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BCHD
Bioactive Compounds in Health and Disease

In silico analyses of bioactive compounds extracted from ziziphus jujuba using supercritical CO_2 extraction: Potential anti-anxiety and anti-Alzheimer's disease.

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ABSTRACT

Background: Jujube (Chinese date) is a fruit with a pleasant, sweet taste, traditionally used as sedative to treat insomnia and anxiety.

Objective: The study aims to explore the potential of these bioactive compounds in exerting anti-anxiety and anti-Alzheimer's disease effects.

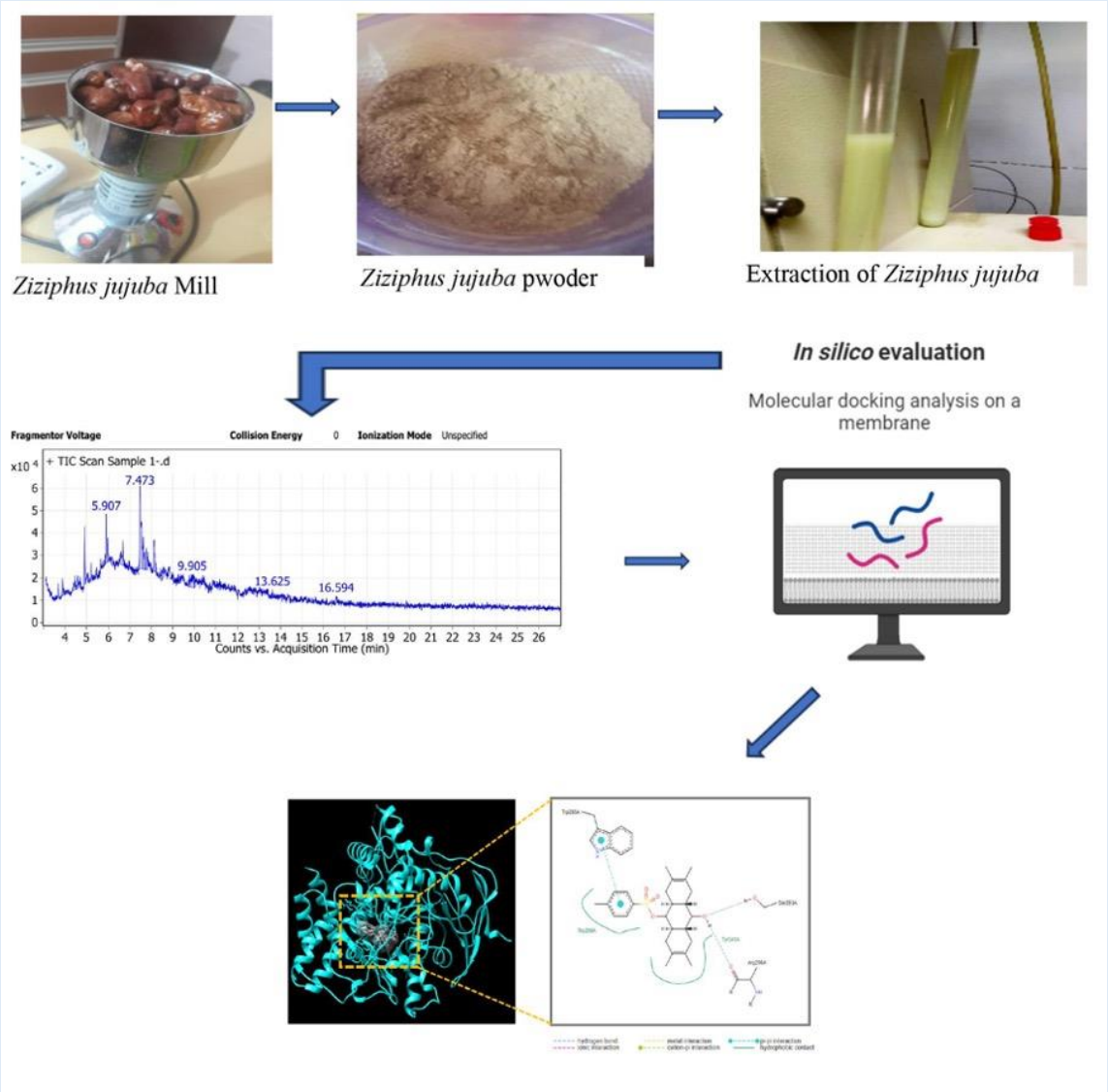
Methods: Molecular docking, ADME, and molecular dynamics studies were carried out for the green-extracted phytochemicals against acetylcholinesterase and Beta-amyloid proteins for Anti-Alzheimer's disease effect and serotonin receptor for anti-anxiety activity. Our results suggest that compound (2,3,6,7-tetramethyl-10-(4-methylphenylsulfonyloxy)-1,4,4.alpha.,5,8,8a.beta.,9.b) showed good binding affinity of -10.3 with acetylcholinesterase (4EY7) as anti-Alzheimer's disease and the compound andrographolide with serotonin transporter (6VRH) showed

binding affinity of -9.7 for anxiety. Compounds with the best docking scores were subjected to molecular dynamic simulations.

Results: These compounds revealed the best stability.

Conclusion: These findings are promising for manufacturing new functional foods, nutraceuticals, food supplements, and/or pharmaceuticals which may play a good and safe role in treating Alzheimer disease and anxiety. While further biological studies must be carried out, our computational studies suggest that these compounds have potent activity.

Keywords: Ziziphus jujuba; Bioactive compounds; Neurological disorders disease; Molecular docking, ADME and Molecular dynamics.



INTRODUCTION

Since ancient times, traditional or herbal medicine has been used to treat different diseases, and numerous studies have been carried out to know the efficacy and safety of phytochemicals. The use of active phytochemical constituents to treat diseases is one of the trends in the drug discovery process, and computational studies like molecular modeling are constituting this process [1-3]. Phytochemicals derived from plants have a great interest in developing new therapeutic strategies because of their vast range of biological activities [4-6]. Phytochemicals that are consumed in one's diet could decrease the risk of numerous diseases and does not cause any side effects [7-10]. In terms of neurological disorders, plant-based compounds have emerged and shown suitable inhibitory activities to cure diseases like Alzheimer's and Parkinson's disorders [11-13].

Ziziphus jujuba Mill. Commonly called Chinese date, belongs to the family of Rhamnaceae. Jujube is generally found in the Asian continent, mainly in China, Korea, India, and Japan. Jujube fruits are used as dietary supplements and have promising medicinal benefits. Due to the presence of vitamins, sugars, and minerals, the nutritional value of the plant was well known. Therapeutically, jujube helps with sleep deprivation and improves digestion [14,15]. The proven medicinal values of the jujube plant led to increased cultivating areas, and genetic engineering technologies were used to increase production in South and North China [16]. The potential health benefits of the plant made Western countries take an interest in cultivating jujube in their native regions. There are more than 100 cultivators of jujube in the United States [17]. Jujube showed pharmacological benefits in neurological disorders, like maintaining sleep quality by increasing the neurotrophic factor's expression [18]. It also has a wide variety of neurological protective activities and therapeutic effects against disorders like Alzheimer's [19] and anxiety [20]. Mental health or mental well-being are crucial aspects of a high-quality life. Neurological diseases cause severe cognitive, sleep, and memory-related problems. Factors like age, environmental conditions, dietary habits, etc., can cause neurodegenerative diseases [21]. Recent studies have revealed that phytochemicals are essential in developing new treatments for neurological disorders [22]. The present study mainly focuses on Alzheimer's and anxiety.

Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by dementia. AD causes cognitive impairment and mental illness. Despite many ongoing research efforts to treat AD, unfortunately there is no permanent cure for the disease [23]. Tau-protein hyperphosphorylation due to the accumulation of β-amyloid (βA) and neurofibrillary tangles in the intercellular region of the brain causes AD [24]. The role of acetylcholinesterase was studied extensively; the conversion of acetylcholine by hydrolysis to choline and acetic acid is controlled by acetylcholinesterase; this process reduces the acetylcholine content in the brain regions like the hippocampus and cortex, which maintains physiological functions of the brain [25, 26]. Inhibiting acetylcholinesterase was proven to show reducing the effects of AD. Drugs like donepezil have inhibiting properties of acetylcholinesterase and the FDA approved donepezil to treat Alzheimer's disease [27]. We are trying to focus on inhibiting acetylcholinesterase by using phytochemical extracts of jujube by taking donepezil as standard [28].

Neurological disorders like anxiety and depression contribute to disturbances in mental health [29]. Development of early age stress is the main reason to develop anxiety biologically due to the neural structures and the defects in their functions [30]. Behavioral changes, anxiety, and mood-related parts are affected by serotonergic transmission. Serotonin transporter plays a significant role in serotonergic transmission, aiding serotonin clearance in synaptic cleft [31]. Inhibition of serotonin receptors with phytochemicals may help in reducing anxiety and aid in maintaining mental well-being [32].

In the new trends of drug discovery, computational methods are playing a vital role by aiding the drug discovery process by reducing the time of screening molecules, scaffold identification, and predicting toxicity parameters [33, 34]. Using methods like molecular docking, finding affinities between ligand and target protein was made easy, and molecular dynamics was used to give the change in stabilities in terms of structure by the ligand binding [35- 38]. In phytochemical/natural product screening, computational drug discovery tools are useful for the fast and easy screening of compounds or phytochemicals [39-41]. The present study aims to evaluate the phytochemical green-extracted Ziziphus Jujuba Mill computationally. Functional foods and nutraceuticals manufactured with jujube fruit could be involved in treating neurological disorders.

The study aims to explore the potential effects of exerting the jujube's bioactive compounds as anti-anxiety and anti-Alzheimer's disease natural remedy by employing in silico methods, such as molecular docking, molecular dynamics, and ADME. The researchers aim to identify specific compounds within Ziziphus jujuba that have the potential to interact with relevant molecular targets associated with anxiety and Alzheimer's disease. The results of this research can be the nucleus of other future in vitro, in vivo, and clinical research in order to verify the possibility of its clinical implementation.

MATERIALS AND METHODS

Plant material preparation: The dried Jujube fruits purchased from the local market in Baghdad city were milled for a short time (1-3min) using a high-speed multi-functional electric crusher (650W, 22000r/m, Beem, Germany). The ground fruits were sieved to isolate the corners. The milled jujube was ground again to make it smoother, then stored in sealed plastic bags in a cold, dark place until further extraction.

Plant Extraction: The Jujube powder (300g) was subjected to green extraction by supercritical CO_2 extraction technique using (Supercritical CO_2, Green Extraction, Spe-ed TM SFE-2/4 system, Applied Separation, USA) with 90% ethanol as a co-solvent. The extraction employed as reported by Song et al., [42] with some modifications as follows: dynamic extraction time: 3 hours; static time: 1 hour; CO_2 flow rate: 50g/min; CO_2 pressure: 270 bar (10 MPa); vessel (oven) temperature: 52°C. The Co-solvent was evaporated using a rotary evaporator (BÜCHI Labortechnik AG, Flawil, Switzerland).

Gas chromatography mass spectrometry (GC–MS): GC-MS analysis of fruits of jujube was carried out by using Perkin Elmer Auto System XL Equipped with flame ionization detector (FID), column type of ZB-5, fused silica capillary column (60m, 0.32mm, i.e., 0.25µm film thickness). The oven temperature was sustained initially at 50 °C for 5 min and then automated from 50 to 240°C at a 3°C/min rate. A sample size of 2µl with a split ratio of 1:10. The injector temperature was maintained at 2300C, and the detector temp was. 2500C. Helium gas was used as a carrier at a 1 ml/min flow rate [42].

In silico studies: To evaluate in-silico studies, we have used various tools like PyRx, AutoDock Vina 4.2, Discovery studio visualizer, PyMol, GROMACS and web servers including Swiss ADME and Pose viewer.

Preparation of Ligand: All the structures were evaluated from the GC-MS, and known structures were downloaded from PubChem in the 3D orientation of SDF format. Structures that are unknown were drawn using Chem Draw and converted into 3D. Clustering of ligands to screen was done using Discovery studio visualizer [43].

Preparation of Protein: Acetylcholinesterase(4EY7), Beta-Amyloid Fibril (2LMN), and Serotonin receptor (6VRH) are the proteins used in the study, which were downloaded from Protein data bank (PDB) in PDB format. Each protein was prepared using discovery studio by

removing the water molecules to prevent the interaction of water with the system while docking, based on the standard pockets binding sites identified. Additional ligand groups present in the protein were removed. Finally, the protein was saved in PDB format.

Molecular docking: The docking procedure for silica compounds in the molecule was done using AutoDock vina4.2, since the Si atoms were not defined in the PyRx setting [44]. Compounds without silica were docked using PyRx software [45]. First, energy minimization of ligands was done and converted in PDBQT format. The protein was loaded after specifying the grid parameters based on the co-crystals present, and proteins which do not have co-crystal like 2LMN. Prior studies involving in docking of 2LMN were referred to, and the pocket from those studies were taken into consideration, then docking was initialized.

2D and 3D interactions: 3D images of the complexes were generated by using chimera and saved in PDB format [46]. 3D complexes of the ligands and proteins were uploaded to the pose viewer website to get the 2D interaction images [47].

ADME Studies: ADME studies were carried out using Swiss ADME website by converting the structures into smiles format and uploaded to the website to predict the ADME properties.[48]

Molecular Dynamics: Best ranked proteins were performed for MD Stimulation using GROMACS software. Time of stimulation is for 100ns. CGenFF was used for topology generation and minimization of ligand. CHARMM 36 forced field was used to for the stimulation. TIP3P water model was used. RMSD, RMSF, hydrogen bond graphs were generated after the stimulation.[49]

RESULTS AND DISCUSSION

Supercritical CO_2-extraction revealed a greenish white extract as shown in Figure 1 having the same odor of the jujube fruit.

