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Deciphering curcumin and piperine's anti-amyloidogenic capabilities using an *in silico* method: A look at ligand-target complex formation

[Descifrando las capacidades anti-amiloideogénicas de la curcumina y la piperina utilizando un método *in silico*: Una mirada a la formación del complejo ligando-diana]

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Abstract: Amyloid-β (Aβ) peptides play pivotal role in the pathogenesis of Alzheimer Disease (AD) and exert toxic effects on neurons. Current treatment options available to AD patients, such as AChE inhibitors, only provide symptomatic relief. Dietary phytochemicals are currently being used as adjuvant therapy to expedite their therapeutic efficacy. This study was designed to investigate potential bioactive compounds of curcumin and piperine as anti-amyloidogenic agents. Molecular docking was applied to conduct screening process in silico to predict the most probable conformation of curcumin and piperine ligand when they bind to Aβ42 peptide. Molecular docking screening showed that curcumin and piperine could bind to Aβ42 peptide with different binding affinities. Curcumin possessed binding affinity to Aβ42 peptide with binding energy of -5.6 kcal/mol while piperine had binding energy of -5.4 kcal/mol. Considering binding affinities, intermolecular interactions with amino acids, hydrogen bonding and hydrophobicity, curcumin emerged as potential lead compound to treat AD.

Keywords: Curcumin; Piperine; Anti-amyloidogenic; Molecular docking; *In-silico*

Resumen: Los péptidos amiloide-β (Aβ) son actores clave en el desarrollo de la enfermedad de Alzheimer (EA) y causan daño a las neuronas. Los tratamientos actuales para la EA, como los inhibidores de la AChE, solo ofrecen alivio de los síntomas. Los fitoquímicos dietéticos se están explorando como terapias complementarias para mejorar su eficacia. Este estudio investigó el potencial de la curcumina y la piperina como agentes anti-amiloideogénicos. El acoplamiento molecular se utilizó para simular la interacción de la curcumina y la piperina con el péptido Aβ42. Los resultados mostraron que ambos compuestos se unen al péptido Aβ42 con diferentes afinidades. La curcumina mostró una energía de unión de -5,6 kcal/mol, mientras que la piperina tuvo una energía de unión de -5,4 kcal/mol. Basándose en las afinidades de unión, las interacciones intermoleculares con aminoácidos, los enlaces de hidrógeno y la hidrofobicidad, la curcumina surgió como un compuesto líder prometedor para el tratamiento de la EA.

Palabras clave: Curcumina; Piperina; Anti-amiloideogénico; Acoplamiento molecular; *In-silico*

INTRODUCTION

In the 21st century, Alzheimer's disease (AD) is a representation of fundamental challenge for public health. This progressive neurodegenerative disorder was named after Dr. Alois Alzheimer who discovered changes in a 51-year-old women's brain tissue suffering from unknown mental illness in the year 1906 (US Department of Health and Human Services, 2019). This disease is the most prevalent form of dementia, which accounts for up to 60-80 % of cases (Cuomo). It is estimated that there are at least 50 million persons worldwide living with AD or the other dementias, and this figure is expected to skyrocket to about 152 million by the year 2050 (Patterson, 2018). These cases have incurred huge cost of worldwide dementia care, which is about US$ 1 trillion. Apparently, AD has caused huge economic impacts on the developed and developing countries and AD is still on the rise throughout the world.

While the detailed etiopathogenesis of this multifactorial disease has not been fully explained, it is described that one of the classic hallmarks of AD is extracellular amyloid-β (Aβ) plaques (Giuffrida *et al*., 2009). The pathological production of Aβ peptide is the prime factor of neuronal death and dysfunction, leading to dementia, as stated in amyloid cascade hypothesis. Most of the researchers endorse amyloid cascade hypothesis because of the recent genetic linkage studies and the tendency of Aβ peptide to aggregate that lead to neurotoxicity (Verdile *et al*., 2004). After conducting the complete sequencing of this peptide, it is revealed that the amount of amino acid residues fell between 39 and 43. Aβ42 peptide, the target used in this study, is made up of 42 amino acid residues and amphipathic due to the presence of hydrophilic N-terminal segment and hydrophobic 12-14 C-terminal amino acids (Finder & Glockshuber, 2007). Hence, Aβ42 peptide is the focus of this study due to the peptide's pathogenicity.

To date, there is no treatment available to slow or stop the damage or destruction of neurons. The FDA-approved drugs for AD, including donepezil, memantine, memantine combined with donepezil, rivastigmine, galantamine and tacrine, treat the AD symptoms only without curing the AD patients. To develop pharmacologic treatment that treats the underlying causes of AD, an attempt was made to assess the ability of curcumin and piperine as a potential lead candidate targeting Aβ42 peptide in the drug discovery process of AD via molecular docking. Researchers nowadays have great interests in inherent bioactive compounds that have biological effects like antioxidant, and anti-inflammatory properties. Curcumin from *Curcuma longa* and piperine from *Piper nigrum* were proven to provide these cognitive benefits. These compounds have been used as memory-boosters and anti-aging tonics (Mao *et al*., 2017). Since loss of memory is one of the features of AD, preliminary screenings of these natural products are important in this study to determine the viabilities to be a potential lead compound which targets Aβ42 peptide in the drug discovery process of AD. Curcumin has been applied in the medical and culinary field for a long period of time in Asia. Based on the recent epidemiological reports, individuals who used curcumin in their normal diet had better cognitive performance than those who did not (Ng *et al*., 2006). In a study, KLVFF motif in Aβ42 peptide had strong interaction with curcumin through hydrophobic forces and hydrogen bonds (Kumaraswamy *et al*., 2013). In addition, Chaiwiang *et al*. (2016), described that the spatial memory in mice has greater improvement by using piperine than by donepezil in a Morris water maze test. Molecular docking was applied to conduct the screening process *in silico* in this study. This computational method can predict the most probable conformation of a ligand when the ligand binds to a protein (Quiroga & Villarreal, 2016). The data obtained on affinity and mechanistic aspects of an interaction between a lead candidate and Aβ42 peptide can provide better comprehension of structure-activity relationships and mechanistic aspects of actions, which are important to select the best chemical class for further optimization (Erlanson *et al*., 2004). This study is significant in discovering the bioactive compounds that can slow or stop AD progression. This study may also be able to expand the range of available options to help the patients and the caregivers to cope with AD and improve the quality of life.

MATERIALS AND METHODS

Figure No. 1 depicts the research workflow summary. In summary, two ligand molecules, curcumin and piperine, were tested for their binding patterns using the Aβ receptor as a target. The investigation of their binding sites was conducted using molecular surface and intermolecular interaction analyses.

