INTERACTIONS OF OLIVE OIL POLYPHENOLS WITH Aβ OLIGOMERS

Jois Moriani Jacob\textsuperscript{1)} dan Erda Eni Rame Hau\textsuperscript{2)}

\textsuperscript{1,2)}Jurusan Peternakan, Politeknik Pertanian Negeri Kupang
Jl. Prof. Dr. Herman Yohanes, Lasiana, Kota Kupang P.O.Box. 1152, Kupang 85011
Korespondensi: rdahau@gmail.com

ABSTRAK
Neurotoksisitas terutama mempengaruhi patogenesa penyakit Alzheimer (AD). Mekanisme yang diprediksi adalah pembentukan dan pengendapan plak beta amiloid (Aβ) dalam keadaan agak terlarut dalam jaringan otak. Senyawa polifenol yang dihasilkan oleh minyak zaitun telah dihubungkan dengan treatment dari pembentukan plak Aβ dengan cara mengikat dan menghambat pembentukan agregat oligomerik beracun yang terlarut. Studi ini bertujuan untuk menganalisis afinitas pengikatan Aβ (11-42)- oligomer dengan beberapa senyawa polifenol dan menentukan senyawa yang paling mampu untuk menghambat agregasi Aβ-oligomer. Metode komputasi digunakan untuk memprediksi kemampuan senyawa polifenol untuk berikatan dengan Aβ (11-42)-oligomer. Metode ini termasuk docking, visualisasi 3D, dan analisis struktural. Hasil menunjukkan hubungan positif yang kuat antara server docking dan skor afinitas pengikatan yang dihasilkan. Hasil lebih lanjut diilustrasikan polifenol dengan kelompok O-H tinggi dari struktur kimia mereka berkorelasi dengan ikatan afinitas yang lebih tinggi untuk Aβ (11-42)-oligomer. Hesperidin memiliki jumlah kelompok O-H tertinggi dalam studi dan juga memiliki salah satu nilai ikatan afinitas tertinggi di ketiga server docking. Hal ini menunjukkan hubungan timbal balik antara struktur polifenol dan ikatan afinitas yang dihasilkan.

Key words: Alzheimer, Senyawa polifenol, beta amyloid, docking, visualisasi 3D, Hesperidin.

INTRODUCTION

Alzheimer’s disease (AD) is a neurological disorder affecting an estimated 36 million people worldwide (Lundkvist et al., 2014). Clinical manifestations commence with discrepancies in memory but inevitably progress to irreversible cognitive decline. A key neuropathological feature of AD is the accumulation of amyloid plaques in the parenchyma of the neocortex, amygdala and hippocampus (Huang et al., 2000). The main constituent of these plaque deposits is the \(~4\) kDa amyloidbeta peptide (Aβ), which is produced through the sequential cleavage of amyloid precursor protein (APP) via βsecretase and γsecretase.

Current treatment for AD provides a delayed progression of symptomatology at various stages of the disease; However such treatments do not stop the advancement of AD. As such, novel therapeutics for the treatment and prevention of AD are urgently required. Since the initiation of amyloid formation by Aβ is dependent on interactions involving the central hydrophobic region (residues 17 to
(Hilbich et al., 1992), many researchers have focused their attention on blocking this region. Docking programs are now extensively used to test for ligand-peptide binding properties and experimental research is only conducted after extensive in silico experimental research has produced satisfactory results. Thus, the focus of attention for many researchers in AD therapeutics is the use of small molecules to target the misfolding and/or aggregation phase of amyloidogenesis, with the aid of computational software. The screening process of computational docking helps narrow down compounds that have structural compatibility with the Aβ fibre pharmacophore (Jiang et al., 2013).

The most extensively studied small molecules that impact Aβ aggregation are the polyphenols (Gauci et al., 2011). Though polyphenols have been shown to inhibit Aβ aggregation, it is still not clear whether they can reach the brain in sufficient concentrations or in a form capable of acting on Aβ oligomers in vivo (Singh et al., 2008). Hence it is vital to do further research on their intracellular and molecular targets to determine the role of polyphenols in neuroprotection. More research is also needed to investigate the pharmacokinetics of polyphenols.

