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Determination of Candidate Alternative Plant Actives for Dementia and Alzheimer's Disease Proteins through Docking Studies

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Plant actives,
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Docking

Abstract: Alzheimer's disease (AD) is a common type of dementia, which is a progressive brain disorder causing memory, thought and behavioral issues. Effective therapeutic treatments for AD and/or Dementia have not yet been developed. In this study select transcriptomic datasets were analyzed and disease proteins that comply with selection criteria were identified. These proteins were then docked with Donepezil, Galantamine, Memantine and Rivastigmine drugs as well as *Thymus cilicius*, *Melissa officinalis*, *Salvia sclarea*, *Linum usitatissimum* and *Curcuma longa* plant actives. Resulting binding energy values for mutant proteins are significantly different from wild type, especially in MET (MET proto-oncogene, receptor tyrosine kinase) (PDB ID: 3ZXZ). The plant actives showed notable Relative Stability values when docked with wild type proteins in comparison to drug molecules. To conclude, Alpha-Muurolene, Alpha-Atlantone, Alpha-Cadinene, Beta-Bourbonene, Beta-Cubebene and Germacrene-D as candidate alternative plant actives have been suggested for these diseases.

Demans ve Alzheimer Hastalığı Proteinlerine Yönelik Aday Alternatif Bitki Aktiflerinin Kenetlenme Çalışmaları ile Belirlenmesi

Anahtar Kelimeler
Demans,
Alzheimer hastalığı,
Hastalık proteinleri,
Bitki aktifleri,
İlaçlar,
Kenetlenme

Öz: Alzheimer hastalığı (AD), hafıza, düşünce ve davranış sorunlarına neden olan ilerleyici bir beyin bozukluğu olan yaygın bir demans türüdür. AD ve/veya Demans için etkili terapötik tedaviler henüz geliştirilememiştir. Bu çalışmada, bazı transkriptomik veri kümeleri analiz edilmiş ve seçim kriterlerine uyan hastalık proteinleri belirlenmiştir. Bu proteinler Donepezil, Galantamine, Memantine ve Rivastigmine ilaçlarının yanı sıra *Thymus cilicius*, *Melissa officinalis*, *Salvia sclarea*, *Linum usitatissimum* ve *Curcuma longa* bitki aktifleri ile kenetlenmiştir. Mutant proteinler için ortaya çıkan bağlanma enerjisi değerleri, özellikle MET'de (MET proto-onkogen, reseptör tirozin kinaz) (PDB ID: 3ZXZ) olmak üzere, vahşi tipten önemli ölçüde farklıdır. Bitki aktif maddeleri, ilaç molekülleri ile karşılaştırıldığında vahşi tip proteinlerle kenetlendiğinde dikkate değer Göreceli Stabilite değerleri göstermiştir. Sonuç olarak, bu hastalıklar için Alpha-Muurolene, Alpha-Atlantone, Alpha-Cadinene, Beta-Bourbonene, Beta-Cubebene ve Germacrene-D aday alternatif bitki aktif maddeleri olarak önerilmiştir.

1. Introduction

Dementia is a disease caused by mental dysfunction. It is usually a chronic or progressive disorder resulting from a variety of brain diseases that affect memory, thought, behavior and ability to perform daily activities [1, 2]. Individuals with one of several forms of dementia experience neurological decline, including difficulty and worsening in cognitive, psychological, and physical activities [3]. The differentiation between

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a variety of dementia syndromes can be hard due to similar clinical features. Nevertheless, some of the common forms of dementia as follows; Alzheimer's disease (AD), Lewy Body Dementia and Mixed Dementia. Huntington's disease, Parkinson's disease and Multiple Sclerosis can be listed as other forms of dementia [4, 5].

AD is a neurodegenerative disease that generally effects elderly people. AD ranges from early memory changes to functional dependence and death [6]. The global burden of the disease is expected to increase further due to the aging of the population. There is currently no cure. Current therapies for AD provide only a slight improvement in symptoms. The development of amyloid - β (Aβ) plaques is the most prominent feature of this disease. An intrinsic outlook of the molecular pathogenesis of AD may contribute to a new viewpoint in perception of the disease, hence offering improved opportunities for timely diagnosis and treatment [7]. Genome-wide gene expression studies have changed the prospect of medicinal studies [8]. The aim of gene expression studies is to focus on target/biomarker genes in dementia and AD that may function in AD signaling pathways [9].