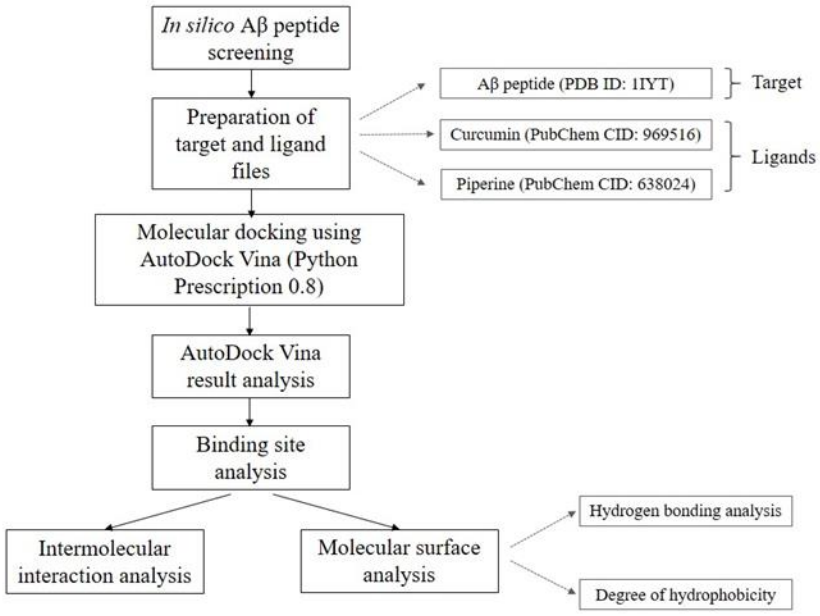

Figure No. 1
Summary of computational approach conducted in this study

In silico Aβ42 peptide screening preparation of target and ligand files

The structure of Aβ42 peptide (PDB ID: 1IYT) was downloaded from Protein Data Bank (PDB) in RCSB PDB format. The retrieved pdb file (PDB ID: 1IYT) was modified and stabilized by BIOVIA Discovery Studio Visualiser (DSV) 2019. Any water and heteroatom (HETATM) detected from the window were deleted. Since water molecules were not involved in the binding process, the ligand pose predicted may be affected by the presence of water around the binding regions of the target. Water molecules were loosely bound and can be easily displaced by ligands (Koellner *et al*., 2000). The computational work was made easier and the docking accuracy was improved by removing the water molecules (Wong & Lightstone, 2011). Polar hydrogen atoms were added to the protein because hydrogen atoms were important to the protein *in vivo* but was failed to be captured by x-ray crystallography (Woińska *et al*., 2016). The addition of polar hydrogen atoms rectified the 'lost' atoms to better mimic the *in-vivo* structure in this study. Polar hydrogen atoms were also crucial for the formation of hydrogen bond between ligand and target (Patrick, 2023). The processed protein was then saved in PDB format. The structures of curcumin (PubChem CID: 969516) and piperine (PubChem CID: 638024) were downloaded from PubChem in three-dimensional (3D) structure data format (SDF). The 3D conformer files of curcumin and piperine were converted to .pdb format in PyMOL software. PyMOL is a source-available molecular visualization system created by Warren Lyford DeLano. It is commercialized initially by DeLano Scientific LLC, as a private software company dedicated to creating useful tools that become universally accessible to scientific and educational communities. The PDB files of ligands were submitted to DSV. The removal of water from the ligands and the addition of hydrogen atoms to the ligands were performed in similar fashion as the protein PDB file to stabilize the ligands and allow the ligands to fit into the active site easier. The processed ligands were also then saved in .pdb format.

Molecular docking using AutoDock Vina

Docking of the ligand to Aβ42 peptide was performed in PyRx – Python Prescription 0.8. PyRx is a program designed for virtual screening in computational drug discovery. It is useful for screening chemical libraries against putative targets for drugs. It is a useful tool for computer-aided drug design since it has a docking wizard and an intuitive user interface. In addition, PyRx has a strong visualization engine and features akin to a chemical spreadsheet, which are crucial for structure-based drug creation. The protein and the compound to be used in docking were set as macromolecule and ligand respectively in PyRx and

the output files were automatically converted to AutoDock.pdbqt format files, whereby 'q' indicated the added charged whereas 't' indicated the stored information of atom types which were conformed to AutoDock's algorithm (Rizvi *et al*., 2013). AutoDock Vina and AutoDock 4.2 were used as the docking software in PyRx. Software for simulating molecular modeling is called AutoDock. It works particularly well for protein-ligand docking. AutoDock 4 is offered under the terms of the General Public License. It is among the most frequently used docking software programs. On the other hand, among the most popular and quick open-source docking engines is AutoDock Vina. It is a readymade computational docking application built on a fast gradient-optimization conformational search and a straightforward scoring algorithm.

Docking process was conducted in PyRx after completing the setting of macromolecule and ligand. The grid was adjusted prior to docking, and the center position and dimensions of the grid box for each ligand were recorded, as shown in Table No. 1. The docking space on the target was defined by a 3D cuboidal grid box and ligands would explore possible binding interactions within the space. The accuracy of pose prediction could be enhanced by optimizing the grid box size. Irrelevant binding conformations would be generated in an excessively large space. A too small area would omit the key amino acids during the docking process (Feinstein & Brylinski, 2015).

The exhaustiveness level was set to 10 so that 10 times of scoring calculations were performed concurrently, which was equivalent to 10 runs for every execution of the docking process with the starting conformation of ligand randomized at each run. The total number of simulations for each ligand-target pair was 100 after repeating the docking process for 10 times. The results were shown after each docking and the time taken for each docking process was different for each ligand-target pair, depending on the input file complexity and the bond formation (Lindstrom *et al*., 2008).

AutoDock Vina result analysis

Three types of information were obtained from AutoDock Vina, including the binding energy, the visualization of binding conformation, and the lower and the upper bound root mean square deviation (RMSD) value of each docking mode. The root mean square deviation (RMSD) between corresponding atoms of two protein chains is a commonly used measure of similarity between two protein structures. In bioinformatics, RMSD is the measure of the average distance between the atoms (usually the backbone atoms) of superimposed molecules. The RMSD is 0 for identical structures, and its value increases as the two structures become more different. The RMSD is being computed for two groups of atoms and all frames in the trajectory belonging to atom group, and it can be calculated by finding the square root of the mean square error. The values of the RMSD between the crystal and the predicted structures are widely used to confirm whether a close-match docked pose was predicted or not by the docking simulation, whereas, an RMSD value ≤ 2 Å is fairly good (Raschka, 2014). The binding energy values with the corresponding RMSD values, and the docked complex structure were saved as comma- separated values (CSV) and SDF respectively for further analysis.

Table No. 1
The centre position and dimension of the grid box for each ligand prior to docking in PyRx

Ligands	Centre	Dimensions (Angstrom)
Curcumin	X = -5.1041 Y = -0.0652 Z = 1.1930	X = 37.2304 Y = 25.0000 Z = 51.9781
Piperine	X = -4.2491 Y = -0.0652 Z = -0.0399	X = 37.2244 Y = 25.0000 Z = 50.0292

The centre position and dimension of the grid box for each ligand prior to docking in PyRx

Binding site analysis

The docked complexes obtained from AutoDock Vina were converted to files in PDB format by PyMOL and then the PDB files were open in DSV 2019. The 3D docked complex was shown and the binding site was analyzed in DSV 2019.