This project aims to analyse the binding affinity of Amyloid βeta (1142)-oligomer with several polyphenol compounds from olive oil and determine the most capable compound able to inhibit Aβ oligomer aggregation. Establish anti-amyloidogenic properties and binding locations and predict affinities of polyphenols to Aβ oligomers through the use of 3dimensional computational methods. This project will deduce the effectiveness of the predicted binding affinity of Aβ (1142)-oligomers with polyphenol compounds across the three docking servers. Finally, determine correlations between affinity score, Molecular Weight, Hydrogen Bond count and topology polar surface area of each ligand through statistical analysis.

**METHOD**

The following five steps were undertaken to predict the binding affinity of 60 polyphenolic compounds with Aβ (1142)oligomer, to determine and correlation between docking servers, to examine structural similarities and to determine the best regression model for binding affinity prediction.

**Ligand Retrieval**

The 3D structures of 60 polyphenolic compounds (ligands) were retrieved from the National Centre for Biotechnology Information (NCBIPubChem) in a spatial
data file (SDF). PubChem is a compound database containing authorised chemical description information that has been successful in guiding scientists (Wang et al., 2014). The SDFs were then converted to protein data bank (PDB) format using the online Simplified Molecular Input Line Entry System (SMILES) translator. This conversion was necessary as SDFs are not recognised by most docking tools. SMILES aids in converting the 2D system molecules into 3D system molecules (Achary, 2014).

**Docking**

The second part of the project involved docking all 60 ligands with the Aβ-oligomer using three docking tools; PyRx 0.8 (Vina) with exhaustiveness of 8 and maximized dimensions (Angstrom), SwissDock server, and HexServer with default settings. These docking tools provided effective oligomer-ligand docking schematics, enabling full comprehension of the docking process and methods. In addition, they assisted in docking the ligand to create a set of grids used in describing the targeted protein. AutoDock Vina (0.8) is a virtual screening program that is used for computational drug discovery and uses the creation of screen libraries consisting of various compounds usable against possible drug targets (Dallkyan & Olson, 2015). The output files of AutoDock Vina were in SDF format for all 60 ligands. Only the highest binding affinities of the top 18 ligands were then converted into PDB format using Chem3D (Avogadro), also known as The Avogadro Project. Avogadro has been used to achieve crossplatform assignments in computational chemistry, bioinformatics, molecular modelling and materials science (Hanwell et al., 2012). SwissDock server and HexServer produced PDB files instantly for all 60 ligands.

**Visualization**

The 10 highest oligomer-ligand binding affinities from each docking tool were then visualized using Visual Molecular Dynamics (VMD 1.9.2). VMD is often described as a molecular visualisation program that is used for displaying, analysing and animating large biomolecular systems through the use of 3D graphics and a built-in script (Krone et al., 2012). PDB format was used to facilitate the processing of file editing in TextEdit (Macintosh system)/ Notepad (Windows system). In these text editing tools, the PDB text of the Aβ (1142)oligomer (2mxuabetafibril-oligomer.pdb) was then combined with the conformational data (PDB files) of each highest binding affinity of the 10 ligands from each docking tool. These combined
files were then visualized in VMD to see how those top 10 ligands interact with the oligomer with the representations of: style (NewRibbons) and colour (Secondary Structure) for the protein, style (VDW) for the ligand, and style (Licorice) for the residues within 2.5 Angstrom. Cyan colour and white background was used for the protein structure. This visualization provided vast amounts of data about binding, such as type of binding (i.e., hydrogen, van der Waals) and residues involved in the oligomer-ligand interaction. AutoDock Vina gave more accurate conformations in VMD, as such they have been used in this paper.