Although the prevalence of dementia and AD continues to increase, successful symptomatic treatments for these diseases have not yet been developed [10]. Demonstration of damage to cholinergic pathways in the brain is of great interest in drug development. Acetylcholinesterase inhibitors such as Galantamine and Memantine are generally prescribed to treat dementia and AD [11]. These medications help improve cognitive functions such as memory and thoughts [12].

Multitude of pharmaceutics are developed through the synthesis of naturally existing compounds of plants. Scientific interest in the medicinal use of plants, which often have no significant side effects, for disease and health improvement has increased. Herbal and natural products are the oldest remedies known to man. Medicinal plants have been used by all cultures throughout history. The demand for herbal products is growing exponentially worldwide. Herbal medicines are believed to have a crucial role in the treatment of dementia, AD and memory deficit [13]. Herbs such as *Curcuma longa*, *Melissa officinalis* and *Thymus cilicius* have been reportedly used in the alternative treatments for these disease [14–16]. Various studies investigate the acetylcholinesterase inhibitory effects of herbal medicines used in alternative treatments. Kindl and coworkers researched the antioxidant and anticholinesterase potential of several Thymus species and indicated these species could be used in the prevention and treatment of AD [17]. Dastmalchi and coworkers analyzed the acetylcholinesterase inhibitory effects of *Melissa officinalis*. Their results showed that acetylcholinesterase enzyme was inhibited in a time and dose dependent manner [18]. Kennedy and coworkers identified the cholinesterase inhibitory effects of *Salvia officinalis*. Their results indicated *Salvia officinalis* exhibited inhibition of acetylcholinesterase [19]. Another study done by Teh and coworkers identified the antioxidant, antimicrobial and acetylcholinesterase inhibitory effects of flax seed (*Linum usitatissimum*) [20].

Molecular docking has become a prominent tool in exploring interactions between a protein and a molecule, which may be used in drug development studies. The docking process is comprised mainly of predicting conformation, position and orientation of the ligand and assessing the binding affinity [21]. In drug design, it is important to calculate the interactions between the protein active site and candidate molecules to construct three-dimensional structures. There are many physicochemical parameters in drug design, moreover, the importance of calculating binding energies has been emphasized. Various docking softwares/tools are being used to this end such as Autodock Vina [22–24]. The "force field" used for free energy calculations in Autodock Vina program evaluates bonding in two steps. First, the ligand and the protein start in unbound free conformation and their interaction energies are calculated, which includes the estimated loss of entropy, for each of the ligand and proteins' bound and unbound conformations. Then the lowest energies are computed for the interaction. Studies utilizing docking softwares/tools are diverse. Pradeepkiran and coworkers tried to resolve the issue of inadequacy of Aβ-targeted therapeutics in AD by recommending an alternate drug target [25]. They implemented molecular docking and simulation studies for p-tau to determine hyperphosphorylated and identified five ligands with high docking scores and optimal protein-ligand interactions of p-tau. Another study by Monteiro and coworkers evaluated 39 flavonoids through in silico docking studies by comparison to reference compounds [26]. Their results demonstrated seven of the flavonoids presented no toxicity risks, and had favorable absorption rates for the investigated targets. Shamsi and coworkers aimed at deciphering the molecular basis of interaction between Donepezil and human transferrin which is of importance in iron metabolism, to understand the activity and mechanism of drug binding through docking studies as well as other methods [27]. In a study done by Saleh and Sadeghi it was revealed through docking studies that Tetrahydrodeoxycorticosterone which is reduced in AD patients bound tightly to the catalytic site of enzyme and inhibit substrate binding [28].

In this study, we explore new plant active alternatives for AD and Dementia treatments through docking analysis by the use of force field free energy calculations in Autodock Vina program. Selected plant actives were compared with drug molecules Donepezil, Galantamine, Memantine and Rivastigmine

that are still in use for these diseases. The disease proteins were obtained through statistical analysis of gene expression datasets and listed according to selection criteria. Active sites of these proteins were detected. Conformer distribution and equilibrium geometry of the selected plant actives and drug molecules were calculated. Docking studies were accomplished accordingly and the selected amino acids were mutated.

2. Material and Method

2.1. Analysis of dementia and AD gene expression data sets

High throughput gene expression data (Alzheimer: GSE28146, GSE1297 and E-MEXP-2280; Dementia: GSE5281 and GSE13162) was obtained from Gene Expression Omnibus (GEO) [29] and analyzed using Bioconductor platform [30]. RMA normalization method and linear methods for microarray data (LIMMA) were used for statistical analysis [31]. The decision thresholds for differentially expressed genes were: downregulated: P-value < 0.05, FC <0.5 and upregulated: P-value < 0.05, FC >2 for all the datasets. Proteins of these differentially expressed genes were identified by DAVID (The Database for Annotation, Visualization and Integrated Discovery) Explanations, Visualization and Integration Database [32].