Intermolecular interaction analysis

A 2D diagram and a 3D interaction diagram of each ligand-target pair of the lowest binding energy were created by DSV 2019. Information about type of bonds, the interacting residues involved in the bond formation, and bond distance obtained from DSV 2019 were recorded.

Molecular surface analysis

Docked compound of the lowest binding energy was subjected to hydrogen bonding and hydrophobicity surface analyses in DSV 2019. The binding pocket with the docked ligand was colored based on the type of surface analysis conducted. Purple and green colors were applied in hydrogen bonding analysis whereas brown and blue colors were used in hydrophobicity map.

RESULTS AND DISCUSSION

Computational approach

Analysis of binding affinity and binding energy

The results for the binding energies of the highest frequency for each ligand-target pair were listed after conducting 100 docking runs for each ligand and data sorting (Table No. 2). The binding energy values were recorded when both root mean square deviation (RMSD) upper bound (U.B.) and lower bound (L.B.) values of each ligand were 0. All the docked ligands showed RMSD values of 0, which were lower than 2.0 Å, indicating that all the dockings were successful (Allen & Rizzo, 2014). The binding energies of both ligands were negative, revealing that generally, they had favorable and stable binding to Aβ42 peptide. The binding energy of curcumin docked to Aβ42 peptide was recorded as the lowest (-5.6 kcal/mol), followed by piperine (-5.4 kcal/mol).

Two-dimensional (2D) interaction diagram analysis

The flattened graphical views of ligands docked to Aβ42 peptide, created by BIOVIA DSV 2019, were displayed (Figure No. 2 and No. 3). The intermolecular interactions and the interacting residues from Aβ42 peptide were displayed as dashed lines and colored discs respectively. Curcumin interacted with Tyr10, His6, His13, His14, Asp7, Glu3, Gly9 and Leu17 from Aβ42 peptide (Figure No. 2). Docking results also revealed that Leu34, Phe20, Val24, Ala21, Ile31, Ala30 and Gly33 from Aβ42 peptide involved in the intermolecular interactions formation with piperine (Figure No. 3), and color codes of non-covalent interactions (Figure No. 4).

Table No. 2

Binding energy of curcumin and piperine docked to Aβ42 peptide, with RMSD upper bound (U.B.) and lower bound (L.B.) values of 0

Target	Ligand	Binding energy (kcal/mol)	RMSD U.B. (Å)	RMSD L.B. (Å)
Aβ42	Curcumin	-5.6	0.0	0.0
	Piperine	-5.4	0.0	0.0

Binding energy of curcumin and piperine docked to Aβ42 peptide, with RMSD upper bound (U.B.) and lower bound (L.B.) values of 0

Three-dimensional (3D) interaction diagram analysis

The 3D interaction views between Aβ42 peptide and each ligand were created by BIOVIA DSV 2019 and the magnified views were displayed (Figure No. 5 and No. 6). It was shown that Aβ42 peptide had 2 helical segments interrupted by a 'kink' region that adopted disordered or β-turn conformation. This morphology was normally observed in water-alcohol mixtures or micelle solubilizations (Zhang *et al*., 2000). The intermolecular interactions were displayed with different colors in these figures and in the lists of the interactions (refer to Supplementary File).

Molecular surface analysis

Molecular surface analysis was categorized into two sections, namely hydrogen bonding and degree of hydrophobicity.

Hydrogen bonding analysis

The location of hydrogen bond donor and acceptors were determined by analyzing Figure No. 7 and No. 8 created by BIOVIA DSV 2019. The surfaces were colored at regions that hydrogen bonds may be formed. Hydrogen bond donor and hydrogen bond acceptor were labelled as purple and green respectively. A greater amount of hydrogen bond donors was discovered in molecular surface of Aβ42 peptide docked with curcumin (Figure No. 7)

than piperine (Figure No. 8) and thus, oriented in a manner that the polar groups were concentrated in the green regions and maximized the hydrogen bond formation. Piperine had lower probability of forming hydrogen bonds with Aβ42 peptide because of the scarcity of purple or green colors on the surface (Figure No. 8).

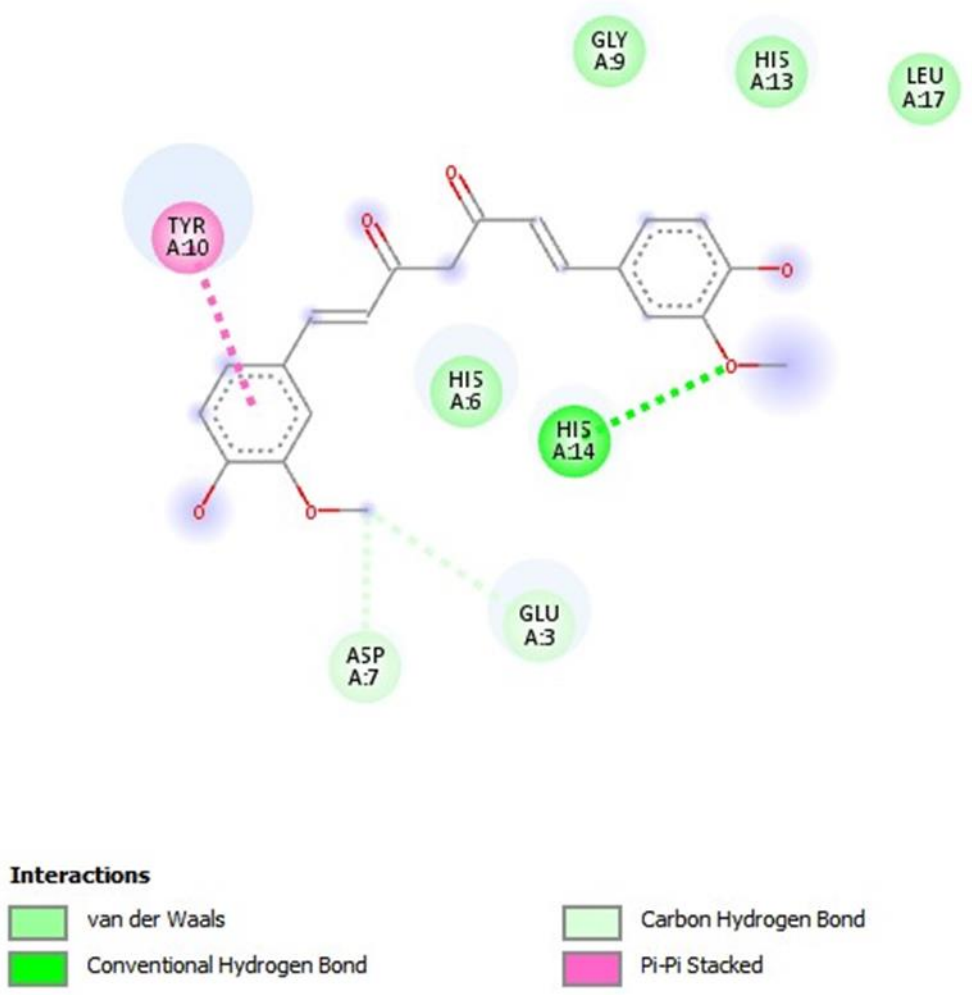

Figure No. 2
The 2D view and the list of intermolecular interactions between Aβ42 peptide and curcumin were created using BIOVIA DSV 2019. The chemical structure shown was curcumin which interacted with Tyr10, His6, His13, His14, Asp7, Glu3, Gly9 and Leu17 from Aβ42 peptide