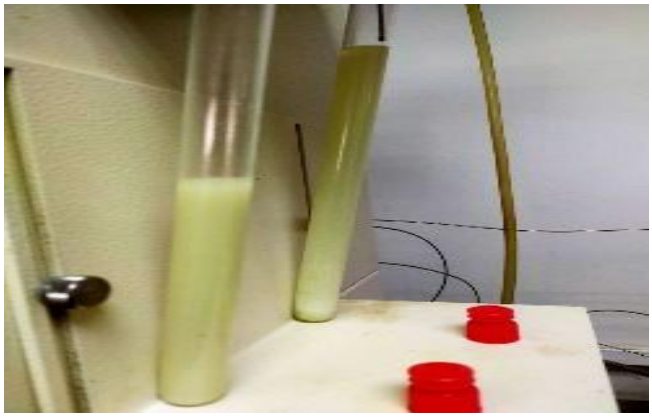


Figure 1. Jujube-Supercritical-CO2-extract

Gas chromatography mass spectrometry (GC–MS) analysis: GC-MS technique is used for the analysis of phytochemical constituents. The presence of bioactive compounds may be responsible for the therapeutic properties of the jujube fruits. GC-MS analysis of jujube fruits revealed that 16 compounds are present in the extract, 15 known and 1 unknown. Results from the GC-MS analysis are given in Figure 2 chromatogram and Table 1.

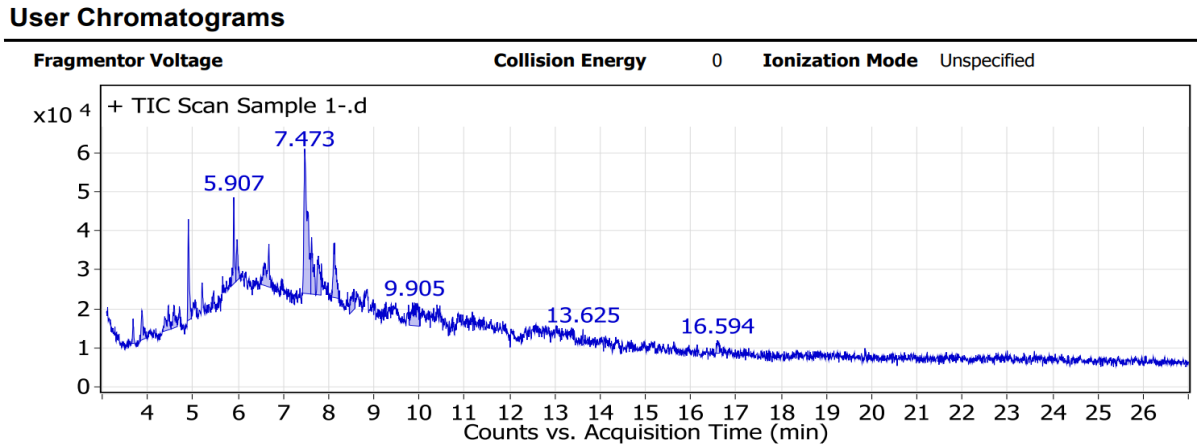
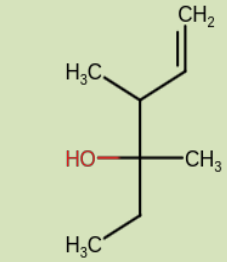
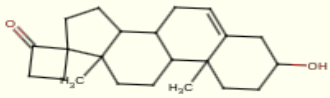
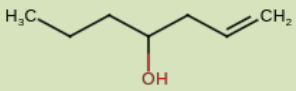
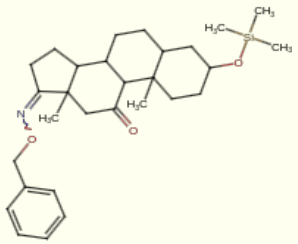
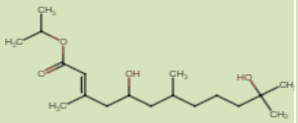
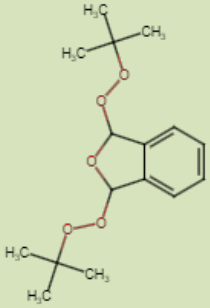
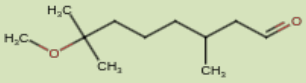
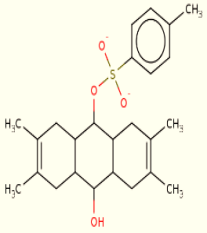
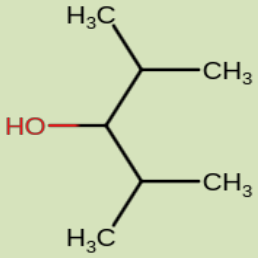
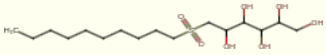
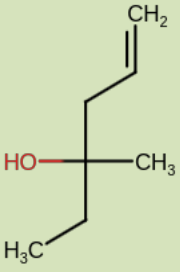
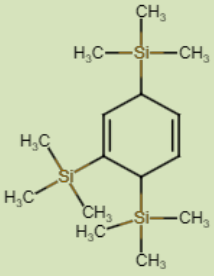
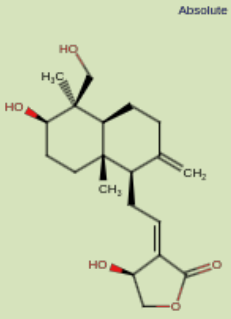
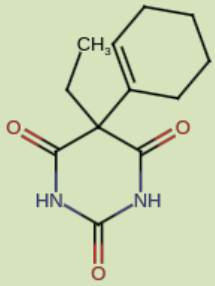


Figure 2. GC-MS chromatograph

Table 1. Phytochemical constituents identified in the Supercritical-CO_2-extract of jujube fruits

