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# Molecular Docking Studies of Nigella Sativa Linn Seed Compound Against Alzheimer's Disease: An *in silico* Study

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Alzheimer's disease is a neurological disorder that can lead to brain atrophy and dementia, affecting cognitive, social, behavioral, and emotional abilities. Medicinal plants have proven to be effective in addressing Alzheimer's disease. *Nigella sativa* Linn seeds have been extensively utilized in managing a range of nervous system conditions, including AD, epilepsy and neurotoxicity. In this study, it has been reported that the evaluation of the anti-Alzheimer potential of phytoconstituents in *Nigella sativa* Linn seeds was done using molecular docking analysis. The AutoDock 4.2.6 software has used the chemical Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside) to identify and fulfill the Lamarckian genetic algorithm. The binding affinity of

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Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside) with key targets: acetylcholinesterase, amyloid beta peptide, and beta-secretase, represented by PDB IDs 7E3H, 2M4J, and 1FKN, respectively. Remarkably, *Nigella sativa* L., seeds exhibit a superior binding affinity and inhibitory effect on acetylcholinesterase (AChE), amyloid beta peptide (Aβ or APP) and beta-secretase (BACE) compared to conventional medication. Donepezil was employed as a positive control in this investigation. This study delves into the evaluation of *Nigella sativa* Linn's phytoconstituents for their potential in combating Alzheimer's disease through molecular docking analysis. Among the bioactive components of *Nigella sativa* Linn, kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside) and the standard drug Donepezil stand out as promising candidates for therapeutic purposes. The affinity of Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside (Binding energy = -7.84 kcal/mol) to bind with acetylcholine esterase significantly surpasses that of the standard drug Donepezil, as indicated by the higher binding energy. The ligand efficiency of Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside, calculated as the binding energy per non-hydrogen atom (-0.37 kcal/mol), is also greater than that of the standard drug Donepezil. These findings underscore the importance of further comprehensive research on Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside) as a potential treatment for Alzheimer's disease, owing to its superior efficacy compared to existing medications.

*Keywords: Kaempferol 3-(2''-galloyl-alpha-L-arabinopyranoside); donepezil; Alzheimer's disease; in silico; Nigella sativa Linn; Molecular docking.*

## 1. INTRODUCTION

*Nigella sativa* Linn is a perennial herbaceous flowering plant belonging to the Ranunculaceae family, native to South and South-West Asia [1]. A frequent name for this plant seed is "Kalonji" in southern Asia, "habbat us sauda" in the Middle East, and "black cumin" in English. South-west Asia, Europe, the Mediterranean region, and India are the main regions for cultivating the *N. sativa* L., plant. It is between 20 and 30 cm tall and has linear, finely divided leaves [2]. The naturally occurring seed components have been widely used as food preservatives. The fruit is a large, inflated capsule with three to seven connected follicles each of which contains a seed. *N.sativa* L., seeds have a corrugated integument and come in sizes ranging from 1 to 5 mm [3]. Especially for cheese and baked items, the seeds have been frequently used as a spice to flavor cuisine. *N.sativa* L., seeds are used in a traditional sweet dish, eaten with honey and syrup, and sprinkled on toast. Black caraway seeds pungent aroma and bitter flavor are somewhat reminiscent of oregano, black pepper, and onions; the dry-roasted seeds are added to spice blends and used to flavor curries, vegetables, and pulses in bread items [4].

The medicinal herb *N.sativa* L., known as the black seed, is utilized globally. Oil and seeds have a long history of use in folklore for both food and medicine [5]. The seeds have multiple therapeutic uses to treat cough, fever, asthma, bronchitis, hypertension, diabetes, and inflammation. Murine cytomegalovirus (MCMV) viral suppression using black seed oil has successfully led to decreased titers in the infected person's liver and spleen [6]. It is one of the best medications for healing in both Christian and Islamic traditions. In "Tibb-e-Nabwi," the constant eating of black seed is advised. Both the accessibility and efficacy of antiviral drugs fall short of those of antibacterial medications [7].