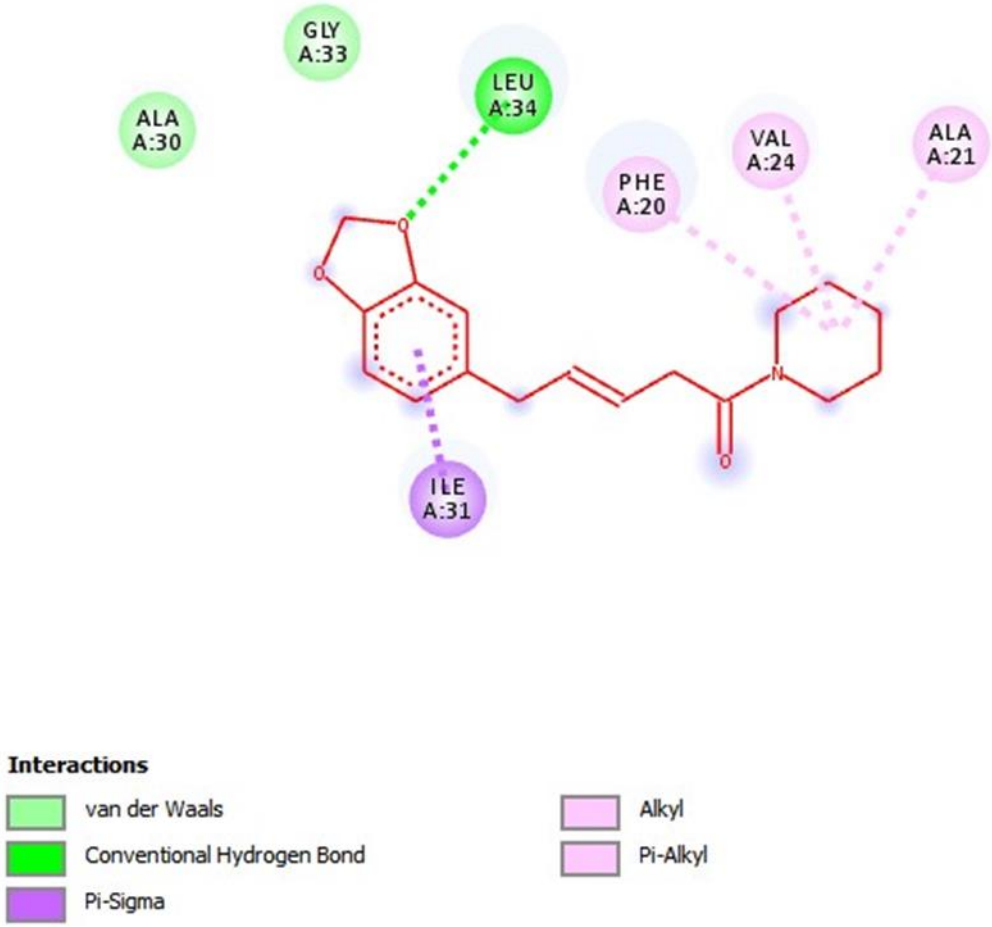

Figure No. 3
The 2D view and the list of intermolecular interactions between Aβ42 peptide and piperine were created using BIOVIA DSV 2019. The chemical structure displayed was piperine which interacted with Leu34, Phe20, Val24, Ala21, Ile31, Ala30 and Gly33 from Aβ42 peptide

Figure No. 4
Color codes of non-covalent interactions which were classified into hydrogen bond, electrostatic, hydrophobic, and unfavorable interactions in BIOVIA DSV 2019

