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Evaluating candidate IRAP inhibitors in Gingko biloba extract and their potential for cognitive enhancement

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Abstract

Ginkgo biloba is commonly used as an alternative medicine and is known for its nootropic effects. It contains many secondary metabolites, including terpene lactones and flavone glycosides. However, the molecular mechanisms of its cognitive enhancement are not well understood. Inhibition of Insulin regulated aminopeptidase (IRAP) has been shown to improve memory in previous studies. Here, we aim to compare the binding affinities of bioactive molecules in *Ginkgo biloba* extract to known IRAP inhibitors. Using an in silico approach, we found that Ginkgolide B had a similar result to AngIV, yet what surprised us was the difference between the structures. Furthermore, Luteolin and HFI-142 almost identical predicted binding affinities and full fitness values. These findings provide new insight into the possible molecular underpinnings of *Ginkgo biloba*'s memory-enhancing effects.

Keywords: Ginkgo biloba, IRAP, nootropics, cognitive enhancement

Introduction

Ginkgo biloba has many reported health benefits. It's often used to treat mental health conditions, Alzheimer's disease, and fatigue (Smith and Luo, 2004). It's been used in traditional Japanese medicine for about 1,000 years. It came on the Western culture scene a few centuries ago, but has enjoyed a surge of popularity over the last few decades. *Ginkgo biloba*, also known as the maidenhair tree, belongs in the family Ginkgoaceae. The trees grow up to 35-50 meters or more. Their products are most popular during the fall season; it can be used to make tea or be added to various Japanese dishes.

Alzheimer's disease (AD) is known for reducing cognitive function and negatively impacting memory. There are still many mysteries of what triggers it, but now we are more aware that it starts long before any symptoms appear. There's a theory that it is caused by an abnormal build-up of proteins in the brain. Two of the proteins in this process are called amyloid beta and tau. Amyloid beta aggregate forms around brain cells and the Tau would tangle with the brain cell (Selkoe, 2001; Ballatore et al., 2007). After the proteins are in contact the brain cells become affected and decrease chemical messengers which are called neurotransmitters. These messengers are the key for signaling or sending messages between brain cells. According to the National Health Service (2019), throughout time, different areas of the brain shrink. Usually the first area that's exposed is the memories, which is the main symptom of Alzheimer's disease, but there are cases in which it affects the vision or language. Sometimes there are more unusual versions of Alzheimer's disease that affect different parts of the brain first.

Although the causes of Alzheimer's disease are not fully understood, there is evidence on what would increase or speed up the development of AD. Some of these causes are age, genetics, Down's syndrome, head injuries and cardiovascular disease. Age is one of the leading causes, for it doubles the chances of getting AD

every five years on average after you reach 65 (National Health Service, 2019).

The leaf extract is usually from leaves 15 cm wide and 8 cm long that remain on the branch 2 weeks after turning color. *Ginkgo biloba*'s standard extract includes 24% flavone glycosides and 6% terpene lactones; this is known as EGb 761 (Smith and Luo, 2004). EGb 761 has several demonstrated neuroprotective effects, including the inhibition of amyloid beta aggregation. As such, EGb 761 shows a potential to become a preventative treatment for Alzheimer's disease (Smith and Luo, 2004). *Ginkgo biloba* extract has potential side effects, such as allergic reactions, bleeding disorders, diarrhea, dizziness, headaches, and constipation (Mayo Clinic, 2020). Most of these side effects are caused by overconsumption of the *Ginkgo biloba* leaf extract.

The *Ginkgo biloba* extract contains several primary terpenoids like bilobalide and ginkgolides A, B, and C. The primary flavonoids include quercetin, kaempferol, and isorhamnetin (Dziwenka and Coppock, 2016). Terpene lactones are one prominent metabolite found in the *Ginkgo biloba* extract. They are commonly used for an additional treatment for therapy in patients with ischemic cardiovascular and cerebrovascular diseases (Xin-wei et al., 2018). Furthermore, flavone glycosides are reported to have anti-inflammatory and antioxidant properties (Mahmoud et al., 2013).

There are a number of alternative medicinal products that reportedly improve cognitive function, known as nootropics, many of which include *Ginkgo biloba* extract. For example, Doctor's Best Extra Strength Ginkgo, 120 mg, is a best seller on iHerb. In the bottle, there is *Ginkgo biloba* leaf extract, which contains a minimum of 24% flavonol glycosides (28.8 mg) and 6% terpene lactones (7.2 mg) (iHerb). It states that the product increases memory and brain capacity and helps healthy mitochondrial and nerve cell function. However, the actual molecular mechanisms of this and similar *Ginkgo biloba*-based products are unclear. Specifically, which molecules in the extract confer the memory enhancing effects- and how- is poorly understood.