Alzheimer's disease (AD) is a neurological ailment that progressively impairs the capacity of an individual to do even the most basic tasks and to develop memory and thinking skills [8]. The majority of the disease's victims are those with late-onset symptoms, which typically start showing signs in their mid-60s [9]. Unlike other illnesses, Alzheimer's disease frequently shows symptoms between the ages of 30 and 60 (WHO, 2020) [10]. Alzheimer's disease is the most common cause of dementia in older people, and researchers have shown that the brains of those patients exhibit unusually high levels of tau tangles, neurofibrillary plaques, amyloid plaques, and clusters of twisted nerve bundles [11]. These brain tangles and plaques are still considered to be the primary signs of Alzheimer's disease. A lack of connections between nerve cells in the central nervous system that include neurons is another identifying feature. Along with the brain, neurons also communicate with muscles and organs throughout the body [12].

Additionally, it is considered that there are various more complex brain changes contributing

to Alzheimer's disease [9]. The entorhinal cortex and hippocampus, which are involved in memory, are the first to suffer damage. Later, it has an effect on the parts of the cerebral cortex that control thought, language, and social behavior [13]. Eventually, numerous additional areas of the brain become damaged. It is believed that Alzheimer's disease is brought on by an abnormal protein buildup in and around brain cells [14]. A specific protein of concern is amyloid, which builds up in plaques surrounding brain cells. Tau protein is another protein that accumulates inside brain cells to form tangles [15]. A few of the proteins involved in the processes that lead to Alzheimer's disease (BACE) are acetylcholinesterase (AChE), amyloid beta peptide (Aβ or APP), and beta-secretase [16].

Acetylcholinesterase (AChE) is the primary cholinesterase in the human body. This enzyme degrades choline esters, including the neurotransmitter acetylcholine. AChE is primarily found in neuromuscular junctions and cholinergic chemical synapses, where it blocks synaptic transmission through its activity [17]. ACh produced by the presynaptic neuron binds to ACh receptors on the post-synaptic membrane, which then delivers the nerve signal. AChE, which is also present on the post-synaptic membrane, prevents signal transmission by hydrolyzing Ach. Acetylcholine transferase takes up the choline as it is released by the presynaptic neuron and uses it to make acetyl-CoA and Ach [18].

By functioning as a cholinergic neurotransmitter that inhibits the lysing activity of acetylcholinesterase, cholinomimetic medications halt this process [19]. The highest amounts of ACh persist inside synapses when AChE is inhibited by medicines, improving cholinergic signaling in the brain's autonomic ganglia, neuromuscular junctions, and central nervous system [20]. By momentarily occupying the static site, reversible inhibitors have been utilized to treat several central nervous system illnesses. FDA-approved drugs like donepezil and tetrahydroacridine (THA) help Alzheimer's disease patients think more clearly [21].

Amyloid plaques have been discovered in the brains of Alzheimer's patients. A peptide from APP, a precursor to amyloid plaques, is digested by gamma and beta secretases to create Aβ [22]. This process is dependent on cholesterol and substrate presentation. Several molecules can bond collectively to create flexible, soluble oligomers. It is currently believed that some misfolded oligomers might set off other events in a chain reaction mechanism similar to the prion disease, in which Aβ molecules become misfolded oligomeric and poison nerve cells [23].

Aβ is essential to understanding the pathogenesis of Alzheimer's disease and supports several genetic, cell biological, biochemical and animal studies [24]. Brain Aβ levels are higher in people with spontaneous Alzheimer's disease [25]. During APP-mediated axonal transport, beta-secretase and presenilin-1 may be produced in the axonal membranes of neurons in the central nervous system [26]. "The 'amyloid hypothesis,' which posits that plaques are responsible, is widely accepted as the pathophysiological explanation for Alzheimer's disease." According to a different theory, amyloid oligomers rather than plaques cause the illness. In humans, beta-secretase one and beta-secretase two belong to the beta-secretase protein family [27]. The aspartic acid protease BACE1 is necessary for peripheral nerve cells to produce myelin sheaths. BACE1, the main beta-secretase in neurons, has generated amyloid beta-peptides [28].