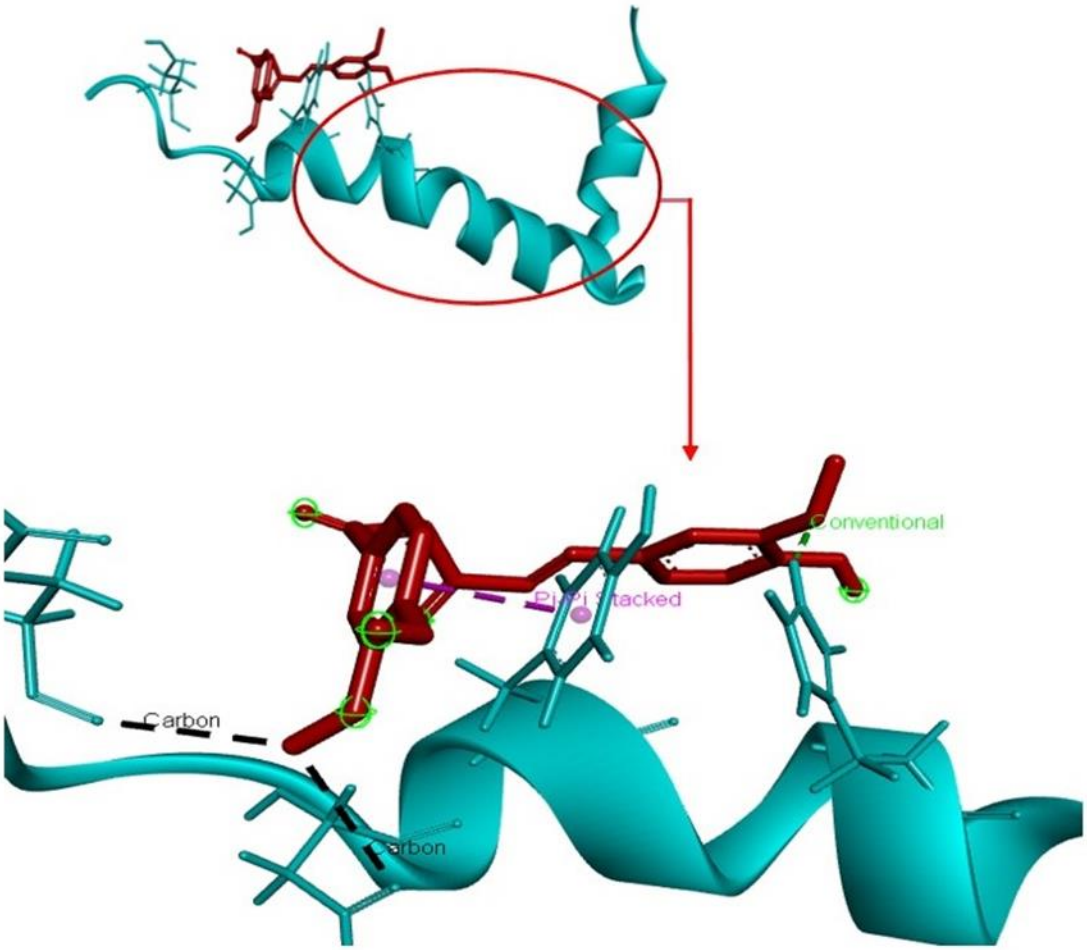

Figure No. 5
The 3D and zoomed-in view of intermolecular interactions between Aβ42 peptide and curcumin using BIOVIA DSV 2019

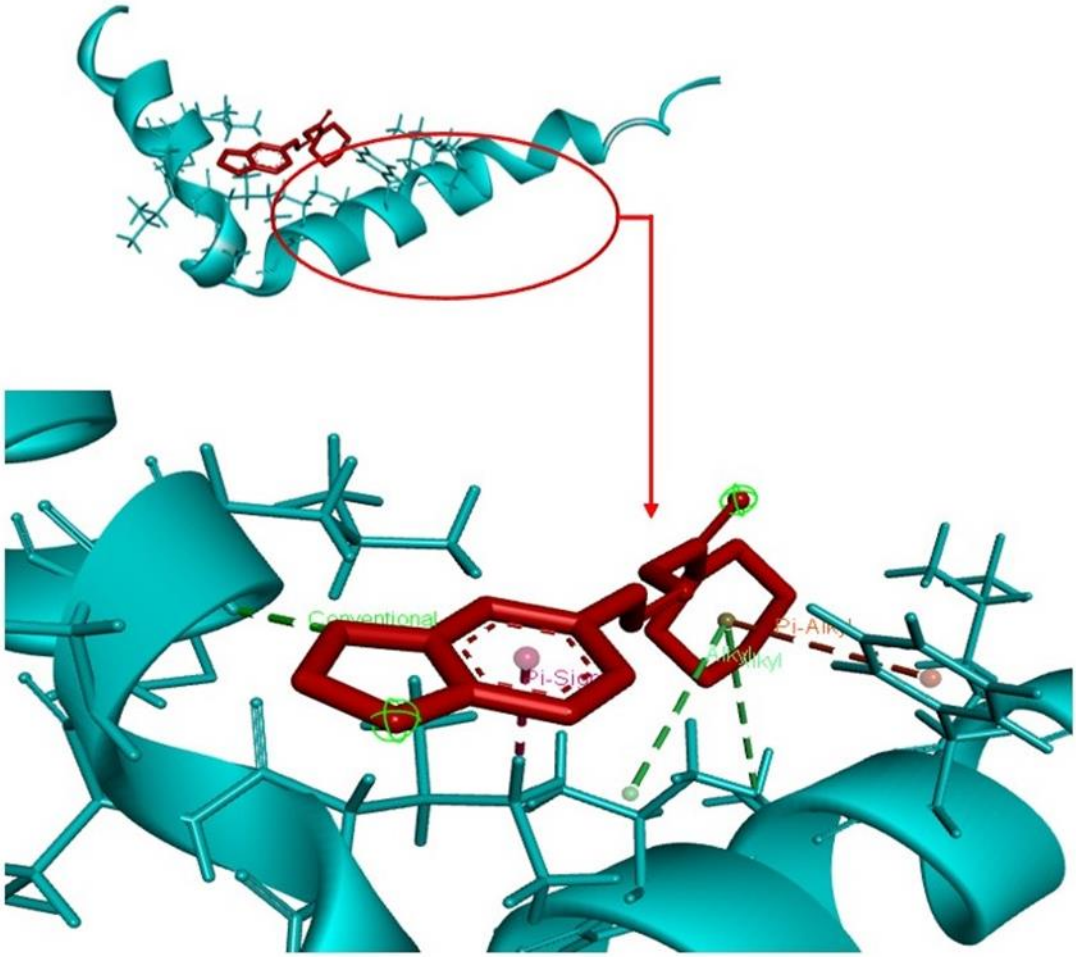

Figure No. 6
The 3D and zoomed-in view of intermolecular interactions between Aβ42 peptide and piperine using BIOVIA DSV 2019.

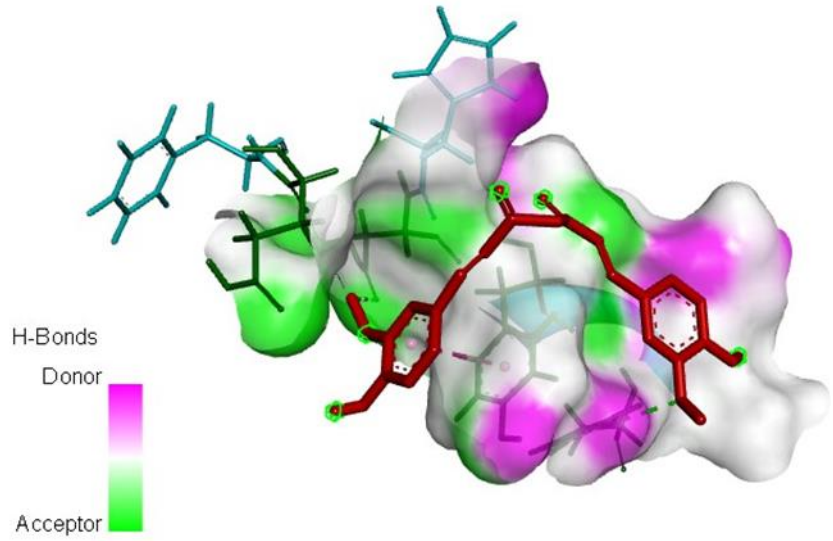

Figure No. 7
The hydrogen bonding of the molecular surface of Aβ42 peptide docked with curcumin using BIOVIA DSV 2019