2.2. Identification of disease proteins used in the study

The disease proteins that fit the following selection criteria; proteins with Protein Data Bank IDs having a resolution of 2 Å and less, and those that have at least one ligand and being defined as Homo sapiens, were selected for docking studies. Protein symbols were converted to PDB IDs using Biological Database Network [33]. 3D structures of proteins' selected for both diseases were obtained from PDB [34], crystallizing ligands were cleaned from these proteins' x-rays.

2.3. Plant and drug lists related to dementia and AD

Five plants that are commonly used in alternative treatment for Dementia and AD (*Thymus cilicius, Melissa officinalis*, *Salvia sclarea*, *Linum usitatissimum* and *Curcuma longa*) were selected. Phytochemicals of these plants were obtained from Duke's Phytochemical and Ethnobotanical Databases - USDA [35]. The number of active molecules for these plants are 42, 66, 60, 149 and 100, respectively. A total of 117 plant actives were chosen to be used in the docking studies according to the following criteria for active molecules; approximate volume (Å3), functional groups (including hydroxy, carbonyl, amine groups) and having the same dipole moment.

Four of the most widely used FDA approved drugs for these diseases were obtained through literature review (Figure 1).

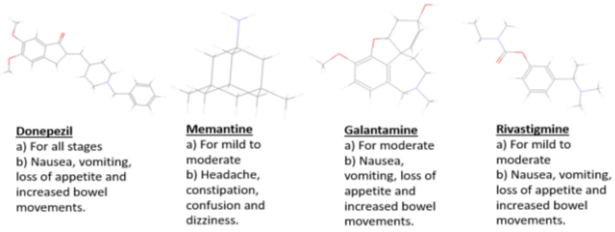

Figure 1. 2D structures of the studied drug molecules, a) usage areas and b) side effects

2.4. Conformer distribution and geometry optimizations

The 2D structures of the selected plant actives and drug molecules were obtained from PubChem [36]. The most stable conformer was determined by Spartan'14 V1.1.4 program [Spartan'14 Wavefunction Inc. Irvine, CA, 2006] in gas phase. Conformer distribution was performed using the Molecular Mechanics MMFF method [37]. Physicochemical properties such as Dipole moment (debye), weight (amu), volume (Å3) and logP values were also calculated.

2.5. Docking studies

A grid box was formed such that the active site amino acids of the proteins were in the cube measured at 45 x 45 x 45 Å3 using Biovia Discovery Studio Visualizer [38]. The x, y, z coordinate of the box is determined as the coordinate of the corresponding atom of the amino acid to which the inhibitor from the existing PDB ID is bound.

Each of the four drug molecules and the selected plant actives were separately docked using Autodock Vina (v1.1.2) [39] to the disease proteins and their binding energies were calculated. The molecules that interacted with amino acids were determined from docking studies.

The crucial amino acids that were identified as suitable for bonding were then mutated with Biovia Discovery Studio Visualizer (v17.2.0.16349). The changes in binding energies as a result of mutations depending on the binding regions of the enzymes are obtained for enzyme-based ligand design.

2.6 Mutation Studies

Mutants for the amino acids with crucial interactions in wild type docking studies were built and all docking studies were repeated for each drug molecule and the selected plant active to determine the essential role of the mutant proteins.

3. Results

In this study, the proteins of Dementia and AD were obtained through statistical analysis of gene expression datasets. The PDB ID's for these proteins were determined and the proteins with the desired specifications (resolution under 2 Å, organism: Homo sapiens and at least 1 unique ligand) were selected (Table 1).

After the proteins were stripped from their crystallized inhibitors and water molecules, the x, y, z coordinates were established to determine the center of the grid box (45x45x45 Å3). The interactions between active site amino acids and the ligands are given in Figure 2.

Accordingly, the drug molecules and the plant actives were docked to these proteins, respectively. The amino acid interactions with drug molecules and plant actives were listed (Table 2).

