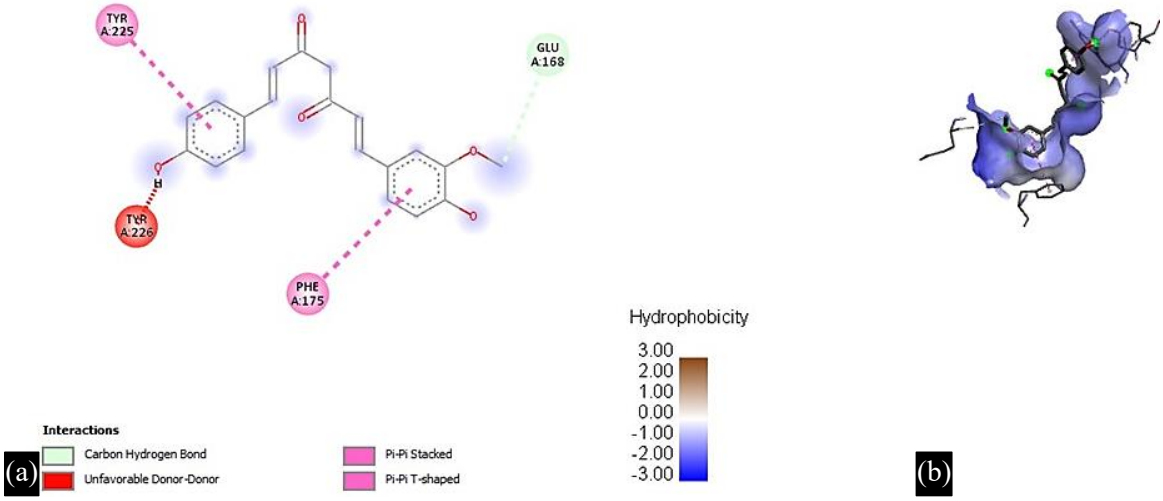

Figure 10. Visualization of Demethoxycurcumin-prion protein interaction, (a) 2-dimensional, (b) 3-dimensional.

The botanical species Curcuma phaeocaulis belongs to the Zingiberaceae family (ginger family) [24]. The name Curcuma phaeocaulis Valeton is an accepted name. The species in the genus Curcuma has this name as its recognized name [25]. This species' natural habitat extends from Jawa to Yunnan, China. It is typically found in the wet tropical habitat and is a rhizomatous geophyte [26]. In vitro cytotoxic tests and in vivo zebrafish xenograft assays reportedly demonstrated significant antitumor action within the extract of ethanol of CPV's root [27]. It has been found that Curcumin and demethoxycurcumin (DMC) of CPV have inflammasome inhibiting effects [28].

Uncovering the molecular process causing the physical modification in PrP(C) towards its pathogenic variant, PrP(Sc), is a prime objective of prion structural biology investigations [29]. The target protein selected for this experiment was *"Solution-state NMR structure of prion protein mutant V210I at neutral pH" (2LV1).* It is a membrane protein by classification [30].

The 20 phytocompounds of Curcuma phaeocaulis Valeton selected for the in-silico analysis were Curcumin, Curdione, Germacrone, Curzerene, Beta elemene, Demethoxycurcumin, Bisdemethoxycurcumin, Curlone, Alpha-Tumerone, Zingiberene, alpha-Curcumene, Cyclocurcumin, Nerolidol, Campesterol, beta-Pinene, Camphene, Limonene, Kaempferol, Myrcene, and beta-Farnesene (Figure 7). These phytochemicals of CPV encase majorly tannins, phenolic compounds, flavonoids, organic acids, and inorganic compounds, and anthocyanin [31]. Owing to their physiochemical properties, drug-likeliness, ADMET, pharmacokinetics, and toxicity predictions, these ligands were instrumental in the creation of targeted therapies (Tables 2–5).