IRAP stands for insulin regulated aminopeptidase. IRAP removes amino acids from extracellular signaling peptides. It also it helps with antigen presentation and cellular glucose uptake (Barlow and Thompson, 2020). IRAP inhibition is associated with improved cognitive function (Albiston et al., 2008). AngIV is a peptide and a well-known inhibitor of IRAP (Lew at al., 2003). IRAP has also been shown to be inhibited by non-peptide molecules, such as, 4H-benzopyrans, bosphinic pseudopeptides, 3,4-diaminobenzoic acid derivatives, aryl sulfonamides, and spiro-oxindole dihydroquinazolinones (Georgiadis et al., 2020).

Ginkgo biloba has shown many promising reasons to suggest that it has the ability to increase brain activity significantly. On the other hand, many students and researchers have indicated that it doesn't improve memory and brain activity. Here, we aim to determine whether molecules within the *Ginkgo biloba* extract can bind IRAP. Furthermore, we hope to understand whether *Ginkgo biloba* extract has the potential to serve as a cognitive enhancer. We use in silico methods to determine the binding affinity of two key molecules: luteolin and ginkgolide B. We hypothesize that luteolin and ginkgolide B will bind to IRAP and have similar binding affinities to known IRAP inhibitors.

Methods

To test our hypothesis, we used computer simulation to model IRAP and potential inhibitors using a similar approach to Nakajima (2020). We used PDB structure 6YDX as our template for IRAP (Mpakali et al., 2020). From our literature, we found multiple ligands to choose from within the *G. biloba* extract. We chose luteolin and ginkgolide B (Li et al., 2018). For the positive control, the choice was AngIV (Barlow and Thompson, 2020). HFI-142 is a benzopyran that

represents our second positive control (Albiston et al., 2008).

During our research we've come across multiple software such as Chimera 1.14. After researching we designed the ligands in ChemSketch and exported each molecule as a .mol to Chimera 1.14. In Chimera, we selected Dock Prep and executed the following modifications to each protein: Delete solvent, Add Hydrogens, Mutate incomplete sidechains to ALA or GLY, Add charges using AMBER ff14S. Net charges for non-standard residues were assigned using the Gasteiger method (Junmei et al., 2006). Protein-ligand pairs were submitted to SWISS-DOCK.

Subsequently, we determined the proper docking by comparing the predicted clusters to the corresponding crystal structure for IRAP-AngIV. Next, we went to ViewDock and changed the Chain IDs by Edit text file to rewrite chain IDs for ligands. Finally, we used PRODIGY-LIGAND to calculate the change in Gibbs free energy, ΔG (kcal/mol). The more negative the number, the more favorable the binding energy. Full fitness is calculated as the average of the top 30% predicted effective energies for a given cluster; effective energy is the sum of the total energy of the system (Grosdidier, et al., 2011). The best predictions for each ligand docking with IRAP were modelled and captured in Chimera.

Results

The results of simulations in SWISS-DOCK and PRODIGY-LIG are shown in Table 1. Luteolin has the most negative full fitness value. However, AngIV has the lowest predicted ΔG (-8.68 kcal/mol SWISS-DOCK; -9.6 kcal/mol PRODIGY-LIG). The difference of ΔG between Ginkgolide B and AngIV is 1.3 kcal/mol; the difference of ΔG between HFI-142 and Luteolin is much smaller (0.1 kcal/mol).

As shown in Figure 1, the structure of Luteolin (A) is similar to that of HFI-142(B). For instance, Luteolin and HFI-142 have three aromatic rings each, though HFI-142 contains an amine group.

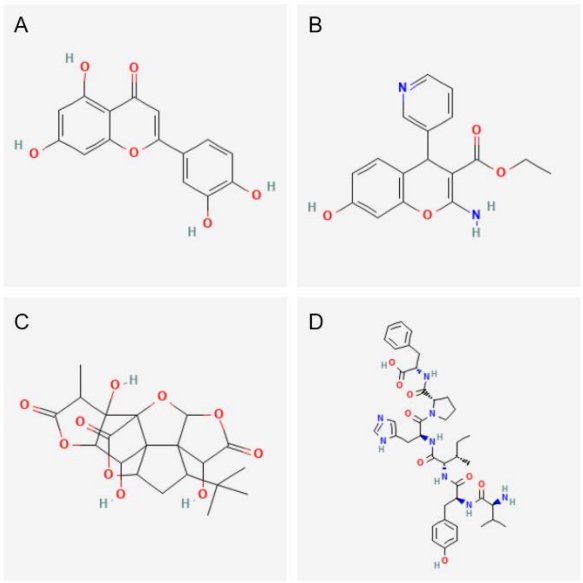

FIGURE 1: 2-dimensional chemical structures of Luteolin (A), HFI-142 (B), Ginkgolide B (C), and AngIV (D).