**Structural Analysis**

PubChem bioAssay was used to conduct a structural analysis in order to determine any type of relationship between the polyphenol compounds. Fifty eight compounds (ligands) were submitted for analysis using their compound identifier (CID) number. A dendrogram tree was obtained based on their molar refractivity and topological indices. Two ligands were unable to be submitted for structural analysis due to unidentified CID number. The non-included compounds were Hydroxytyrosol diglucoside and Hydroxytyrosol rhamnoside.

**RESULTS AND DISCUSSION**

A total of 60 ligands were analysed in the docking part of the study. The top 6 ligands and their VMD representation can be seen in Figures 1-6.

*Hydroxyltyrosol diglucoside*

Hydroxyltyrosol diglucoside attained binding affinity scores of 5.7, 8.19 and 66.22 for Vina, Swissdock and Hex Server, respectively. Figure 1 shows the ligand interacting with the Aβ (1142) oligomer through Glycine, Leucine and Glutamine residues. Hydrogen bonding occurs between the amine and carboxylic groups of LEU17 with the hydroxyl groups from the ligand’s glucoside and hydroxyltyrosol regions. Van der Waal interactions also occurred between methyl groups from LEU34 and the aromatic ring in the hydroxytyrosol region of the ligand. There is also Hbond interaction in similar fashion like LEU17 with the ligands glucoside regions. Studies from Serpell (2000) found that electrostatic interactions involving His-Asp/Glu salt bridges along with hydrophobic contacts in the region LEU17ALA21 stabilises an antiparallel arrangement of βstrands. Furthermore there are suggestions that hydrophobic residue, such as LEU17 is important in interactions in
the intersheet direction of abetafibril formation.

Figure 1. Hydroxyltyrosoldiglucoside interacts with Aβ oligomer through glutamine (GLN), leucine (LEU) and glycine residues (GLY).

Figure 2. Three dimensional representation of Gentisic acid interacting with residues; leucine (LEU), lysine (LYS) and glutamine (GLN) originating from Aβ oligomer. Within the three docking servers used; Swissdock, Vina and Hex, Gentisic acid (Figure 2) showed to have the predicted binding affinities of: 6.2, 4.7, 39.43 to Aβ (1142) oligomer. This polyphenol interacted with Aβ oligomer residues; Leucine (LEU17), Lycine (LYS16) and Glutamine (GLN15). Studies have shown that these three residues enhance the fibrillation process of Aβ oligomer. Gentisic acid is a polyphenol composed of three OH groups on the aromatic ring, providing a three-dimensional conformation that is able to interact with amyloidogenic aromatic residues (Garai & Frieden, 2013).

**Neonuezhenide**

For docking servers Vina, Hex and Swiss Dock, Neonuezhenide attained binding affinity scores of 7.10, 82.93 and 8.87, respectively. As shown in figure 3, LEU17, LEU34, GLN15 and ILE32 residue groups interacted with Aβ (11-42)-oligomer. Isoleucine and leucine contains methyl groups, hydrophobic side chain and would usually been seen buried inside Aβ (1142)-oligomer. With the combination of hydrophobic and electrostatic interactions, it can determine the level
of the fibrils aggregating. The hydrophobicity of the residues is vital as it is a dominant factor in determining the amyloidogenesis of the protein.

![Figure 3. Three dimensional representation of Neonuezhenide interacting with Aβ amyloid oligomer residues; Isoleucine (ILE), leucine (LEU) and glutamine (GLN).](image)

Just like the other residues, glutamine (GLN15) contains hydrogen bonding via its amide group and ester region of Neonuezhenide. A hydrolysis of amide group on glutamine (NH2) and OH group forms glutamine 15 (Schmid et al., 2011). With hydrogen bonding in between side chains and amide chains, poly glutamine can aggregate into beta sheets, which will result to polar zippers. The polar zippers can join specific transcription factors to different DNA segments and this can increase nonspecific affinity of affected proteins. Furthermore glutamine secretase can influence glutamine supply to neurons that can result to failures in the synaptic connectivity and transmission, which will affect brain functions (Nawrot et al., 2013).