Table 1. Dementia and AD disease proteins

	Dementia				**Alzheimer**			
Protein Symbol	PGK1		MET		FKBP1B		UBE2N	
PDB ID	3C39	5O7D	3CD8	3ZXZ	5HKG	4IQ2	4ONN	4ONM
Resolution (Å)	1.85	1.84	2	1.8	1.5	1.7	1.5	1.35

Table 2. The amino acid - plant active/drug molecule interactions (plant actives are written as italic and drug molecules are in bold character)

FKBP12B protein with PDB ID: 5HKG and 4IQ2												
Memantine					V55	I56						
Cadinene	Y26			F46	V55	I56	F59					F99
Isospathulenol	Y26			F46	V55	I56	F59					F99
Alpha-Cadinene	Y26	F36		F46	V55	I56	F59		H87			F99
Alpha-Muurolene	Y26	F36		F46	V55	I56	F59					F99
Beta-Elemene	Y26	F36		F46	V55	I56	F59	Y82		V90	I91	F99
Copaene	Y26	F36		F46	V55	I56	F59			V90		F99
Galantamine	Y26		D37	F46	V55							
(+)-(S)-Ar-Turmerone	Y26	F36	D37	F46	V55		F59					F99
Alpha-Curcumene	Y26		D37	F46	V55		F59			V90	I91	
Zedoarondiol	Y26		D37	F46	V55	I56	F59	Y82				F99
Spathulenol	Y26		D37	F46	V55	I56	F59					
Tryptophan	Y26		D37	F46	V55	I56						
Rivastigmine	Y26	F36					F59	Y82			I91	
Alpha-Bisabolol	Y26	F36		F46	V55		F59	Y82	H87	V90	I91	
Beta-Elemene	Y26	F36		F46	V55	I56	F59	Y82			I91	F99
UBE2N protein with PDB ID: 4ONM and 4ONN												
Galantamine						K68	V69					
2-Hydroxy-Methyl-Anthraquinone		Y34			F57	K68	V69	R70				
Rhamnazin					F57	K68	V69	R70	R85			
Memantine					F57	K68		R70				
Alpha-Atlantone		Y34			F57	K68		R70				
Spathulenol		Y34			F57	K68		R70				
Cadinene		Y34			F57	K68		R70				
Isospathulenol		Y34			F57	K68		R70				
Alpha-Cadinene		Y34			F57	K68		R70				
Alpha-Cubebene		Y34			F57	K68		R70				
Beta-Cubebene		Y34			F57	K68		R70				
Beta-Elemene		Y34			F57	K68		R70				

Beta-Selinene					F57	K68		R70	R85			
Rivastigmine		Y34	E55		F57	K68						
Galantamine			E55			K68						
Alpha-Atlantone	N31	Y34	E55	L56	F57	K68	V69	R70				
Apigenin		Y34	E55		F57	K68						
Tryptophan		Y34	E55		F57	K68						
Vicenin-1		Y34	E55		F57	K68	V69					
Memantine			E55		F57	K68						
Rhamnocitrin		Y34	E55		F57	K68	V69					
Rivastigmine		Y34	E55		F57	K68		R70				
PGK1 protein with PDB ID: 5O7D and 3C39												
Galantamine		A214			F291				P338			
(+)-(S)-Ar-Turmerone		A214	F241	L256	F291				P338			
Alpha-Curcumene		A214	F241	L256	F291				P338			
Alpha-Cadinene		A214		L256	F291				P338			
Beta-Elemene		A214		L256	F291			L313	P338			
Memantine									P338			
Beta-Elemene		A214		L256	F291			L313	P338			
Copaene		A214							P338			
Delta-Cadinol		A214							P338			
T-Muurolol		A214							P338			
Galantamine		A214			F291							
(+)-(S)-Ar-Turmerone		A214		L256	F291	M311			P338			
2-Hydroxy-Methyl-Anthraquinone	G213	A214		L256	F291							
Alpha-Atlantone		A214		L256	F291							
Alpha-Curcumene		A214		L256	F291	M311			P338			
Zingiberene		A214		L256	F291	M311			P338			
Cadinene		A214		L256	F291			L313	P338			
Isospathulenol		A214	G238	L256	F291				P338			
Nerolidol		A214		L256	F291	M311						
Apigenin		A214		L256	F291		G312					
Tryptophan		A214		L256	F291	M311	G312					
Vicenin-1		A214		L256	F291							
Vitexin	G213	A214		L256	F291					G340		
Alpha-Cadinene		A214		L256	F291	M311		L313				
Alpha-Cadinol		A214		L256	F291			L313				
Beta-Elemene		A214		L256	F291			L313	P338			
Cadina-1,4-Diene		A214		L256	F291			L313	P338			
MET protein with PDB ID: 3CD8 and 3ZXZ												
Donepezil									H1162	G1163	M1211	
Rivastigmine		V1092									M1211	
1,7-Bis-(4-Hydroxy-Phenyl)-Hepta-1,4,6-Triene-3-One		V1092	A1108						H1162	G1163	M1211	
Beta- Bourbonene	I1084	V1092	A1108		L1140	L1157		M1160			M1211	
Germacrene D, 1,10-epoxide	I1084	V1092	A1108		L1140	L1157					M1211	A1221
Germacrene-D		V1092	A1108					M1160			M1211	
Alpha-Bisabolol	I1084	V1092	A1108				Y1159				M1211	
Cadinene	I1084	V1092	A1108								M1211	A1221
Isospathulenol		V1092									M1211	A1221
Alpha-Cadinol		V1092	A1108	K1110		L1157					M1211	A1221
Alpha-Cubebene	I1084	V1092	A1108	K1110		L1157					M1211	A1221
Alpha-Muurolene	I1084	V1092									M1211	
Beta-Cubebene	I1084	V1092									M1211	A1221
Copaene		V1092									M1211	
Delta-Cadinol	I1084	V1092	A1108			L1157					M1211	
T-Muurolol	I1084	V1092	A1108								M1211	A1221
Donepezil		V1092							M1211		A1226	Y1230
Beta-Cubebene		V1092	A1108		L1140	L1157			M1211	A1221	A1226	Y1230
Galantamine	I1084	V1092	A1108						M1211			Y1230
Germacrene-D	I1084	V1092	A1108		L1140		Y1159	M1160	M1211			Y1230
Alpha-Muurolene	I1084	V1092	A1108	K1110		L1157			M1211			Y1230