Lipinski's Rule of 5 was developed to meet "drugability" criteria based on oral bioavailability of small doses. When the compound has two or more 5 violations, the compound should have low solubility and/or low permeability [32]. 20 selected field crops were analyzed by ADME according to Lipinski's five rules. (Table 3). It evaluated the permeability and solubility of the compounds (Table 4). Drug manufacturers can evaluate a drug candidate's safety and effectiveness attributable to ADME features, which are essential for supervisory clearance [33]. The ADME analysis was done using SwissADME. The ligands were evaluated to determine whether or not they could be easily eliminated from the system in order to gauge medication excretion (Table 4 and Figure 6). The ligands were evaluated to check if they accumulated at the peptide location and all the molecules had their toxicity estimated in order to assure safe medication intake (Table 5). After the pharmacological studies, the twenty compounds were monitored to identify the associations amid the ligand and protein complex. Germacrine, cyclocurcumin, and demethoxycurcumin were found to be the three ligands with the most effective binding affinities, with binding affinities of -7.1, -6.7, and -6.5, respectively (Table 6). In silico assessments were carried out in order to visualize their interactions with the target protein and analyse the characteristics of their amino acids in Biovia Discovery Studio. They are represented through the 2d and 3d interaction diagrams (Figures 8–10).

Signaling, immunity, and regulation of genes are dependent on protein and ligand interactions. [34]. During the interactions, the amino acids induce Van der Waals interactions, hydrogen bonds interconnections, alkyl groups, and also Pi-alkyl associations, and also the Pi-sigma bonds, which all contribute significantly in the protein-ligand stability seen during docking when the ideal configurations of each docking are attributed to molecular dynamic simulation research to study the molecular processes at work in the molecular interactions [35].

CONCLUSION

This analysis investigated the potential of using phytocompounds from the Curcuma phaeocaulis Valeton plant as a cutting-edge medication to treat Creutzfeldt-Jakob disease. The study's findings suggested that germacrone, cyclocurcumin, and demethoxycurcumin could be possible new medication candidates since they exhibited the highest binding affinities to the 2LV1 PDB ID protein. The revelation of these pharmacological qualities indicates that natural compounds could serve to be a useful foundation for the discovery and development of novel drugs to combat this crippling ailment. To ascertain the compounds' effectiveness and safety, more research is required. The work emphasizes the value of multidisciplinary research and molecular docking analysis in the hunt for new Creutzfeldt-Jakob disease drugs.

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Abbreviations

CJD:	Creutzfeldt-Jakob disease
sCJD:	Sporadic Creutzfeldt-Jakob disease
CPV:	Curcuma phaeocaulis Valeton
PrPC:	Soluble Cytoplasmic Prion Proteins
PrPSc:	Scrapie Variant Prion Proteins
BBB:	Blood-Brain Barrier
ADME:	Absorption, Distribution, Metabolism, Excretion
MR:	Molar Refractivity