For amyloid peptides to build up in Alzheimer's patients' brains, the amyloid precursor protein (APP) has to be cleaved twice in a row. The C99 fragments have been linked to the cell membrane, and soluble extracellular fragments have been produced as a result of BACE1's extracellular cleavage of APP [29]. Gamma-secretase uses the cleavage of C99 inside its transmembrane region to release the APP's intracellular domain and produce amyloid protein [30]. An amyloid-peptide fragment has been eliminated because gamma-secretase cleaves APP closer to the cell membrane than BACE1. BACE1 and alpha-secretase compete for APP-processing beta-secretase, and its absence causes P3 because it prevents the growth of amyloid [31].

Contrary to the presenilin proteins implicated in beta-secretase and APP, familial Alzheimer's disease is an uncommon form of the disease with an early start and is brought on by mutations in the BACE1 gene [32]. On the other hand, this enzyme has been linked to sporadic late-onset Alzheimer's disease, which is more common. Even though BACE2 and BACE1 are closely related, no in vivo proof of APP cleavage has been found [33]. Recent studies reveal that

BACE1 is important in myelination, although APP and other transmembrane proteins are broken down by BACE. The beta peptide of the VGSC subunit began processing similarly to APP as a substrate for BACE1 [34].The risk of Alzheimer's disease and other cognitive impairments is reduced by a single APP residue mutation, which reduces BACE1's ability to cleave it and produce beta-amyloid. Acetylcholinesterase (AChE), amyloid beta peptide (Aβ or APP), and beta-secretase (BACE) are three proteins that have been inhibited at different stages to stop the progression of Alzheimer's disease [35].

To halt the advancement of Alzheimer's disease, these three key proteins have been targeted for inhibition at distinct stages. The present work is to identify the phytoconstituents present in the seeds of Nigella sativa Linn as well as assess their potential anti-Alzheimer agents through a molecular docking approach. Furthermore, the analysis was compared with that of the standard medication, Donepezil.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of Ligand

Based on the LC-MS findings [36] the flavonoid Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside) was chosen as a ligand and its structure was optimized using Chem Draw Professional 16.0 software with the standard drug Donepezil [37].

### 2.2 Preparation of Target Protein

The chosen target proteins for this study were stable human acetylcholinesterase, amyloid beta peptide, and beta-secretase (PDB IDs: 7E3H, 2M4J, and 1FKN, respectively). The survey shows that these protein structures were determined through X-ray diffraction with resolutions of 2.45 Å and 1.90 Å and the solid-state NMR method (PDB ID: 2M4J), each consisting of a single chain (A). The corresponding PDB format files for these selected target proteins were obtained from the RCSB Protein Data Bank database [38] To prepare the target proteins for molecular docking analysis, an optimization process was carried out, involving the removal of heteroatoms and water molecules. The optimized acetylcholinesterase, amyloid beta peptide, and beta-secretase targets, each with a single chain (A), were utilized for the molecular docking study [39].

### 2.3 Molecular Docking Study

The optimized ligand molecule, Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside) [40] was utilized for molecular docking with the chosen protein using AutoDock 4.2.6 software [41]. Flexible docking was executed, utilizing the pdbqt format for the ligand, a rigid macromolecule, and flexible residues. The grid parameters employed in the molecular docking analysis are outlined in Table 1, while Fig. 3a, 3b and 3c illustrate the grid boxes covering the predicted active flexible residues for each target. The Lamarckian genetic algorithm (LGA), with a maximum of 2.5 million energy evaluations, was employed to explore the molecular docking analysis. Ligand parameters specific to Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside) for AutoDock were considered, as were the molecular docking parameters for the interaction of the selected ligand with the target proteins. Furthermore, the 2D Ligplot and 3D protein-ligand binding interactions for this topmost conformation of the docked complex were analyzed utilizing the EMBL-EBI PDB sum generator web-based tool [42].