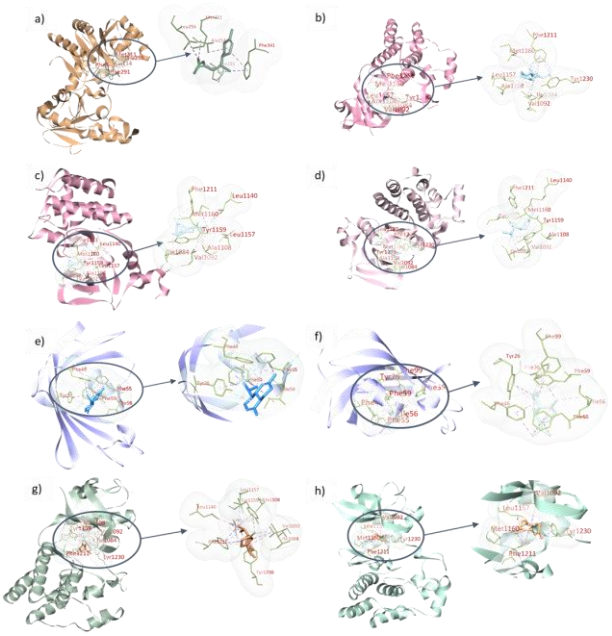

Figure 2. The graphical images of the protein structures with their active sites: a) 3C39, Alpha-Atlantone b) 3CD8, Beta-Bourbonene, c)3CD8, Beta-Cubebene, d) 3CD8, Alpha-Muurolene, e)5HKG, Alpha-Cadinene, f)5HKG, Alpha-Muurolene, g)3ZXZ, Beta-Cubebene, h)3ZXZ, Germacrene-D

Aromatic amino acids have been replaced with aliphatic amino acids and vice versa. V, K, Y, F and M are replaced with F, Y, I, I and F respectively (Table 3). The repetitive amino acids were selected, mutated and re-docked with the same approach (Table 4).

Table 3. Generated mutations for the selected disease proteins

Generated Mutations			Protein Name	PDB ID
Wild Type	**Amino Acid Number**	**Mutant Type**		
V	55	F	FKBP1B	5HKG
K	68	Y	UBE2N	4ONM, 4ONN
Y	26	I	FKBP1B	4IQ2
F	342	I	PGK1	3C39
V	341	F	PGK1	3C39
M	1211	F	MET	3CD8,3ZXZ
F	291	I	PGK1	5O7D

To detect which amino acid is more effective on the binding energy, the following formula was used:

Relative Stability (RS) = Post Mutation Binding Energy (PMBE) - Wild Type Binding Energy (WTBE)

Table 4. Dementia and AD disease protein's wild type and post-mutation docking results that include plant actives that are more stable than drug molecules according to RS values (kcal mol-1) (plant actives are written as italic and drug molecules are in bold character).