REFERENCES

1. *Prion Diseases | NIH: National Institute of Allergy and Infectious Diseases*. (2020, May 22). Web.archive.org. https://web.archive.org/web/20200522095052/https://www.niaid.nih.gov/diseases-conditions/prion-diseases
2. Prusiner S. B. (1998). Prions. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(23), 13363–13383. https://doi.org/10.1073/pnas.95.23.13363
3. Shim, K. H., Sharma, N., & An, S. S. A. (2022). Prion therapeutics: Lessons from the past. *Prion*, *16*(1), 265–294. https://doi.org/10.1080/19336896.2022.2153551
4. Hermann, P., Appleby, B., Brandel, J. P., Caughey, B., Collins, S., Geschwind, M. D., Green, A., Haïk, S., Kovacs, G. G., Ladogana, A., Llorens, F., Mead, S., Nishida, N., Pal, S., Parchi, P., Pocchiari, M., Satoh, K., Zanusso, G., & Zerr, I. (2021). Biomarkers and diagnostic guidelines for sporadic Creutzfeldt-Jakob disease. *The Lancet. Neurology*, *20*(3), 235–246. https://doi.org/10.1016/S1474-4422(20)30477-4
5. Sitammagari, K. K., & Masood, W. (2023). *Creutzfeldt Jakob Disease*. PubMed; StatPearls Publishing. https://pubmed.ncbi.nlm.nih.gov/29939637/
6. Salehi, P., Clark, M., Pinzon, J., & Patil, A. (2022). Sporadic Creutzfeldt-Jakob disease. *The American journal of emergency medicine*, *52*, 267.e1–267.e3. https://doi.org/10.1016/j.ajem.2021.07.038
7. *Creutzfeldt-Jakob Disease Treatment*. (n.d.). Ucsfhealth.org. https://www.ucsfhealth.org/conditions/creutzfeldt-jakob-disease/treatment
8. Lee, S., Lee, H., Kim, J., Kim, J. H., Gao, E. M., Lee, Y., Yoo, M., Trinh, T. H. T., Kim, J., Kim, C. Y., & Ryou, C. (2022). The Effect of *Curcuma phaeocaulis* Valeton (Zingiberaceae) Extract on Prion Propagation in Cell-Based and Animal Models. *International journal of molecular sciences*, *24*(1), 182. https://doi.org/10.3390/ijms24010182
9. *Curcuma phaeocaulis in Flora of China @ efloras.org*. (n.d.). Www.efloras.org. Retrieved April 1, 2023, from http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200028371
10. Behzadi, P., & Gajdács, M. (2021). Worldwide Protein Data Bank (wwPDB): A virtual treasure for research in biotechnology. *European journal of microbiology & immunology*, *11*(4), 77–86. https://doi.org/10.1556/1886.2021.00020
11. Mohanraj, K., Karthikeyan, B. S., Vivek-Ananth, R. P., Chand, R. P. B., Aparna, S. R., Mangalapandi, P., & Samal, A. (2018). IMPPAT: A curated database of Indian Medicinal Plants, Phytochemistry And Therapeutics. *Scientific reports*, *8*(1), 4329. https://doi.org/10.1038/s41598-018-22631-z
12. Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J., & Bolton, E. E. (2021). PubChem in 2021: new data content and improved web interfaces. *Nucleic acids research*, *49*(D1), D1388–D1395. https://doi.org/10.1093/nar/gkaa971
13. Pinzi, L., & Rastelli, G. (2019). Molecular Docking: Shifting Paradigms in Drug Discovery. *International journal of molecular sciences*, *20*(18), 4331. https://doi.org/10.3390/ijms20184331