**Isoverbascoside**

Isoverbascoside attained binding affinity scores of 8.75, 8, and 79.11 for Swissdock, Vina and Hex Server, respectively. The interaction between isoverbascoside with Aβ-(1142)-oligomer was represented in VMD as shown in Figure 4. Isoverbascoside interacts with beta amyloid fibril through 4 residues of the protein, leucine (LEU17 and LEU34), isoleucine (ILE32), and glutamine (GLN15). Leucine and isoleucine residues are hydrophobic therefore hydrogen bonds is the major interaction between beta amyloid fibril isoverbascoside. Glutamine as polar residue also interacts with this ligand through hydrogen bonds by donating and accepting a proton.
The side chains of ILE32 and LEU34 interaction with the aromatic rings is the most important hydrophobic which forced the layer of β2 sheet to be contorted, and partially opened the tightly compacted structure of the crossβ subunit (Buchete & Hummer, 2007). The compactness of the hydrophobic core comprising the β2 portion of the fibril can be a crucial stabilization element in aggregation and elongation of Aβ aggregates. According to Doig et al. (2002), NmethylLEU34 alter the fibril morphology and resulting in the reduction of the toxicity. Nmethylated derivatives of beta (2535) prevent the aggregation and inhibit the toxicity of the wild-type peptide by blocking hydrogen bonding on the outer edge of the assembling amyloid.

**Hesperidin**

Hesperidin attained binding affinity scores of 7.7, 79.25 and 8.90 for Vina, Hex Server and Swissdock, respectively. Figure 5 shows a VMD representation of hesperidin interacting with isoleucine, leucine and glycine on the Aβ (1142) oligomer. ILE32 interacts with hesperidin at three distinct locations. The alkyl group of two ILE32 residues can be seen interacting with the disaccharide portion of the ligand (rutinose). That is, the methyl groups of ILE32 interact with rhamnose and glucose, respectively. The third interaction of ILE32 is with the distal benzene ring on the ligand.
Glycine (GLY33) interacts with the ligand at two distinct locations; the first is proximal to the disaccharide rutinose and the second is adjacent to the hydroxyl group on the distal benzene ring. GLY33 is a hydrophobic residue in the Aβ (1142) oligomer (Ahmed et al., 2010). Studies of Kanski et al. (2002) suggest that GLY33 may aid in the toxicity of the Aβ (1142) oligomer by contributing free radical propagation processes. Thus, disruption of GLY33 may reduce toxicity.

**Ligstroside**

LYS16 mainly interacts via hydrogen bonding formation between lysines carboxylic and amine groups with the hydroxyl group attached to the aromatic ring of Ligstroside. ILE32 formed an interaction with Ligstroside via van der waal interactions, the methyl groups of ILE32 interacted with the aromatic, alkane and alkene regions of the ligand. ILE32 and GLY33 interaction has been studied and
there was interruption in the structure of Aβ oligomer, however this interruption cannot disaggregate the structure of Aβ oligomer (Crescenzi et al., 2002).

### Structural Analysis

A total of 58 ligands were analysed in the structural analysis. Table 1 lists the ligands and their associated CID. The dendrogram shows that heavier polyphenols with high OH group counts have similar structural activity. Compounds which gave high affinity scores in the docking study displayed a large number of OH groups in their chemical structure and showed similar structural activity. For example high affinity polyphenols such as Hesperidin (CID: 10621), Apigenin6,8diCglycoside (CID: 52808805), Isoverbascoside (CID: 6476333) and Cyanidin3Orutinoside (CID: 29232) showed similar structural activity. Juxtapose, molecules with less to no hydroxyl groups such as 1,3Dimethylox2imidazolidinone (CID: 8704), Cinnamic acid (CID: 44539) and Hydroxytyrosol acetate (CID: 7174906) were clustered distal to the latter compounds; these compounds attained weak affinity scores from all three docking servers.