Peak	RT	Name	Formula	Area	Area Sum %
1	3.682	3,4-Dimethyl-5-hexen-3-ol	$C_8H_{16}O$	9675.58	1.38
2	3.876	1-Hepten-4-ol	$C_7H_{14}O$	24148.89	3.45
3	4.465	Isopropyl 5,11-dihydroxy-3,7,11-trimethyl-2-dodecenoate	$C_{18}H_{34}O_4$	26812.41	3.83
4	4.581	Octanal, 7-methoxy-3,7-dimethyl-	$C_{11}H_{22}O_2$	20760.35	2.96
5	4.905	Oxirane, [(hexadecyloxy)methyl]-	$C_{19}H_{38}O_2$	53376.45	7.62
6	5.907	3-Pentanol, 2,4-dimethyl-	$C_7H_{16}O$	43368.8	6.19
7	5.979	5-Hexen-3-ol, 3-methyl-	$C_7H_{14}O$	24714.77	3.53
8	6.684	Andrographolide	$C_{20}H_{30}O_5$	52446.92	7.49
9	7.473	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3β,17β)-	$C_{22}H_{32}O_2$	204706.35	29.23
10	7.628	Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-,17-[O-(phenylmethyl)oxime], (3α,5α)-	$C_{29}H_{43}NO_3Si$	48186.36	6.88
11	7.777	1,3-Bis-t-butylperoxy-phthalan	$C_{16}H_{24}O_5$	39157.07	5.59
12	8.133	1-Heptatriacotanol	$C_{37}H_{76}O$	58519.27	8.36
13	8.56	2,3,6,7-tetramethyl-10-(4-methylphenylsulfonyloxy)-1,4,4.alpha.,5,8,8a.beta.,9.b	$C_{25}H_{34}O_4S$	25488.65	3.64
14	9.905	d-Mannitol, 1-decylsulfonyl-	$C_{16}H_{34}O_7S$	52389.12	7.48
15	13.625	1,4-Cyclohexadiene,1,3,6-tris(trimethylsilyl)-	$C_{15}H_{32}Si_3$	4844.93	0.69
16	16.594	Cyclobarbital	$C_{12}H_{16}N_2O_3$	11753.64	1.68

Table 2. 2D structures of phytochemical compounds from of Ziziphus jujube green extract

Compounds			
 **3,4-Dimethyl-5-hexen-3-ol**	 Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3β,17β)-	 **1-Hepten-4-ol**	 Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl) oxime], (3α,5α)-
 **Isopropyl 5,11-dihydroxy-3,7,11-trimethyl-2-dodecenoate**	 1,3-Bis-t-butylperoxy-phthalan	 **Octanal, 7-methoxy-3,7-dimethyl-**	1-Heptatriacotanol
Oxirane, [(hexadecyloxy)methyl	 2,3,6,7-tetramethyl-10-(4 methylphenylsulfonyloxy)-1,4,4.alpha.,5,8,8a.beta.,9.b	 **3-Pentanol, 2,4-dimethyl-**	 d-Mannitol, 1-decylsulfonyl-
 **5-Hexen-3-ol, 3-methyl-**	 1,4-Cyclohexadiene, 1,3,6-tris(trimethylsilyl)-	 **Andrographolide**	 Secobarbital

The 2D chemical structures were mentioned in Table 2. The compounds that are identified in GC-MS were further carried out in silico studies for neurological disorders.

Molecular docking: Molecular docking studies were carried out for the 16 compounds for 2LMN (Beta-Amyloid Fibril) and 4EY7 (Acetylcholinesterase) standards curcumin and donepezil [50,51]. The best docking scores were obtained for the compound 2,3,6,7-tetramethyl-10-(4-methylphenylsulfonyloxy)-1,4,4. alpha.,5,8,8a. beta.,9. b (Compound 13) with both the proteins -9 for 2LMN and -10.3 for 4EY7. In order to get the interactions, 3D protein-ligand complex structures were generated for compound 13 with both proteins. With the Beta-Amyloid protein compound, 13 was unable to form an interaction between the Amyloid fibrils to prevent aggregation (Figure 3). Compound 13 with Acetylcholinesterase showed a good pose compared to the standard pose, and compound 13 showed good interactions, Hydrogen bonding with amino acids Ser293, Arg296, and pi-pi interaction with Trp286. Hydrophobic interactions were also observed with Trp286 and Tyr341 (Figure 4). These results are compared with standard compound interactions Trp286 pi-pi interactions were observed in both the compounds, and the standard has 2 more pi-pi interactions with Trp86 and 1 hydrogen bond with Phe295, and hydrophobic interactions with Try337, Try341, Phe336, Trp66, and Trp286 (Figure 5). Major interactions were similar when compared, so further MD analysis, the protein Acetylcholinesterase was chosen and explored for its stability with molecular dynamics studies.

Table 3. Binding affinities of 16 phytochemicals and respective standards against 2LMN (Beta-Amyloid Fibril) and 4EY7 (Acetylcholinesterase)

Number	Compound	2LMN (Beta-Amyloid Fibril) **Binding Affinity** (kcal\mol)	4EY7 (Acetylcholinesterase) **Binding Affinity** (kcal\mol)
1	3,4-Dimethyl-5-hexen-3-ol	-4.9	-5.7
2	1-Hepten-4-ol	-4.4	-5.1
3	Isopropyl 5,11-dihydroxy-3,7,11-trimethyl-2-dodecenoate	-6.7	-8.3
4	Octanal, 7-methoxy-3,7-dimethyl-	-5	-6.4
5	Oxirane, [(hexadecyloxy)methyl]-	-5.3	-7.1
6	3-Pentanol, 2,4-dimethyl-	-4.9	-5.2
7	5-Hexen-3-ol, 3-methyl-	-4.6	-5
8	Andrographolide	-7.5	-9.8
9	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3beta,17beta)-	-8.7	-8.6
10	Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl) oxime], (3α,5α)-	-4.2	-8
11	1,3-Bis-t-butylperoxy-phthalan	-6.9	-9.1
12	1-Heptatriacontanol	-5.2	

Number	Compound	2LMN (Beta-Amyloid Fibril) **Binding Affinity** (kcal\mol)	4EY7 (Acetylcholinesterase) **Binding Affinity** (kcal\mol)
13	2,3,6,7-tetramethyl-10-(4-methylphenylsulfonyloxy)-1,4,4.alpha.,5,8,8a.beta.,9.b	-9	-10.3
14	d-Mannitol, 1-decylsulfonyl-	-6	-6.1
15	1,4-Cyclohexadiene, 1,3,6-tris(trimethylsilyl)-	-3.8	-7.6
16	Cyclobarbital	-6.7	-8.6
17	Curcumin/Donepezil(Standards)	-7.6	-11.8

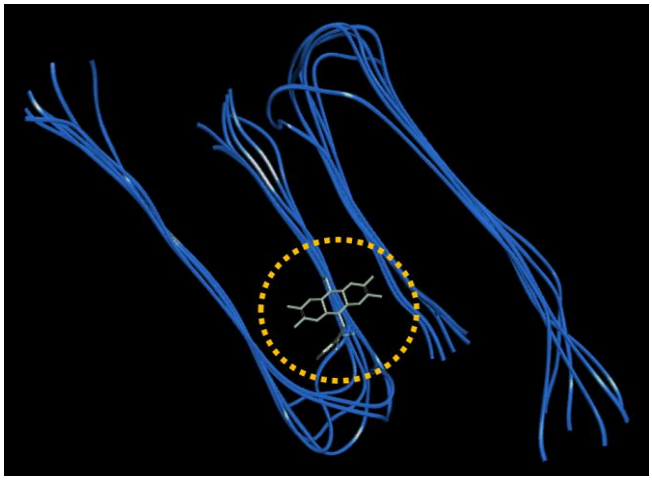


Figure 3. Compound 13 with 2LMN (Beta-Amyloid Fibril)