14. *PyRx Blog*. (n.d.). Pyrx.sourceforge.io. Retrieved March 30, 2023, from https://pyrx.sourceforge.io/blog#:~:text=For%20Vina%20Wizard%2C%20on%20the
15. Xiong, G., Wu, Z., Yi, J., Fu, L., Yang, Z., Hsieh, C., Yin, M., Zeng, X., Wu, C., Lu, A., Chen, X., Hou, T., & Cao, D. (2021). ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties. *Nucleic acids research*, *49*(W1), W5–W14. https://doi.org/10.1093/nar/gkab255
16. (2021). Peptide2.com. https://www.peptide2.com/peptide_calc_note.htm
17. Laskowski, R. A., Jabłońska, J., Pravda, L., Vařeková, R. S., & Thornton, J. M. (2018). PDBsum: Structural summaries of PDB entries. *Protein science: a publication of the Protein Society*, *27*(1), 129–134. https://doi.org/10.1002/pro.3289
18. *Developing Solid Oral Dosage Forms | ScienceDirect*. (n.d.). Www.sciencedirect.com. https://www.sciencedirect.com/book/9780128024478/developing-solid-oral-dosage-forms
19. Scientific American. (2018). *What Is a Prion?* Scientific American. https://www.scientificamerican.com/article/what-is-a-prion-specifica/
20. Jones, E., & Mead, S. (2020). Genetic risk factors for Creutzfeldt-Jakob disease. *Neurobiology of disease*, *142*, 104973. https://doi.org/10.1016/j.nbd.2020.104973
21. NHS. (2019). *Overview - Creutzfeldt-Jakob disease*. NHS. https://www.nhs.uk/conditions/creutzfeldt-jakob-disease-cjd/
22. Ogami-Takamura, K., Saiki, K., Endo, D., Murai, K., & Tsurumoto, T. (2022). The risk of Creutzfeldt-Jakob disease infection in cadaveric surgical training. *Anatomical science international*, *97*(3), 297–302. https://doi.org/10.1007/s12565-022-00662-x
23. De Vries, K., Cousins, E., & Harrison Dening, K. (2021). Palliative care in Creutzfeldt-Jakob disease: looking back, thinking ahead. *BMJ supportive & palliative care*, bmjspcare-2020-002799. Advance online publication. https://doi.org/10.1136/bmjspcare-2020-002799
24. *Curcuma phaeocaulis Valeton - Encyclopedia of Life*. (n.d.). Eol.org. Retrieved March 31, 2023, from https://eol.org/pages/1122299
25. *Curcuma phaeocaulis Valeton — The Plant List*. (n.d.). Www.theplantlist.org. Retrieved March 31, 2023, from http://www.theplantlist.org/tpl1.1/record/kew-235270
26. *Curcuma phaeocaulis Valeton | Plants of the World Online | Kew Science*. (n.d.). Plants of the World Online. Retrieved March 31, 2023, from https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:872383-1
27. Dong, Y., Zhao, C., Wang, X., Xie, M., Zhong, X., Song, R., Yu, A., Wei, J., Yao, J., Shan, D., Lv, F., & She, G. (2022). Lvsiyujins A–G, new sesquiterpenoids, from Curcuma phaeocaulis Valeton root tuber and their preliminary pharmacological property assessment based on ADME evaluation, molecular docking and in vitro experiments. *New Journal of Chemistry*, *46*(18), 8507–8522. https://doi.org/10.1039/D2NJ00101B
28. Nam, Y. J., Choi, J., Lee, J. S., Seo, C., Lee, G., Lee, Y., Kim, J. K., Kim, P., Lim, J. J., Choi, H. S., & Choi, Y. (2022). *Curcuma phaeocaulis* Inhibits NLRP3 Inflammasome in Macrophages and Ameliorates Nanoparticle-Induced Airway Inflammation in Mice. *Molecules (Basel, Switzerland)*, *27*(7), 2101. https://doi.org/10.3390/molecules27072101
29. Biljan, I., Ilc, G., Giachin, G., Plavec, J., & Legname, G. (2012). Structural rearrangements at physiological pH: nuclear magnetic resonance insights from the V210I human prion protein mutant. *Biochemistry*, *51*(38), 7465–7474. https://doi.org/10.1021/bi3009856
30. Bank, R. P. D. (n.d.). *RCSB PDB - 2LV1: Solution-state NMR structure of prion protein mutant V210I at neutral pH*. Www.rcsb.org. Retrieved March 31, 2023, from https://www.rcsb.org/structure/2lv1
31. Yuandani, Yuandani & Jantan, Ibrahim & Rohani, Ade & Sumantri, Imam. (2021). Immunomodulatory Effects and Mechanisms of Curcuma Species and Their Bioactive Compounds: A Review. Frontiers in Pharmacology. 12. 10.3389/fphar.2021.643119.
32. Benet, L. Z., Hosey, C. M., Ursu, O., & Oprea, T. I. (2016). BDDCS, the Rule of 5 and drugability. *Advanced drug delivery reviews*, *101*, 89–98. https://doi.org/10.1016/j.addr.2016.05.007

33. *The Role of ADME & Toxicology Studies in Drug Discovery & Development*. (2020, March 10). The Connected Lab. https://www.thermofisher.com/blog/connectedlab/the-role-of-adme-toxicology-studies-in-drug-discovery-development/#:~:text=ADME%20properties%20allow%20drug%20developers
34. Fu, Y., Zhao, J., & Chen, Z. (2018). Insights into the Molecular Mechanisms of Protein-Ligand Interactions by Molecular Docking and Molecular Dynamics Simulation: A Case of Oligopeptide Binding Protein. *Computational and mathematical methods in medicine*, *2018*, 3502514. https://doi.org/10.1155/2018/3502514
35. Niranjan, V., Rao, A., Janaki, B., Uttarkar, A., Setlur, A. S., Chandrashekar, K., & Udayakumar, M. (2021). Molecular Docking and Interaction Studies of Identified Abscisic Acid Receptors in *Oryza sativa:* An *In-Silico* Perspective on Comprehending Stress Tolerance Mechanisms. *Current genomics*, *22*(8), 607–619. https://doi.org/10.2174/1389202923666211222161006