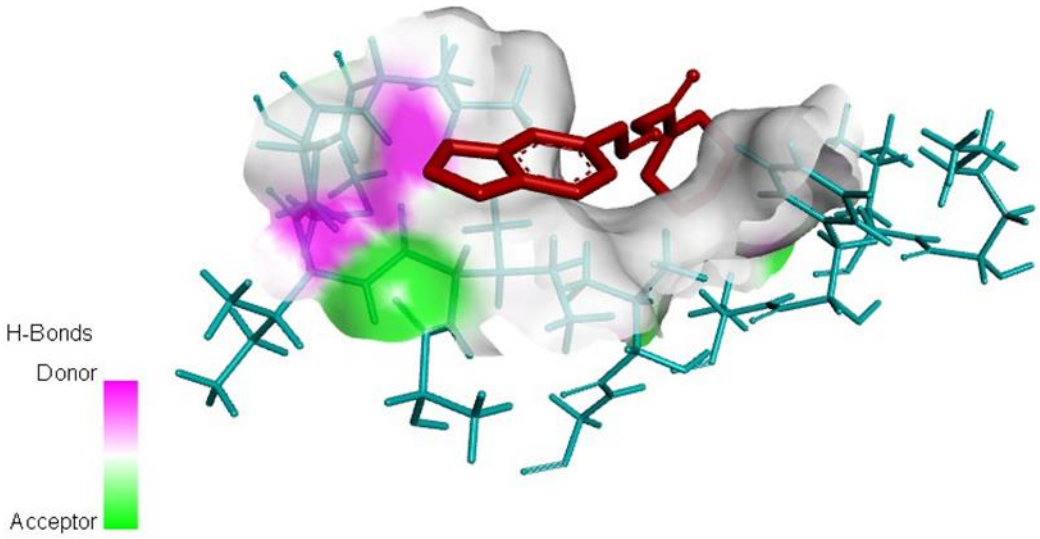

Figure No. 8
The hydrogen bonding of the molecular surface of Aβ42 peptide docked with piperine using BIOVIA DSV 2019

Analysis of hydrophobicity maps

The hydrophobic maps (Figure No. 9 and No. 10) were created by BIOVIA DSV 2019. Largest area of high hydrophilicity was observed on the molecular surface of Aβ42 peptide bound with curcumin (Figure No. 9). On the other hand, biggest area of high hydrophobicity was observed on the molecular surface of Aβ42 peptide bound with piperine (Figure No. 10).

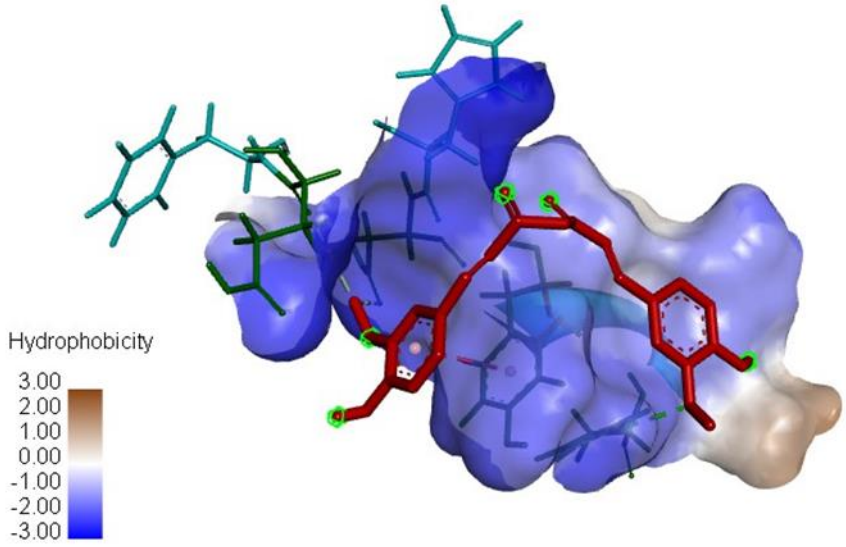

Figure No. 9
The hydrophobicity degree of the molecular surface of Aβ42 peptide docked with curcumin using BIOVIA DSV 2019

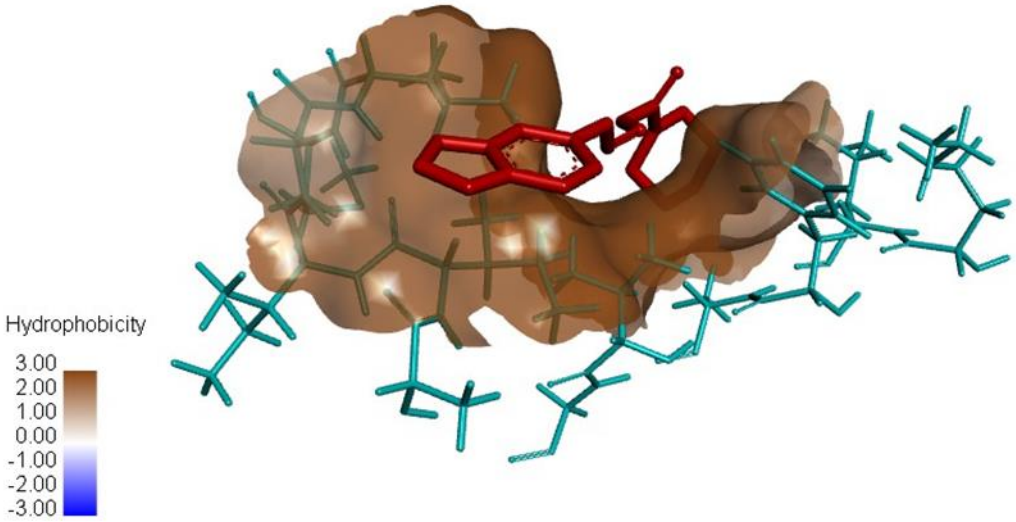

Figure No. 10
The hydrophobicity degree of the molecular surface of Aβ42 peptide docked with piperine using BIOVIA DSV 2019

Medicines nowadays were obtained directly from natural sources or developed from a lead compound that originated from natural sources. It is because natural products are rich sources of biologically active compounds, also known as active principles. Compounds which are principally responsible for the natural extract's pharmacological activity can be isolated and used as a lead compound. Hence, natural products are still the most important root of novel compounds for therapeutic drug development (Patrick, 2023). Since Asia has an immense range of floras and medicinal herbs, explaining the bioactivity of the phytochemicals will help to understand the mechanisms of action and potential adaptations into pharmaceutical drug (Jantan *et al*., 2015). According to the folk literature, curcumin and piperine possess nootropic benefits (Goozee *et al*., 2016). In this study, filtering of potential lead compounds in drug discovery of AD was performed on these compounds by molecular docking, an *in-silico* approach which saved cost and efficient. When the ligands were bound to the target, the energies released or consumed were represented by binding energies (Purich, 2010).

The overall energy of the ligand-target complex decreased because the binding happened spontaneously without consuming energy. The absence of positive score indicated that unfavorable binding, which consumed energy, was not formed. On an arbitrary scale, the lower is the binding energy, the greater the binding affinity and the stability of the ligand-target complex are, and vice versa (Du *et al*., 2016). According to the binding affinities of the ligands, the binding between curcumin and Aβ42 peptide would be more stable than that of piperine.