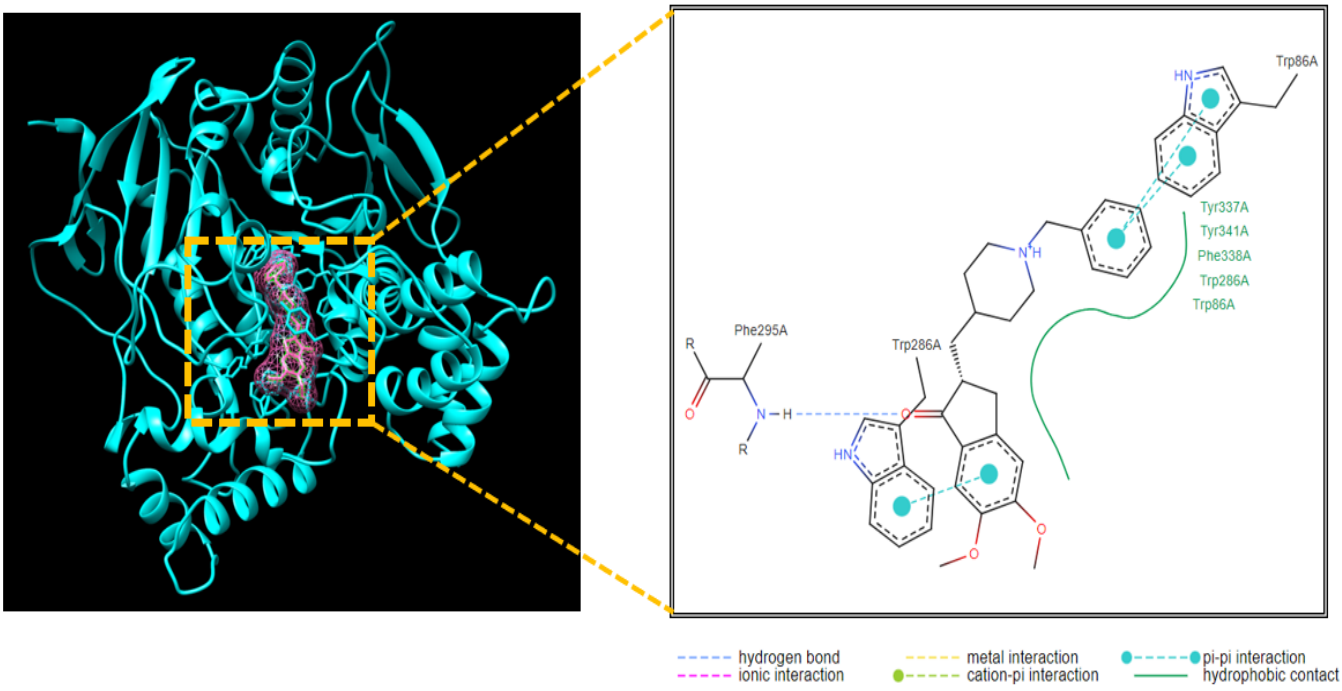


Figure 4. Interactions of Compound 13 with 4EY7 (Acetylcholinesterase)

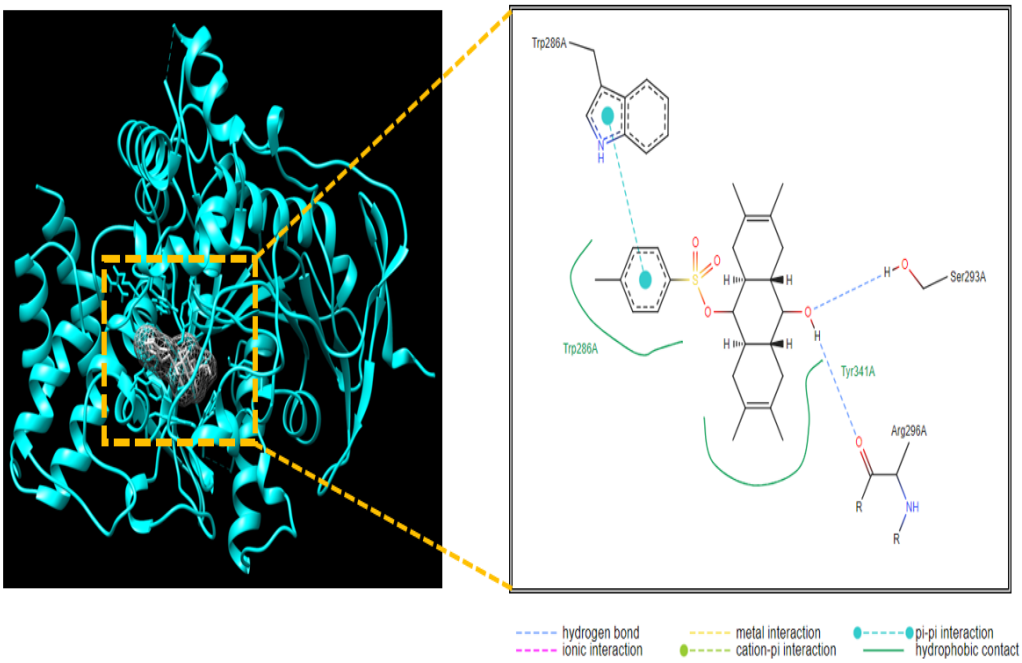


Figure 5. Interactions of Donepezil (Standard) with 4EY7 (Acetylcholinesterase)

Phytochemical compounds and standard (Paroxetine) were docked against 6VRH (Serotonin Transporter) [52]. Docking scores are mentioned in Table 4. Andrographolide showed an excellent binding affinity with protein -9.7; the standard affinity was -10.4. By comparing standard and Andrographolide, we observed that they both sit in the same pocket, and the interactions of Andrographolide have 1 hydrogen bond with Thr497 and 3 hydrophobic interactions were observed with Phe341, Ile172, and Phe335 (Figure 6). Standard showed 1 hydrogen bond with Tyr95, hydrophobic interactions with Tyr95, Phe341, and Ile172, and 1 ionic interaction with Asp98 (Figure 7). Phe341 and Ile172 are the common Hydrophobic interactions observed in standard and Andrographolide. Further, these 2 compounds were analyzed for stability using molecular dynamics.