## 3. RESULTS AND DISCUSSION

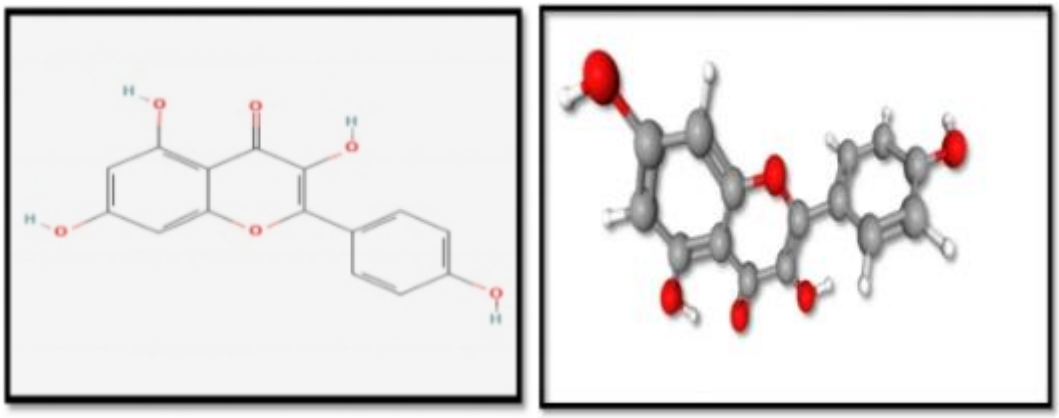
Molecular docking investigations were conducted involving the following targets: protein acetylcholinesterase (PDB ID: 7E3H), amyloid beta peptide (PDB ID: 2M4J), and beta-secretase (PDB ID: 1FKN). Additionally, the phytoconstituent found in *Nigella sativa* Linn seeds, specifically Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside) (Fig. 1 and 2), was also included in the study. Furthermore, docking studies were conducted using the standard drug Donepezil (Fig. 3 and 4).

The evaluation of the drug-likeness and ADME properties of Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside in comparison to the standard drug Donepezil (Table 1) demonstrates that the ligand Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside did not contravene any of Lipinski's rule of five. Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside) possesses a mass of 570.5 Da, slightly greater than that of the standard Donepezil, but comfortably falls within the Lipinski range of 130 to 725 Daltons [43]. Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside) features 8 hydrogen bond donors and 4 acceptors, in contrast to donepezil, which has 4 donors and 0 acceptors. The log P

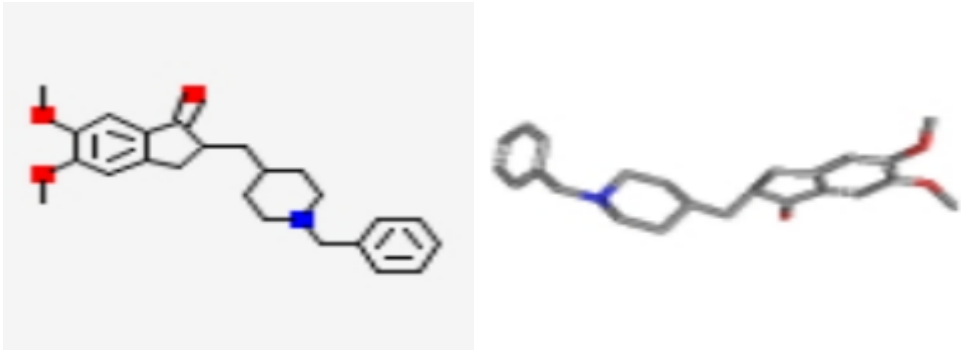
value of 2.57 for Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside) indicates its ideal hydrophobicity for pharmaceutical use. The bond rotation and good human intestinal adsorption parameters further confirm its potential as a drug. Both the standard Donepezil and the ligand Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside) exhibit positive human oral bioavailability [44].

Since Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside adheres to all the rules for an optimal drug, molecular docking was conducted with the designated target proteins to assess its potential as an anti-Alzheimer's agent. The affinity of Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside (Binding energy = -7.84 kcal/mol) to bind with acetylcholine esterase significantly surpasses that of the standard drug Donepezil, as indicated by the higher binding energy. The ligand efficiency of Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside, calculated as the binding energy per non-hydrogen atom (-0.37 kcal/mol), is also greater than that of the standard drug Donepezil. The inhibitory constant, representing the concentration at which half-maximum inhibition occurs for Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside) (7.79 µM), is significantly higher than that of the standard drug Donepezil. The additional internal energy parameters, including intermolecular energy, Vanderwaals energy, electrostatic energy and torsional energy, are also superior for Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside) compared to the standard drug Donepezil (Fig. 5a and 5b).