**Table 1. Ligands analysed and their associated CID**

| CID     | Ligand                      | CID     | Ligand                                      |
|---------|-----------------------------|---------|---------------------------------------------|
| 10621   | Hesperidin                  | 72      | 3,4-Dihydroxybenzoic acid                   |
| 5280443 | Apigenin-6,8-di-C-glycoside | 547     | 3,4-Dihydroxyphenyllactic acid              |
| 5291488 | Luteolin-7-O-glucoside      | 1738    | Homovanillic acid                           |
| 5319116 | Luteolin-4'-O-glucoside     | 370     | Gallic acid                                 |
| 378305  | Luteolin 7-O-rutinoside     | 3469    | Gentisic acid                               |
| 5748483 | Chrysoeriol-7-O-glucoside   | 10394   | Propionic acid                              |
| 5280805 | Rutin                       | 6508    | Quinic acid                                 |
| 29232   | Cyanidin-3-O-rutinoside     | 8655    | Syringaldehyde                              |
| 49852298| Luteolin-6-C-glucoside      | 10742   | Syringic acid                               |
| 25203368| Quercetin-3-O-glucoside     | 8468    | Vanillic acid                               |
| 442831  | Acetoxypinoresinol          | 8704    | 1,3-Dimethylox2imidazolidinone              |
| 3010930 | Hydroxytyrosinol            | 840     | 6-Deoxyhexopyranose                         |
| 6476723 | Neomucunalide               | 6479876| Caffeoylglucuronide                         |
| 91895359| Nuezhenide                  | 131348  | Cornuside                                   |
| 6476722 | Oleoside dimethylester      | 169607  | Elenolic acid                               |
| 5281544 | Oleuropein                  | 71718370| Ligstroside aglycone                        |
| 56842347| Oleuropeinglycon            | 14136859| Ligstroside                                 |
| 73399   | Pinocretin                  | 89640   | Loganic acid                                |
| 24352   | Propionomazine              | 5458792 | Oleoside A                                  |
| 443023  | Siringaresinol              | 11652416| Oleocanthal                                 |
| 44429837| Acetoside                   | 289     | Cathelic                                    |
| 689043  | Caffeic acid                | 16928   | Homovanililly alcohol                       |
| 1794427 | Chlorogenic acid            | 82755   | Hydroxytyrosol                              |
| 444539  | Cinnamic acid               | 7174906 | Hydroxytyrosol acetate                      |
| 445858  | Ferulic acid                | N/A     | Hydroxytyrosol diglucoside                 |
| 6476333 | Isoverbascoside/isoaacteose | 5316821 | Hydroxytyrosol glucoside                    |
| 637542  | o-Coumaric acid             | N/A     | Hydroxytyrosol rhamnoside                   |
| 637542  | p-Coumaric acid             | 10393   | Tyrosol                                    |
| 637775  | Sinapic acid                | 11195402| Tyrosol acetate                             |
| 5281800 | Verbascoside/acteoside      | 159278  | Tyrosol glucoside                           |
Thus, compounds that contain a high number of OH groups may suggest that OH groups are very important in the interaction process with the Aβ oligomer and which research shows may prevent protein aggregation (Stefani & Rigacci, 2013). A limitation of this research was the omission of Hydroxytyrosol diglucoside and Hydroxytyrosol rhamnoside in the structural analysis. This was due to a lack of CID number in the PubChem database. However, as a result of the correlation between hydroxyl groups and high affinity, it is expected that both omitted compounds have similar structural activity to those compounds with high affinity scores.

**CONCLUSION**

Further statistical analysis needs to be conducted to confirm the predictive capabilities of the regression model by testing the model with an independent set of ligands (i.e., predict the binding affinities of the ligands using Mw and compare the results to actual binding affinities for Swissdock). Cross validation is a possible method to evaluate the predictive capabilities of the model by separating the available data into two parts. Modeling of the data uses one part only and the model designated for this part is then utilised to predict the values in the other part of the data. This can deduce the predictive accuracy of the model as good models should have high predictive accuracy.

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