	WTBE	PMBE	RS	WTBE	PMBE	RS
FKBP1B		5HKG			4IQ2	
Memantine	-6.5	-7.2	-0.7			
Galantamine				-6.3	-6.3	0
Rivastigmine				-5.3	-5.1	0.2
Cadinene	-6.7	-7.4	-0.7			
Isospathulenol	-7	-7.8	-0.8			
Alpha-Cadinene	-6.5	-7.6	-1.1			
Alpha-Muurolene	-6.6	-7.8	**-1.2**			
Beta-Elemene	-6.5	-7.2	-0.7	-6.1	-6.6	-0.5
Copaene	-6.8	-7.5	-0.7			
(+)-(S)-Ar-Turmerone				-6.3	-6.6	-0.3
Zedoarondiol				-6	-6.7	-0.7
Alpha-Curcumene				-6.5	-6.3	0.2
Tryptophan				-6.2	-6	0.2
Spathulenol				-7.1	-6.9	0.2
Alpha-Bisabolol				-6.3	-6.3	0
UBE2N		4ONN			4ONM	
Galantamine	-6.2	-6.5	-0.3	-6.6	-6.9	-0.3
Memantine	-5.6	-5.7	-0.1	-6.4	-6.6	-0.2
Rivastigmine	-5.5	-5.6	-0.1	-5.9	-6.1	-0.2
Alpha-Atlantone	-6	-6.6	-0.6	-6.8	-7.2	-0.4
Vicenin-1	-7.4	-7.6	-0.2			
Rhamnocitrin	-6.9	-7	-0.1			
Apigenin	-7.1	-7.2	-0.1			
Chlorogenic-Acid	-6.6	-7.1	-0.5			
Tryptophan	-6.5	-6.6	-0.1			
Rhamnazin				-7.2	-7.5	-0.3
2-Hydroxy-Methyl-Anthraquinone				-8	-8.2	-0.2
Spathulenol				-6.7	-6.9	-0.2
Cadinene				-7.1	-7.3	-0.2
Isospathulenol				-6.6	-6.8	-0.2
Alpha-Cadinene				-7.2	-7.4	-0.2
Alpha-Cubebene				-6.7	-6.9	-0.2
Beta-Cubebene				-6.6	-7	-0.4
Beta-Elemene				-6.4	-6.7	-0.3

Beta-Selinene				-7.1	-7.4	-0.3
PGK1		3C39			5O7D	
Memantine	-5.9	-5.4	0.5			
Galantamine	-5.9	-7.5	**-1.6**	-7.1	-7.6	-0.5
(+)-(S)-Ar-Turmerone	-6.9	-7.5	-0.6	-6.5	-6.1	0.4
Alpha-Atlantone	-6.1	-7	-0.9			
Alpha-Curcumene	-6.7	-7.6	-0.9	-6.3	-5.9	0.4
Isospathulenol	-6.3	-7.1	-0.8			
Apigenin	-8.4	-8.9	-0.5			
Vicenin-1	-7.4	-7.9	-0.5			
Alpha-Cadinene	-7	-8.1	-1.1	-7	-6.8	0.2
Alpha-Cadinol	-6.6	-7.6	-1			
Cadina-1.4-Diene	-6.9	-7.5	-0.6			
Alpha-Muurolene	-5.9	-5.7	0.2			
Copaene	-6.1	-6.4	-0.3			
Delta-Cadinol	-6.2	-5.9	0.3			
T-Muurolol	-6.1	-6.2	-0.1			
2-Hydroxy-Methyl-Anthraquinone	-8.3	-8.5	-0.2			
Zingiberene	-6.9	-7.3	-0.4			
Cadinene	-7.4	-7.8	-0.4			
Nerolidol	-5.9	-6.1	-0.2			
Tryptophan	-6.7	-7	-0.3			
Vitexin	-7.7	-7.9	-0.2			
Beta-Elemene	-6.4	-6.2	0.2	-6.2	-5.9	0.3
MET		3CD8			3ZXZ	
Donepezil	-8	-8.5	-0.5	-8.2	-9.1	-0.9
Rivastigmine	-6.6	-6.6	0			
Galantamine				-6.7	-8.7	**-2**
Beta- Bourbonene	-7.2	-8.1	-0.9			
Germacrene-D	-6.7	-7.5	-0.8	-7	-8.1	**-1.1**
Cadinene	-8.3	-8.8	-0.5			
Alpha-Cubebene	-7.2	-7.8	-0.6			
Alpha-Muurolene	-6.5	-7.4	-0.9	-6.8	-8	**-1.2**
Beta-Cubebene	-6.7	-7.7	-1	-7.3	-8.3	-1
T-Muurolol	-7.2	-7.8	-0.6			
1,7-Bis-(4-Hydroxy-Phenyl)-Hepta-1,4,6-Triene-3-One	-7.2	-7.6	-0.4			
Germacrene D, 1,10-epoxide	-7.3	-7.7	-0.4			
Alpha-Bisabolol	-7.5	-7.6	-0.1			
Isospathulenol	-7.4	-7.8	-0.4			
Alpha-Cadinol	-7.4	-7.8	-0.4			
Copaene	-6.8	-7.2	-0.4			
Delta-Cadinol	-7.4	-7.6	-0.2			