Acetylcholinesterase plays various roles in different disease conditions, and its involvement in Alzheimer's disease is notably harmful. Acetylcholinesterase (AChE) plays a crucial role in the cholinergic system and is responsible for the breakdown of the neurotransmitter acetylcholine (ACh). In Alzheimer's disease (AD), AChE's activity is particularly significant due to its association with cognitive function and memory. Reduced acetylcholine levels impair synaptic transmission, contributing to the cognitive decline observed in AD patients. Despite the beneficial effects of AChE inhibitors in temporarily alleviating symptoms, they do not address the underlying pathology of AD and have limited long-term efficacy.



**Figs. 1 & 2. 2D & 3D structure of Kaempferol 3-(2"-galloyl-alpha-L-arabinopyranoside)**



**Figs. 3 & 4. 2D & 3D structure of Donepezil**

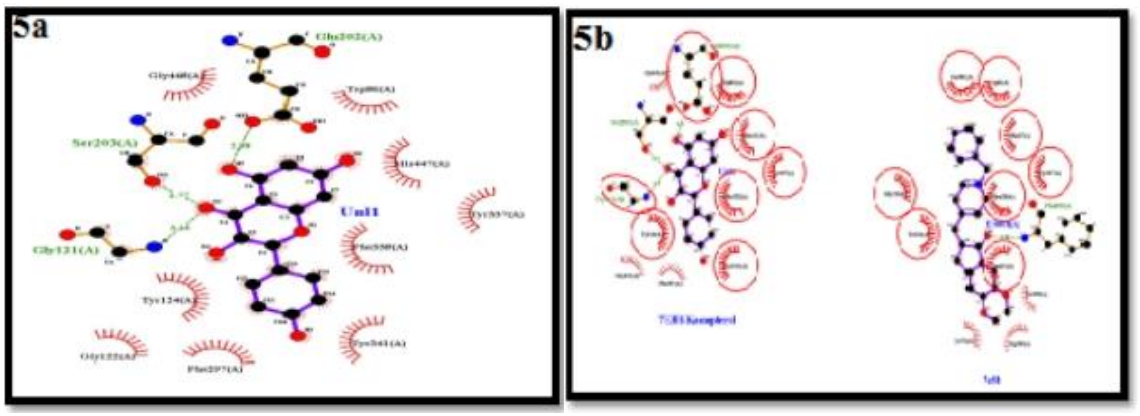



**Fig. 5a & 5b. Residues involved in interactions of Acetylcholinesterase with ligands (7E3H)**

The molecular docking investigations involving acetylcholinesterase and kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside confirms its superiority as an inhibitor compared to the currently available standard, Donepezil. In vitro studies have confirmed the reduced activity of Alzheimer's disease activation with higher levels of acetylcholine esterase. These studies further extrapolate the need to inhibit acetylcholine esterase to treat Alzheimer's disease.

The lower binding energy of Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside (-4.79 kcal/mol) compared to -4.09 kcal/mol for the standard drug Donepezil with the Amyloid beta peptide target presents compelling evidence for the potential superiority of Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside as a better alternative to Donepezil in the treatment of Alzheimer's disease. To provide additional validation, the slightly higher inhibitory constant observed for Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside (19.04 µM) compared to Donepezil (18.5 µM) suggests that it could serve as a superior alternative for the treatment of Alzheimer's disease, potentially requiring a lower dosage (Fig. 6a and 6b). All the internal energy parameters, comprising electrostatic energy, Vander Waals energy, and torsional energy, demonstrate enhancements compared to donepezil. Moreover, the number of hydrogen bonds established by Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside with Amyloid beta peptide is also better than that of Donepezil (Table 2).

*In silico* molecular docking studies have identified one such small molecule, Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside), which exhibits a higher affinity for Amyloid beta peptide compared to the standard Donepezil. The accumulation of amyloid-beta plaques in AD contributes to increased AChE activity, leading to a decline in acetylcholine levels in the brain. The lower binding energy of Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside (-7.28 kcal/mol) compared to -6.81 kcal/mol for the standard drug Donepezil with the beta-secretase target provides promising evidence for the potential superiority of Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside over Donepezil in the treatment of Alzheimer's disease.