Van der Waals (vdW) interactions were depicted in 2D interaction diagrams (Figure No. 2 and No. 3). Curcumin had 4 vdW interactions with the target, which were more than that of piperine by 2 vdW interactions. In fact, vdW is a relatively weak electric force and the ligands must be close enough to the binding region of Aβ42 peptide for interactions to occur (Schaeffer, 2008). Hence, the other types of binding interactions like hydrogen bonds were more significant. The vdW between the ligand and the target arose from the interaction of quantum-mechanical fluctuations in the electronic charge density and formed between hydrophobic regions of the ligand and the target (Stöhr *et al*., 2019). Transient areas of high and low electron densities formed temporary dipoles which contributed to the vdW interactions. This type of interaction was important in defining shape complementarity but was frequently neglected because the slope of Lennard-Jones (L-J) potential was steep and sensitive to steric clashes which would cause penalty of score (Fahmy & Wagner, 2002). vdW was also neglected within (semi-)local density functional approximations or Hartree-Fock theory (Stöhr *et al*., 2019). Molecular size and structure of the ligands were one of the

crucial aspects of influencing the binding energy by affecting the number of intermolecular interactions formed with the target (Atkovska *et al*., 2014). Since curcumin ($C_{21}H_{20}O_6$) has higher number of atoms than piperine ($C_{17}H_{19}NO_3$), it was initially assumed that the lower binding energy observed in curcumin was due to the greater amount of possible intermolecular interactions formed with the target, producing a more stable docked complex. However, the number of intermolecular interactions formed between curcumin and Aβ42 peptide was lesser than that of piperine by 1 interaction. The difference in binding energy observed can be further explained through the type of intermolecular interactions formed with Aβ42 peptide (Abdul *et al*., 2019). An understanding of non-covalent interactions in ligand-target complexes was important for rational drug design and appreciation of drug action mechanisms. The low binding energies of ligands are attributed to the presence of hydrogen bonds which produced a more stable docked complex. Hydrogen bond is a unique dipole-dipole interaction between a hydrogen atom that is strongly bonded to a highly electronegative atom in vicinity (Gauvin *et al*., 2020). According to Schaeffer (2008), the free energy for hydrogen bonding was normally in the range of -12 kJ/mol to -20 kJ/mol and the binding affinity increased by approximately 1 order of magnitude per hydrogen bond Schaeffer. Rashka *et al*. (2018), also concluded that strong hydrogen bonds were responsible for majority of high-affinity ligands by analyzing the type of interactions discovered in 136 non-homologous protein-ligand complexes. As for the small difference in binding energy noted between curcumin and piperine, the greater number of hydrogen bonds formed in curcumin- Aβ42 peptide complex was the prime reason, and this was facilitated by two circumstances. Firstly, curcumin had 1 additional electron-deficient hydrogen atom that can be attracted to an electron pair from the residues of the target, when compared to piperine. More importantly, curcumin had additional 3 highly electronegative oxygen atoms with electron pairs, acting as hydrogen-bond acceptors, which could interact with the electron-deficient hydrogen atom. The strong binding of curcumin to the target was mostly due to the hydrogen bonds between His14 from Aβ42 peptide and functional group UNK0: O (bond length of 2.29681 Å). Pauling (1960), described that any hydrogen bond length shorter than 2.6 Å indicated that the hydrogen bond was noteworthy and strong, forming a more stable docked complex (Pitzer, 1960). Curcumin had a hydrogen bond which fitted the criteria mentioned by Pauling (1960), and thus, the highest binding affinity of curcumin was observed.

Hydrophobicity analysis plays a crucial role of providing a preliminary notion whether the compound possesses the ability to penetrate blood-brain barrier (BBB), in which the barrier has closely packed cells lining the capillaries with few or existing pores, and the capillaries have coatings of fat cells (Galea *et al*., 2007). The relative distribution of drug in an n-octanol/water, also known as partition coefficient (P), is used as a reference to hydrophobicity analysis as log P value is commonly used as a measure of hydrophobicity in drug design (Patrick, 2023). The equation is:

$$p = \frac{\textbf{Concentration of drug in octanol}}{\textbf{Concentration of drug in aqueous solution}}$$

Since P value is directly proportional to the concentration of drug in octanol, piperine with the greatest hydrophobicity will have the highest *p* value and thus the highest log P value whereas curcumin will have the lowest log *p* value

This was deduced because hydrophobic molecules preferred to dissolve in n-octanol layer whereas hydrophilic molecules preferred to dissolve in aqueous layer (Moldoveanu & David, 2016). Piperine with the highest log P value would possess stronger penetrance ability across BBB in the central nervous system (CNS) because piperine was hydrophobic enough to dissolve through the cell membranes of the capillaries and the fatty cells coating the capillaries. However, very hydrophobic drug was expected to dissolve in fat globules in the gut if administered orally and to be poorly absorbed and sparingly soluble in blood if injected, resulting in low bioavailability of the drug (Patrick, 2023). Therefore, the physiochemical properties of the ligands were important and needed to be optimized

for CNS- active drug design, allowing the passive permeation across the BBB. Piperine should also be hydrophilic enough to dissolve in aqueous solutions like blood which distributes piperine to the brain. On the other hand, curcumin should be hydrophobic enough to penetrate the BBB to bind to Aβ42 peptide. If curcumin was too hydrophilic, curcumin may be expelled very fast and could not act on Aβ42 peptide for a desired period to exert the therapeutic effect. For instance, the BBB permeability could be enhanced by increasing the lipophilicity (Gao & Gao, 2018).

Based on Lipinski's Rule of Five, there were 5 integral physiochemical parameters, including molecular weight, lipophilicity, charge, polar surface area and hydrogen bonding. Lipinski's analysis demonstrated that drug with molecular weight lower than 500 g/mol, 5 hydrogen bond donors or less, 10 hydrogen bond acceptors or less and log P value lower than 5, had higher probability to be absorbed orally (Lipinski *et al*., 1997). Curcumin and piperine are good candidates for orally absorbed drug. Curcumin has a molecular weight of 368.4 g/mol, 2 hydrogen bond donors, 6 hydrogen bond acceptors and a log P value of 3.2, that obeyed all the rules (Reyes *et al*., 1923). Besides, curcumin also has similar structure with N-N'-bis(3-hydroxyphenyl)pyridazine-3,6-diamine, a beta-sheet breaker. It is proposed that the symmetric and compact structure of curcumin allows its specific binding to Aβ peptide and any changes in the 2 aromatic end groups of curcumin will alter its activity. In addition, piperine has a molecular weight of 285.34 g/mol, 3 hydrogen bond acceptor and a log P value of 3.5 without any hydrogen bond donor (Reyes *et al*., 1923). Hence, piperine is also suitable for oral consumption.