4. Discussion and Conclusion

In this study, docking and mutation analysis for select Dementia and AD disease proteins were done, plant actives and drug molecules were used as ligands.

One of the disease proteins identified in the analysis, PGK1 (PhosphoglycerateKinase 1) enzyme, is a 417 amino acid-long and ~45 kDa monomer. It is expressed in all somatic cells and premeiotic cells and involved in glycolysis. The three-dimensional fold of PGK is highly conserved among prokaryotic and eukaryotic enzymes, demonstrating a characteristic two-domain structure [40]. Dysfunctional glycolysis takes an active part in formation of AD which is a common form of dementia [41,42]. Altered expression of the PGK1 also causes muscle stiffness, hemolytic anemia, and mental retardation [43]. MET (MET proto-oncogene, receptor tyrosine kinase) protein is a heterodimer made of an alpha chain (50 kDa) and a beta chain (145 kDa) which are disulfide linked. They regulate physiological processes such as proliferation, morphogenesis and survival is dysregulated in various human cancers [44]. MET protein has also been associated with AD in previous studies [45]. FKBP1B (FKBP Prolyl Isomerase 1B), which is from the immunophilin protein family is a 12.6 kDa cis-trans prolyl isomerase and takes part in immunoregulation and key biological processes involving protein folding and trafficking. Gant and coworkers have identified that overexpression of FKBP1B in hippocampal neurons might reverse Ca+2 mediated brain aging [46]. UBE2N (Ubiquitin-conjugating enzyme E2N) plays a role in the regulation of cell cycle and differentiation, DNA repair and survival after DNA damage. It is involved in protein ubiquination pathway as well [47]. In a study done by Kelly and coworkers UBE2N was one of the significant disease proteins mutual in AD and Parkinson's Disease [48].

For FKBP1B protein; considering 5HKG, the RS for Memantine was - 0.7 kcal mol-1 whereas six of the

nine plant actives had RS ≤ - 0.7 kcal mol-1, especially the highest RS was found for Alpha-Muurolene with a value of -1.2 kcal mol-1. However, for 4IQ2, from the remaining drug molecules Galantamine has not been affected and the unstability of Rivastigmine has increased. The RS of Beta-Elemene, Zedoarondiol and (+)(S)-Ar-Turmerone have increased with - 0.5 kcal mol-1, -0.7 kcal mol-1 and - 0.3 kcal mol-1 respectively compared to Galantamine and Rivastigmine.

Concerning UBE2N protein; 4ONN, the RS for Alpha-Atlantone and Chlorogenic-Acid were significant with -0.6 kcal mol-1 and -0.5 kcal mol-1 respectively. For this protein (4ONN) all the studied plant actives in Table 3 have equal to or greater RS values than Rivastigmine and Memantine. For 4ONM, Alpha-Atlantone and Beta-Cubebene have greater RS values (with -0.4 kcal mol-1) then all the drug molecules.

For PGK1 protein; when examining 3C39, Galantamine has the highest value of RS with -1.6 kcal mol-1. Memantine's unstability has increased, all studied plant actives have a greater RS value than this molecule. Despite this, four of the nine plant actives have RS ≥ -0.9 kcal mol-1, highest one is the RS for Alpha-Cadinene had -1.1 kcal mol-1. Only Galantamine had -0.5 kcal mol-1 for 5O7D. However, all the studied plant actives have less RS than Galantamine.

Concerning MET protein; for 3CD8, Rivastigmine has not been affected and Donepezil showed -0.5 kcal mol-1. All the studied plant actives have a better RS values than Rivastigmine, seven of the studied plant actives have a higher RS value than Donepezil molecule. The highest RS value was Beta-Cubebene -1 kcal mol-1 and significantly high RS values were obtained for Beta-Bourbonene and Alpha-Muurolene (-0.9 kcal mol-1) as well. Considering 3ZXZ, Galantamine had -2 kcal mol-1 RS value and it is the highest in all Table 3. Germacrene-D and Alpha-Muurolene have increased their RS values -1.1 kcal mol-1 and -1.2 kcal mol-1 respectively. All the RS for 3ZXZ have shown remarkable values for both drug molecules and plant actives. This result depicts that 3ZXZ mutation is more efficient than the other mutations.