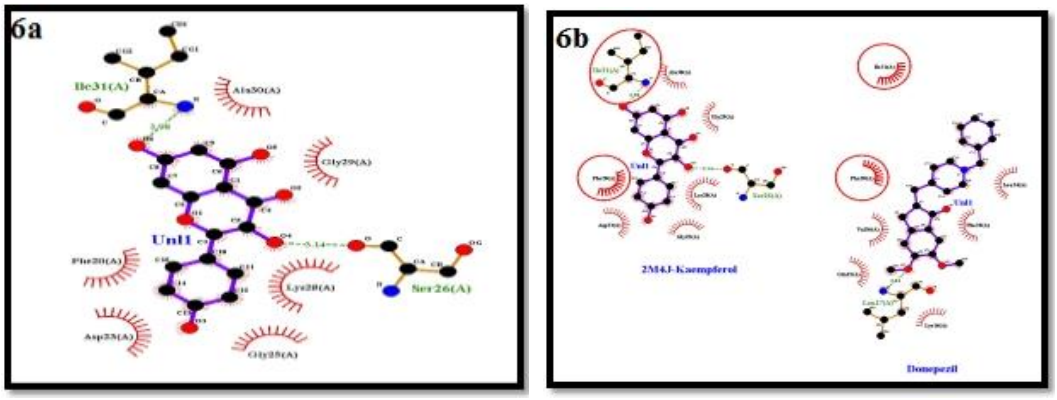



**Fig. 6a & 6b. Residues involved in interactions of Amyloid beta peptide with ligands (2M4J)**

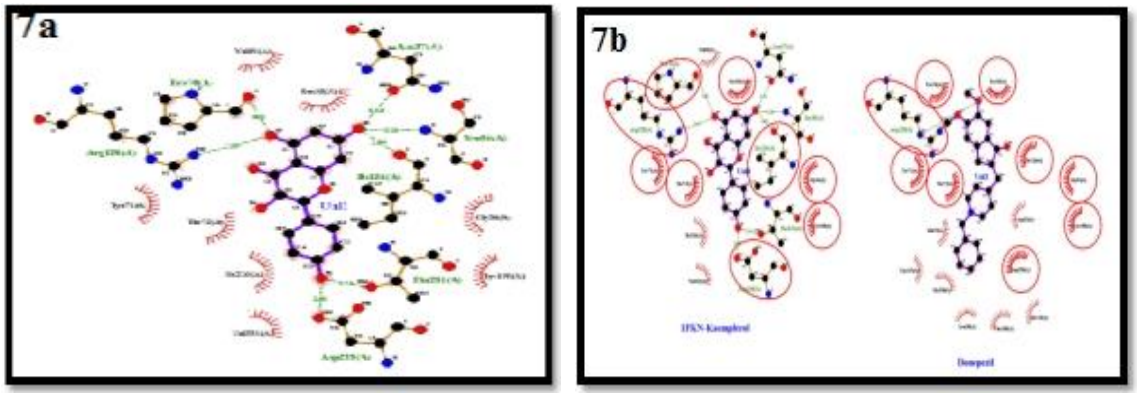



**Fig. 7a & 7b. Residues involved in interactions of Beta-secretase with ligands (1FKN)**

**Table 1. ADME properties of Kaempferol 3-(2"-galloyl-alpha-L-arabinopyranoside) & Donepezil**

| S. No | Description | Standard Values | Kaempferol 3-(2"-galloyl-alpha-L-arabinopyranoside) | Donepezil |
|---|---|---|---|---|
| 1 | Molecularweight (Da) | 130.0 –725.0 | 570.5 | 379.5 |
| 2 | Number of H-bond acceptors | 2.0 - 20.0 | 8 | 4 |
| 3 | Number of H-bond donors | 0.0 / 6.0 | 4 | 0 |
| 4 | QP log P foroctanol/ water | -2.0/6.5 | 2.57 | 3.58 |
| 5 | Lipinski Rule of5 Violations | (maximu mis 4) | 0 | 0 |
| 6 | Number of rotatable bonds | >10 is poor oral availability | 1 | 3 |
| 7 | Human Intestinal Absorption | (<30% is poor) | Good | Good |
| 8 | Human Oral bioavailability | (>0 is positive) | Negative | Negative |