On the other hand, the structures of ginkgolide B (C) and AngIV (D) are quite different in both size as well as charge. As AngIV is a peptide, it is nitrogen-rich, whereas Ginkgolide B contains no nitrogen.

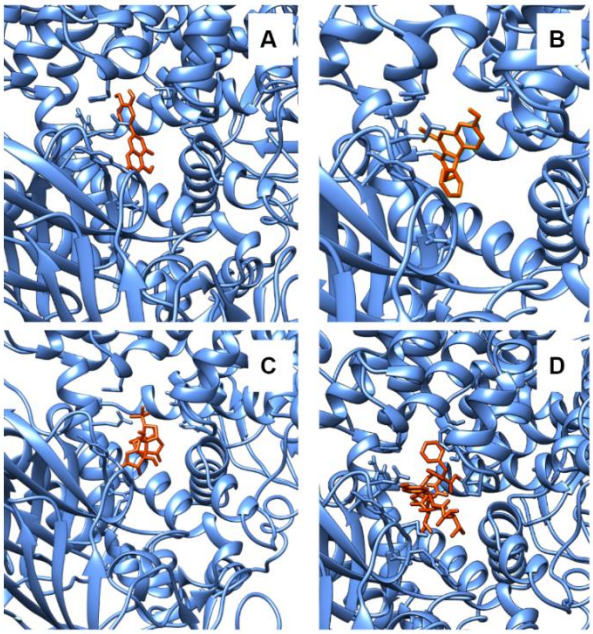

FIGURE 2: The predicted docking positions for each ligand with IRAP: luteolin (A), HFI-142 (B), ginkgolide B (C), and AngIV (D).

TABLE 1: Full fitness and ΔG calculations for each molecule.			
	Full fitness (kcal/mol)	ΔG (kcal/mol) (SWISS-DOCK)	ΔG (kcal/mol) (PRODIGY-LIG)
AngIV	-3873.60	-8.68	-9.6
Luteolin	-3988.18	-7.13	-7.6
Ginkgolide B	-3868.93	-8.44	-8.3
HFI-142	-3978.44	-7.10	-7.5

As shown in Figure 2, SWISS-DOCK correctly predicted the true binding site of AngIV (D). Furthermore, all of the best docking predictions were found to be in the same docking site as AngIV. All ligands interacted with GLU 426, GLN 922, SET 960, and THR 962. Luteolin (A) and HFI-142 (B) have nearly identical orientations within the docking site. HFI-142 was the only ligand to bind with ARG 439. As expected due its size, AngIV has more potential electrostatic interactions within the docking site than the smaller, non-peptide molecules tested; it also interacted with SER 207, GLN 293, ALA 961, and ASN 965.

Discussion

The purpose of this study was to explore whether *Ginkgo biloba* extract can be used for memory enhancement. *Ginkgo biloba* extract contains a range of terpene lactones and flavone glycosides, including ginkgolide B and luteolin, respectively. Full fitness and delta G values tell us the affinity of our molecules to IRAP. When a molecule binds and inhibits IRAP, it has the potential to boost memory in the short- and long-term.

We found that luteolin had the most negative full fitness value of the four IRAP inhibitors tested, and that AngIV had the most negative ΔG. Luteolin had similar predicted binding affinities as the known inhibitor, HFI-142. Interestingly, their chemical structures are also similar. This suggests that luteolin may be a promising memory-enhancing molecule.

AngIV was predicted to be the strongest inhibitor in both SWISS-DOCK and in PRODIGY-LIGAND. Ginkgolide B was found to have a similar full fitness and ΔG values as AngIV in SWISS-DOCK, but a noticeably lower ΔG in PRODIGY-LIG. That said, ginkgolide B had a more negative predicted binding affinity than both luteolin and HFI-142. It's possible that ginkgolide B is a better inhibitor than even HFI-142.

The conclusions that we can draw from this study are somewhat limited in that we only used silico methods. We did not test how the molecule is absorbed, metabolized, or delivered to IRAP in vivo. Future silico research should test additional molecules in *Ginkgo biloba* extract for their ability to inhibit IRAP, as well as test other relevant proteins related to cognitive function. After screening candidate molecules virtually, binding affinity assays could be done *in vitro.*

Ultimately, I would like to focus on how to utilize *Ginkgo biloba* extract safely while minimizing potential side effects. There are numerous nootropic products available that contain *G. biloba* extract, yet the underlying mechanism of actions are poorly understood. Nootropics have the potential to not only help alleviate the symptoms of cognitive decline associated with Alzheimer's Disease, but also provide a "brain boosting" effect for healthy adults and students alike. This work is a step forward towards understanding how natural nootropics, such as *G. biloba* extract, improve cognitive function and memory on a molecular level.

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