The 2D structures of the candidate alternative plant actives that come into prominence; Alpha-Muurolene, Alpha-Atlantone, Alpha-Cadinene, Beta-Bourbonene, Beta-Cubebene and Germacrene-D obtained from PubChem are given in Figure 3. Alpha-Muurolene is the only plant active mutual for the study.

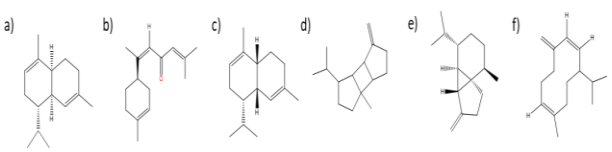

Figure 3. 2D structures of the candidate alternative plant actives a) *Alpha-Muurolene*, b) *Alpha-Atlantone*, c) *Alpha-Cadinene*, d) *Beta-Bourbonene*, e) *Beta-Cubebene* and f) *Germacrene-D*

The candidate alternative plant actives Alpha-Muurolene, Alpha-Cadinene and Beta-Cubebene are actives of *Melissa officinalis*; Alpha-Atlantone is active of *Curcuma longa*; Beta-bourbonene and Germacrene-D are actives of *Thymus cilicus*. Alpha-Muurolene, Beta-Bourbonene and Germacrene-D are sesquiterpenes. Cubebenes are classified as sesquiterpenes as well. Alpha-Cadinene is a bicyclic, Beta-cubebene is a tricyclic sesquiterpene. Sesquiterpenes, which are a class of volatile organic hydrocarbons have antitumor, anti-inflammation, and anti-fungal effects. In a study by Wang and coworkers, the anti-cancer effects of Beta-Bourbonene on prostate cancer were investigated [49]. Their results showed Beta-Bourbonene inhibited the proliferation and induced apoptosis of prostate cancer cells. Beta-Bourbonene is also used as a flavoring agent in food industry. Germacrenes have antimicrobial and intesticidal properties. Germacrene-D is also a carbobicyclic compound. In a study done by Rahali and coworkers, antioxidant and anticholinesterase activities of essential oils which compose of several actives such as Germacrene D, Beta-Bourbonene and Delta-Cadinene have been investigated [50]. A study done by da Silva and coworkers suggests Germacrene D can be used as a model for developing improved anti-cancer agents for leukemia [51]. Lozzio et al. mention the potential effects of Alpha-Muurolene, Alpha-Atlantone and Germacrene-D in the prevention and treatment of Alzheimer's disease [52]. Mawa et al. investigate several biological activities of Beta-Cubebene, Beta-Bourbonene and Germacrene-D such as antioxidant, anti-cancer and antibacterial activities in their study [53]. Alpha-Atlantone is from the class of organic compounds known as sesquiterpenoids. Sesquiterpenoids are a group of terpenoids, which are lipid like molecules. Mara and coworkers have identified antioxidant properties of Alpha-Atlantone in their study [54].

There are several striking outputs of this study. Firstly, the calculated binding energy values from the docking scores of the drug molecules are less favorable than the plant actives in general. This output is of importance for the illumination of candidate natural drug molecules. Secondly, obtained binding energy values for mutant proteins are significantly different from wild type, especially the results for 3ZXZ is striking. The studied drug molecules, Memantine and Galantamine, were found essentially more important than the other drug molecules due to their binding energies and surrounding amino acid interactions. In addition, the plant actives showed notable RS values for wild type proteins through docking studies. Thirdly, the selectivity of the active molecule with the lowest binding energy changes depending on the mutations. It is clearly observed that the targeted mutation of the disease proteins affects the selectivity of the drug molecule and often yield higher binding energies from the wild type similar to plant actives. The change in the amino acid sequence of the active

site residues is a crucial factor in binding energies. Here different molecules have come forth after the mutations as a result of selectivity.

To conclude Alpha-Muurolene, Alpha-Atlantone and Alpha-Cadinene can be candidate alternative plant actives for AD and Beta-Bourbonene, Beta-Cubebene, Germacrene-D and Alpha-Muurolene can be candidate alternative plant actives for Dementia.

Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried out.

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