**Table 2. Molecular Docking parameters for Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside and Donepezil with Acetylcholinesterase, Amyloid beta peptide and Beta secretase**

| Parameters | Acetylcholinesterase | | Amyloid beta peptide | | Beta secretase | |
|---|---|---|---|---|---|---|
| | I | II | I | II | I | II |
| Binding Energy kcal/mol | -7.84 | -6.52 | -4.79 | -4.09 | -7.28 | -6.81 |
| Ligand Efficiency | -0.37 | -0.26 | -0.23 | -0.19 | -0.35 | -0.30 |
| Inhibitory Constant | 7.79 | 9.65 | 19.04 | 18.5 | 4.62 | 6.76 |
| Inhibitory Constant units | µM | µM | µM | µM | µM | µM |
| Intermol energy | -9.33 | -8.65 | -6.28 | -5.91 | -8.77 | -7.33 |
| Vdw_hb_desolv_energy | -8.92 | -8.03 | -7.09 | -5.30 | -8.36 | -7.33 |
| Electrostatic energy | -0.41 | -0.37 | -0.19 | -0.10 | -0.41 | -0.33 |
| Total_Internal | -0.78 | -0.58 | -1.3 | -0.78 | -1.55 | -1.08 |
| Torsional_Energy | 1.49 | 1.49 | 1.49 | 1.49 | 1.49 | 1.49 |
| Unbound_Energy | -0.78 | -0.69 | -1.3 | -0.97 | -1.55 | -1.04 |
| cIRMS | 0.0 | 00 | 0.0 | 00 | 0.0 | 00 |
| refRMS | 64.63 | 56.07 | 102.96 | 91.06 | 10.74 | 10.02 |
| rseed1&2 | None | None | None | None | None | None |

To further validate this, the significantly lower inhibitory constant observed for Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside (4.62 µM) compared to Donepezil (6.76 µM) suggests that it could serve as a superior alternative with a lower dosage for the treatment of Alzheimer's disease. All remaining internal energy parameters, including electrostatic energy, Vander Waals energy, and Torsional energy, exhibit improvements over Donepezil. Furthermore, the number of hydrogen bonds and hydrophobic interactions formed by Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside with beta-secretase exceeds that of Donepezil (Table 3). In silico molecular docking studies have confirmed a small molecule, Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside), which exhibits a superior affinity for beta-secretase compared to the standard Donepezil (Fig. 7a and 7b).

**Table 3. H – bond and hydrophobic interactions of the Targets with the Ligand**

| S. No | Protein | Ligand | No. of Hydrogen bonds | Interacting Residues | No. of Hydrophobic Interactions | Interacting Residues |
|---|---|---|---|---|---|---|
| 1 | Acetylcholinesterase (PDB ID:7E3H) | Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside | 2 | A: Gly 121,<br>A: Glu 202 | 6 | A: Trp 86 (2),<br>A: Tyr 124,<br>A: Tyr 337,<br>A: Phe 338(2). |
| | | Donepezil | 1 | A: Ser 203 | 3 | A: Phe 297,<br>A: Tyr 341(2). |
| 2 | Amyloid beta Peptide (PDB ID:2M4J) | Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside | 2 | A: Ser 26,<br>A: Ile 31. | 2 | A: Lys 28(2) |
| | | Donepezil | 1 | A: Ser 21. | 1 | A: Phe 20. |
| 3 | Beta secretase (PDB ID:1FKN) | Kaempferol 3-(2-galloyl-alpha-L-arabinopyranoside | 5 | A: Gly 36, A: Ser 36,<br>A: Pro 70, A: Thr 72,<br>A: Ile 126. | 2 | A: Arg 128,<br>A: Tyr 198. |
| | | Donepezil | 2 | A: Arg 128,<br>A: Asp 228. | 1 | A: Ile 226. |

## 4. CONCLUSION

In the current investigation, Kaempferol 3-(2''-galloyl-alpha-L-arabinopyranoside, a bioactive constituent derived from *Nigella sativa* Linn, demonstrated a superior binding affinity with acetylcholinesterase, amyloid beta peptide, and beta-secretase. These ligands exhibited notable interactions with the active residues of the proteins. Therefore, based on the insights gained from the *in silico* studies, it is conceivable that Kaempferol 3-(2''-galloyl-alpha-L-arabinopyranoside) may serve as a more promising alternative to Donepezil.

## CONSENT AND ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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