Table 4. Binding affinities of 16 phytochemicals and respective standards against 6VRH (Serotonin Transporter)

Number	Compound	6VRH (Serotonin transporter)
		Binding Affinity (kcal\mol)
1	3,4-Dimethyl-5-hexen-3-ol	-5.1
2	1-Hepten-4-ol	-4.4
3	Isopropyl 5,11-dihydroxy-3,7,11-trimethyl-2-dodecenoate	-7.6
4	Octanal, 7-methoxy-3,7-dimethyl-	-5.7
5	Oxirane, [(hexadecyloxy)methyl]-	-6.3
6	3-Pentanol, 2,4-dimethyl-	-4.5
7	5-Hexen-3-ol, 3-methyl-	-4.9
8	Andrographolide	-9.7

Number	Compound	6VRH (Serotonin transporter) Binding Affinity (kcal\mol)
9	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3beta,17beta)-	-9.5
10	Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3α,5α)-	-7.8
11	1,3-Bis-t-butylperoxy-phthalan	-7.8
12	1-Heptatriacontanol	-7.1
13	2,3,6,7-tetramethyl-10-(4-methylphenylsulfonyloxy)-1,4,4.alpha.,5,8,8a.beta.,9.b	-8.2
14	d-Mannitol, 1-decylsulfonyl-	-6.5
15	1,4-Cyclohexadiene, 1,3,6-tris(trimethylsilyl)-	-7.4
16	Cyclobarbital	-8.1
17	Paroxetine (Standard)	-10.4

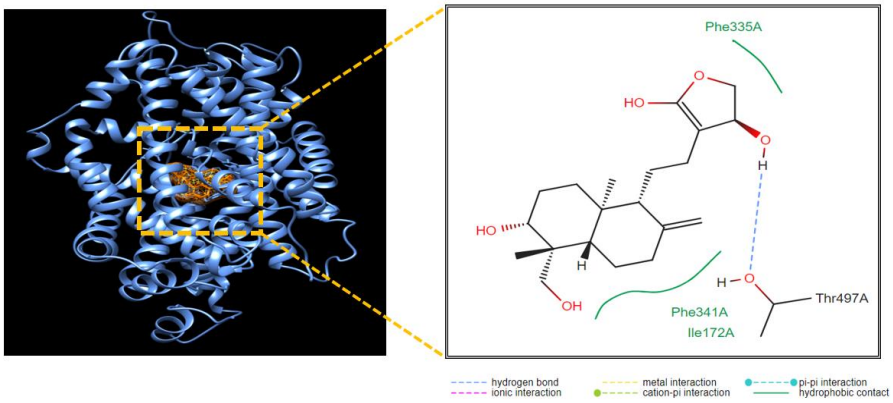


Figure 6. Pictorial representation of Interactions of Andrographolide with 6VRH (Serotonin Transporter)

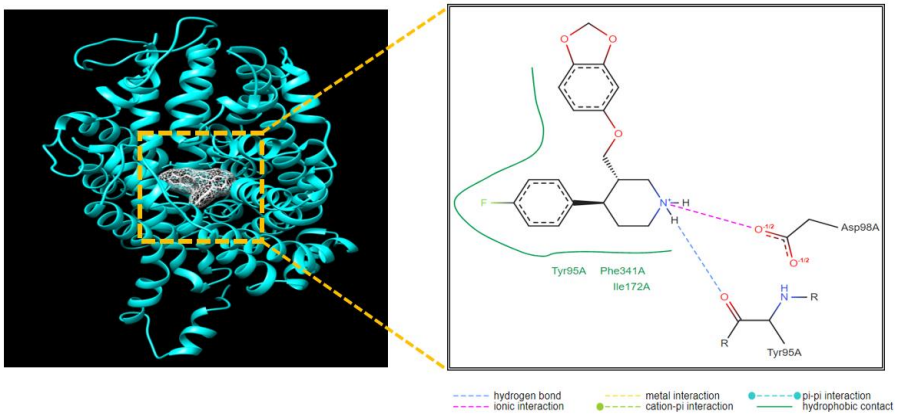


Figure 7. Pictorial representation of Interactions of Paroxetine (Standard) with 6VRH (Serotonin Transporter)

ADME Studies: Drug-likeness or oral bioavailability is the main consideration for evaluating ADME properties. Lipinski's rule of five was considered to predict the drug-likeness of the molecule. According to the rule of five, the molecular mass should be less than 500, the hydrogen bond donor less than five, the hydrogen bond acceptor less than ten, and LogP values should be less than or equal to five. The results obtained indicate within the 16 compounds, 15 molecules are within the parameters of Lipinski's rule of five, but the compound 1-Heptatriacotanol with a molar mass of 537 which violated the parameters. According to the rule of five, 1-Heptatriacotanol molecule has less drug-likeness [53]. The parameters explored here indicated that the extracts or phytochemicals present in the jujube are orally bioavailable. As previously discussed, the jujube was used as a dietary supplement in Chinese culture, which prove the safety and bioavailability of the Phytochemicals.

Table 5. ADME studies of phytochemicals

Number	Name	Mass	Hydrogen bond donor	Hydrogen bond acceptor	Log p
1	3,4-Dimethyl-5-hexen-3-ol	128.21	1	1	2.07
2	1-Hepten-4-ol	114.19	1	1	1.74
3	Isopropyl 5,11-dihydroxy-3,7,11-trimethyl-2-dodecenoate	314.45	2	4	2.84
4	Octanal, 7-methoxy-3,7-dimethyl-	186.29	0	2	2.06
5	Oxirane, [(hexadecyloxy)methyl]-	298.5	0	2	3.85
6	3-Pentanol, 2,4-dimethyl-	116.2	1	1	1.89
7	5-Hexen-3-ol, 3-methyl-	114.19	1	1	1.74
8	Andrographolide	350.45	3	5	1.98
9	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3β,17β)-	328.49	1	2	4.26
10	Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl) oxime], (3α,5α)-	481.74	0	4	4.51
11	1,3-Bis-t-butylperoxy-phthalan	296.36	0	5	3.2
12	1-Heptatriacotanol	**537**	1	1	8.75
13	2,3,6,7-tetramethyl-10-(4-methylphenylsulfonyloxy)-1,4,4.alpha.,5,8,8a.beta.,9.b	410.55	1	4	4.18
14	d-Mannitol, 1-decylsulfonyl-	370.5	5	7	-0.09

Number	Name	Mass	Hydrogen bond donor	Hydrogen bond acceptor	Log p
15	1,4-Cyclohexadiene, 1,3,6-tris(trimethylsilyl)-	296.67	0	0	4.63
16	Cyclobarbital	238.27	2	2	1.12
	Drug likeness (Standard Values)	<500	<5	<10	<=4.15

Molecular Dynamic: The Root-Mean-Square Deviation (RMSD) graph obtained from the molecular dynamic simulations revealed that the Acetylcholinesterase (4EY7) protein showed stability with the test compound over the simulation time of 100ns. The RMSD values are in the threshold range of 1.5 to 0.2 nm, indicating that the protein has experienced a minimal structural deviation from its initial conformation. When compared with the RMSD of the standard drug Donepezil, both compounds displayed RMSD values within the range of 1.5 nm to 0.2 nm, indicating that they could act as stabilizers of the Acetylcholinesterase (4EY7) protein (Figure 8).