Combinatorial treatment using curcumin and piperine may be effective to deliver the drug to the brain across BBB and bind to Aβ42 peptide, and possibly reversed the Aβ-induced up-regulation of neuronal oxidative stress. Combinatorial treatment using curcumin and piperine may be effective to deliver the drug to the brain across BBB and bind to Aβ42 peptide. It was proven that co-supplementation of curcumin with 20 mg of piperine significantly increased the bioavailability of curcumin by 2,000% without any obvious side effect observed (Patil *et al*., 2016). This reduced the toxicity concern even if piperine and curcumin must be consumed in huge amounts to reach the therapeutic effects. What's more, piperine enhanced the neuroprotective effects of curcumin against cognitive dysfunction caused by mild traumatic brain injury in rats, possibly by modulating oxidative-nitrosative stress induced neuroinflammation and apoptosis (Rinwa & Kumar, 2012). Abdul *et al*. (2019), also suggested that synergistic behavior between curcumin and piperine gave a stronger protective effect in cells exposed to cytotoxic Aβ. Hence, it is suggested to conduct docking of complex formed by piperine and curcumin to Aβ42 peptide in the future to determine the underlying mechanisms of the synergistic effect which may be an alternative to treat AD.

Anti-amyloidogenic potential

Anti-amyloidogenic potential of the ligands are crucial in treating patients with AD. It is postulated that binding of curcumin to Aβ42 peptide with high affinity can be useful against Aβ aggregation, either Aβ oligomerization or fibril formation. The successful binding may inhibit the production of insoluble Aβ, and this strategy is important to eliminate the cytotoxic effect caused by insoluble Aβ oligomers and protofibrils in neuronal cells. The induction of reactive oxygen species (ROS) by Aβ can also be avoided. In fact, accumulation of ROS in brain cells will damage deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins and eventually, decrease the number of functional neurons. The binding of curcumin to the target may further disrupt the inflammation related with amyloid deposition, preventing neuronal degeneration and the formation of neurotoxic lesions. Thus, the number of functional neurons and synapses will remain high when compared to untreated AD patients. If this postulation holds true, the use of curcumin as a drug against Aβ42 peptide can prevent or delay the downstream aggregation and the formation of the pathologies. It is expected to detect low level of Aβ plaques in individuals treated with curcumin in positron emission tomography (PET) scan. These treated patients will have uncompromised cognitive function and therefore, he or she can possess the normal mental processes which allow the receiving, selection, storage, transformation, development and recovery of information from the external stimuli. The treated patients also will have no difficulty of making sense of the world around him or her and can accomplish familiar tasks without consuming time longer than the usual. Besides, selectivity acts as an integral parameter to discriminate between interaction partners (Nominé *et al*., 2015). Curcumin with the greater binding affinity to Aβ42 peptide indicated that curcumin would have higher selectivity to Aβ42 peptide and show lesser cross-reactivity than

piperine. Curcumin would have lesser chance to interact with different targets, producing lesser undesirable side effects *in-vivo* than piperine. In the future, it is worth to consider pan-assay interference compounds (PAINS) characteristics of curcumin in the evaluation of curcumin as a suitable lead candidate targeting Aβ42 peptide (Nelson *et al*., 2017). The synergistic behavior from curcumin and piperine should also be considered as the binding energy of curcumin (-5.6 kcal/mol) and piperine (-5.4 kcal/mol) did not differ much from each other. The projected increase of AD incidences creates an impetus for development of effective and new therapeutic strategies, and the findings from this study may serve as a foundation for any related research in the future. It is hoped that these findings will further the study of natural compounds as possible treatments and cures for AD. Overall, this study supports the advantages of co-effects from many medications in the treatment of difficult disorders like AD. AChE inhibitors, one of the current therapy alternatives for AD patients, merely relieve symptoms. In this work, we examined four natural substances, including piperine and curcumin, which are thought to have cognitive effects. Researchers are particularly interested in natural goods, since these items' intrinsic bioactive chemicals are known to have a variety of positive biological benefits (Bui & Nguyen, 2017). This study looks into natural substances that are thought to have advantages for cognition. Curcumin (Curcuma longa) and piperine (Piper nigrum) are two traditional culinary herbs and therapeutic medicines in Asia. Natural products continue to be the most important source of new molecules for the development of therapeutic medications because of their unmatched structural diversity and bioactive components (Yuan *et al*., 2016).

CONCLUSIONS

The screening of curcumin and piperine as a potential lead compound to treat AD was conducted via molecular docking to Aβ42 peptide. Curcumin and piperine could bind to Aβ42 peptide with slightly different binding affinities. Considering binding affinities, number and types of intermolecular interactions with key amino acids, hydrogen bonding and hydrophobicity, curcumin emerged as the most suitable potential lead compound which targeted Aβ42 peptide and became the hopeful drug molecule to treat AD. Curcumin possessed the highest binding affinity to Aβ42 peptide with a binding energy of -5.6 kcal/mol and strongest interactions, mainly contributed by 3 hydrogen bonds. Based on Lipinski's Rule of Five, both curcumin and piperine were suitable to be designed as orally absorbed drug. The findings from this study could be used as preliminary data for in-vitro and in-vivo studies. It is necessary to do more research to determine whether the effects also entail complicated AChE and Aβ interactions, as well as amyloidogenic and antioxidant qualities in various curcumin and piperine routes. Moreover, synergistic behavior suggests that using these two chemicals together at a lower dose might produce better results than using them separately. There are presently ongoing molecular experiments as well as animal research, before it reaches to human patients. It is also suggested to conduct structure-activity relationship (SAR) analysis and identification of pharmacophore in the future to determine which structural part of curcumin is important in the binding with Aβ42 peptide.

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CONFLICT OF INTEREST

There is no conflict of interest for this study as corresponding author is sole author for this study and data collection.

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SUPPLEMENTARY MATERIALS
SUPPLEMENTARY FILE A
Table No. A1
The list of interactions showing the colours of interactions used in Figure No. 5, types and distances of interactions between Aβ42 peptide and curcumin using BIOVIA DSV 2019

From	To	Colour	Category	Types	Distance (Å)
A:HIS14:HE2	:UNK0:O		Hydrogen Bond	Conventional hydrogen bond	2.51681
:UNK0:C	A:GLU3:O		Hydrogen Bond	Carbon hydrogen bond	3.51956
:UNK0:C	A:ASP7:OD1		Hydrogen Bond	Carbon hydrogen bond	3.59385
:UNK0	A:TYR10		Hydrophobic	Pi-pi stacked	3.75019

Table No. A2
The list of interactions showing the colours of interactions used in Figure No. 6, types and distances of interactions between Aβ42 peptide and piperine using BIOVIA DSV 2019

From	To	Colour	Category	Types	Distance (Å)
A:LEU34:HN	:UNK0:O		Hydrogen Bond	Conventional hydrogen bond	2.41182
A:ILE31:HG12	:UNK0		Hydrophobic	Pi-sigma	2.42915
A:ALA21	:UNK0		Hydrophobic	Alkyl	4.88840
A:VAL24	:UNK0		Hydrophobic	Alkyl	4.99355
A:PHE20	:UNK0		Hydrophobic	Pi-alkyl	5.08406