The RMSD analysis of the ligands suggests that test compound 13 has experienced elevated deviations at certain regions between the 40th ns and 80th ns of the simulation time frame, with values exceeding 0.2 nm. The observed fluctuations in certain regions indicate the possible orientations of the ligand within the protein binding site to attain a single stable conformation. More importantly, after the 80th ns time point, the test compound has revealed a drop in the RMSD values below 0.15 nm showing the convergence at the end of the simulations confirming the well-defined binding interactions (Figure 9).

In addition to analyzing RMSD, we further examined the Root-Mean-Square Fluctuation (RMSF) profiles. RMSF studies for both standard (Donepezil) and test compound 13 (2,3,6,7-tetramethyl-10-(4-methylphenylsulfonyloxy) 1,4,4. alpha.,5,8,8a. beta.,9. b) demonstrated similar patterns, with high fluctuations observed at 4000th and between 7000 -8000th atoms within the protein. This implies that they might interact with Acetylcholinesterase comparably, despite being structurally distinct compounds (Figure 10).

The RMSD studies of the protein (PDB ID 6VRH) for the entire simulation of 100ns. The study reveals that the standard and test compound are exhibiting similar stability profiles with the deviation value ranging below the threshold of 0.3 and 0.15 for the protein and ligand respectively, suggesting the test compound might have the potential binding affinities with the protein structure (Figures 11 and 12).

The RMSF graph exhibits minimal fluctuation of the protein residues when the test compound is bound to the protein. Higher fluctuations were not observed throughout the simulations and were highly comparable with the standard drug. Similar patterns between the test and standard showcase strong evidence for the potential binding of the test compound without showing any strong residual fluctuation in the binding site (Figure 13)

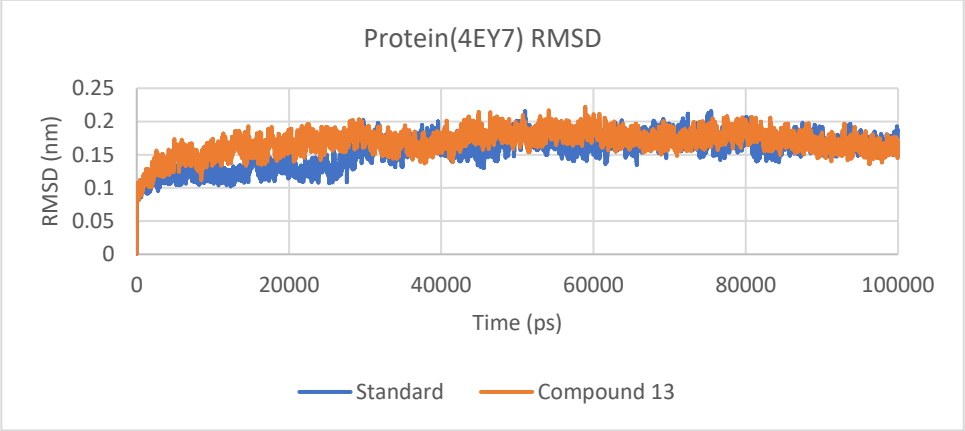


Figure 8. Graphical representation of RMSD for protein (4EY7) for both standard (Donepezil) and test compound 13 (2,3,6,7-tetramethyl-10-(4-methylphenylsulfonyloxy)-1,4,4.alpha.,5,8,8a.beta.,9.b)

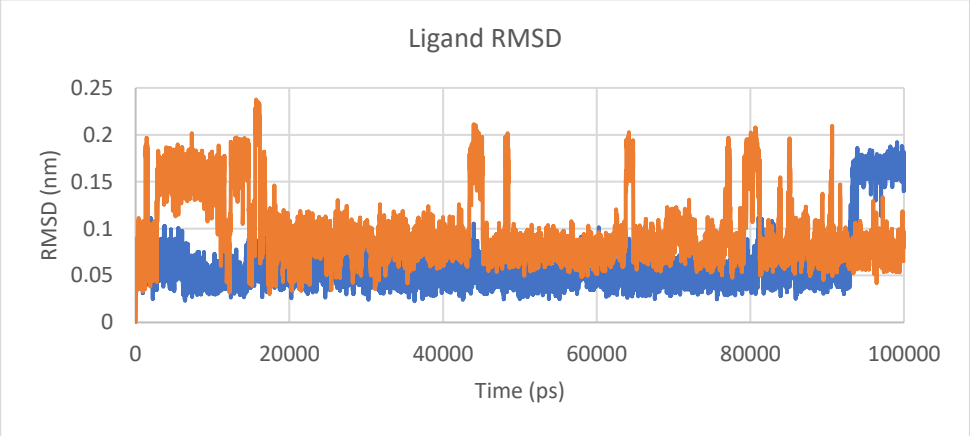


Figure 9. Graphical representation of RMSD for Ligand for both standard (Donepezil) and test compound 13 (2,3,6,7-tetramethyl-10-(4-methylphenylsulfonyloxy)-1,4,4.alpha.,5,8,8a.beta.,9.b)

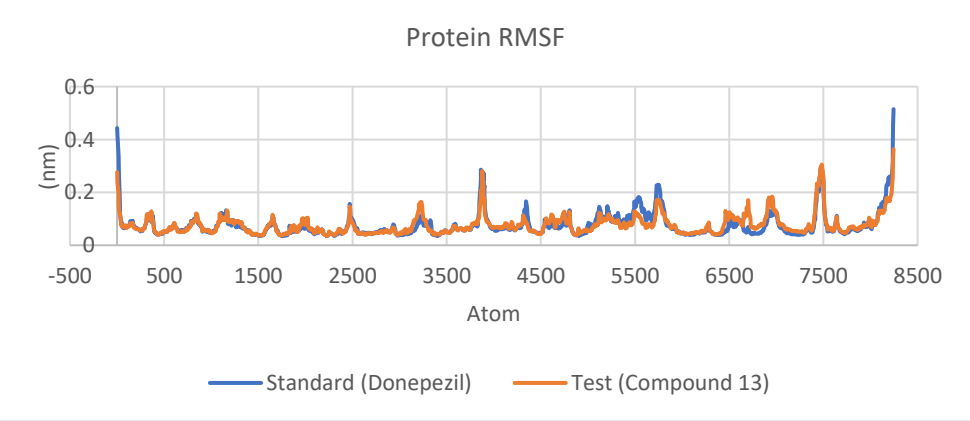


Figure 10. Graphical representation of RMSF for Protein(4EY7) for both standard (Donepezil) and test compound 13 (2,3,6,7-tetramethyl-10-(4-methylphenylsulfonyloxy)-1,4,4.alpha.,5,8,8a.beta.,9.b)

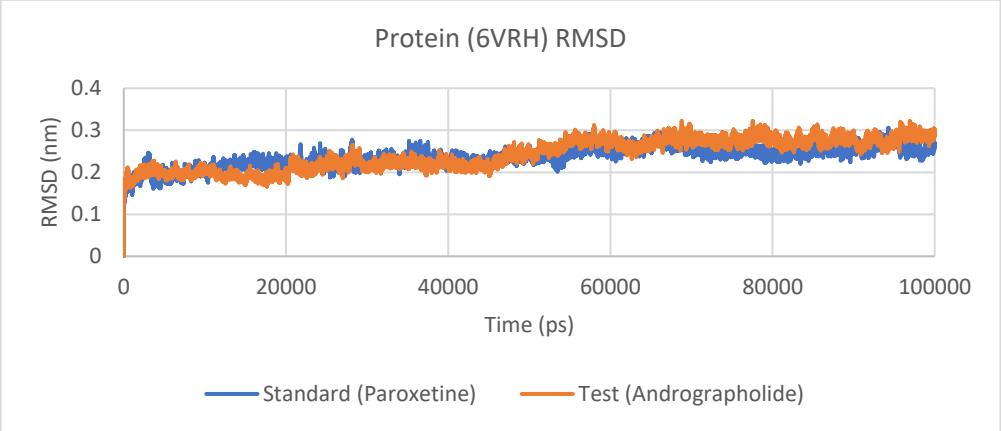


Figure 11. Graphical representation of RMSD for protein (6VRH) for both standard (Paroxetine) and test compound 13 (Andrographolide)

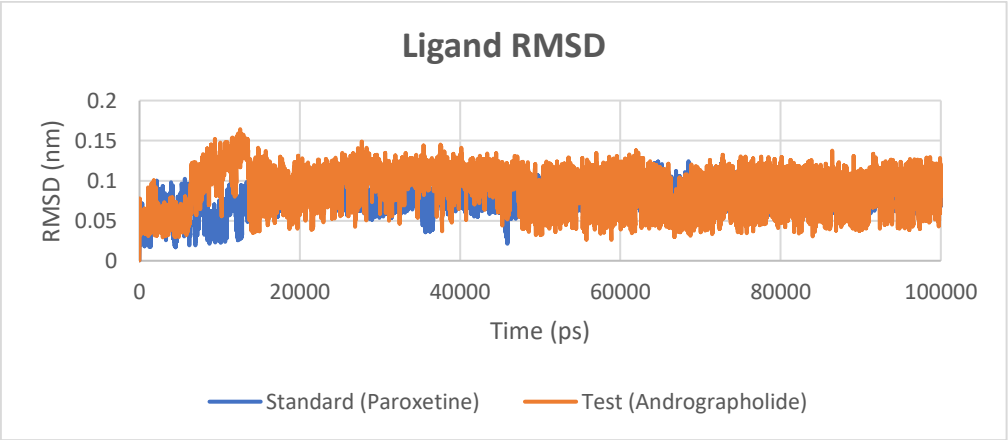


Figure 12. Graphical representation of RMSD for Ligands for both standard (Paroxetine) and test compound 13 (Andrographolide)

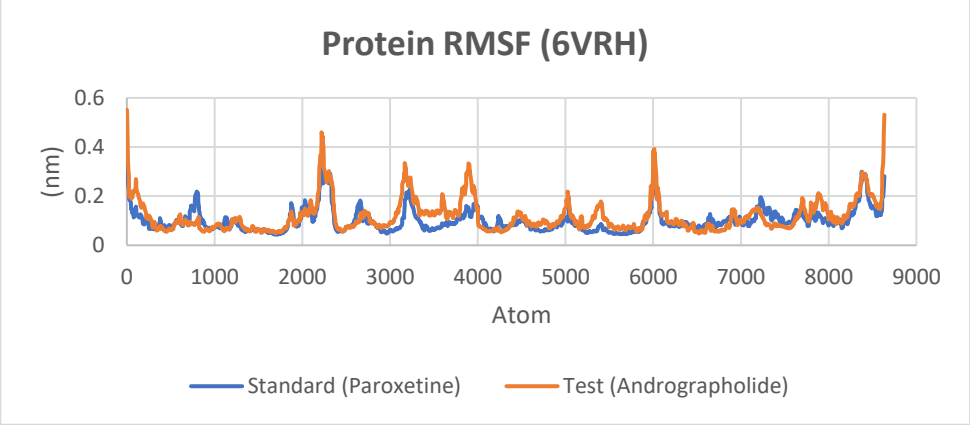


Figure 13. Graphical representation of Protein RMSF for both standard (Paroxetine) and test compound 13 (Andrographolide)

CONCLUSION

The present study was focused on the green extraction of Ziziphus Jujuba Mill. Fruit phytochemicals using supercritical CO_2, analysis by GC-MS, and studying some of their neurological therapeutic effects. The phytochemicals present in the jujube have proven neurological phytochemical properties. These phytochemicals were screened computationally for Alzheimer's and anxiety neurological disorders. Molecular docking studies for proteins 4EY7 (Acetylcholinesterase) for Alzheimer's with (2,3,6,7-tetramethyl-10-(4-methylphenylsulfonyloxy)-1,4,4. alpha.,5,8,8a. beta.,9.b) and 6VRH (Serotonin transporter) for anxiety with Andrographolide revealed that these compounds have good interaction profiles and binding affinity scores. ADME studies of the phytochemicals showed that only 2 molecules, Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl) oxime], (3α,5α)- and 1-Heptatriacotanol has violations against Lipinski's rule of five. Molecular dynamics studies revealed no significant deviations or fluctuations in proteins (4EY7 and 6VRH) compared with the standards. The protein Serotonin receptor (6VRH) has good results regarding RMSD and RMSF. Since the studies were conducted on jujube, a fruit and dietary component, phytochemicals in the fruit have potential health benefits, as they are safe and natural for consumption with fewer side effects and can be utilized in manufacturing functional foods. Based on the results obtained, the compounds (2,3,6,7-tetramethyl-10-(4-methylphenylsulfonyloxy)-1,4,4.alpha.,5,8,8a.beta.,9.b) and Andrographolide can be a potent molecule to treat Alzheimer's and anxiety respectively. Further in vitro and in vivo studies must be carried out to evaluate the biological activities.

Abbreviations: AD: Alzheimer's Disease, βA: β-amyloid, RMSD: Root-Mean-Square Deviation, RMSF: Root-Mean-Square Fluctuations, FID: Flame Ionization Detector, ADME: absorption, distribution, metabolism, and excretion, PDB: Protein data bank, 4EY7: Acetylcholinesterase, 2LMN: Beta-Amyloid Fibril,6VRH: Serotonin receptor, GC–MS: Gas chromatography mass spectrometry.

Authors Contribution: Fadwa W. Abdulqahar: Conceptualization, Methodology, Validation, Formal Analysis, Interpretation of biological data, and Writing – Software Review and Editing. Mohammed A. Morgab: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Software, Writing-Original draft and Visualization. Feryal F. Hussein: Writing-Reviewing and Final Editing. Yulia Apyantseva: Data duration, Methodology and Validation. Tamer M. El-Messery: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Software, Writing-Original draft and Visualization.

Competing Interests: The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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