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Research Article

AZADIRACHTIN, CARDIOFOLIOSIDE, AND KUTKIN CAN BE VITAL PHYTOCHEMICALS FOR THE MODULATION OF SECRETASE ENZYMES FOR THE TREATMENT OF ALZHEIMER'S: AN *IN-SILICO* ANALYSIS

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ABSTRACT

Objective: The aim of this study was to screen for active components from medicinal plants of Uttarakhand that has potential for a therapeutic agent for Alzheimer's disease (AD) by targeting the secretase enzyme using *in-silico* approach.

Methods: We used iGEMDOCK for *in-silico* docking experiments to screen for active components from the medicinal plant can modulate secretase enzyme. Binding energies for active component from 112 medicinal plants were analyzed.

Results: Our *in-silico* analysis suggests that cardiofolioside, azadirachtin, berberine, and kutkin can best possible candidates for modulation of secretase enzyme and thus hold potential to help patients with AD. The results obtained clearly suggests that these active components from plants herbal have the ability to modulate α and β-secretase either as an activator of α-secretase or as an inhibitor of β-secretase when compared with known standard drug.

Conclusion: Cardiofolioside, azadirachtin, berberine, and kutkin can acts as a modulator for secretase enzymes, and these phytochemicals can be exploited for their potential to that of AD.

Keywords: α-secretase, β-secretase, iGEMDOCK, Cardiofolioside, Kutkin, Azadirachtin.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia [1]; AD is associated with the aging population and causes loss of memory in patients. What exactly triggers the onset of AD has still not been pinpointed, but several factors have been shortlisted. Most of these factors ultimately lead to neuronal degeneration and plaque formation which has been also observed as a hallmark in the brain of AD patients. Accumulation of amyloid beta fibrils and tangles of tau in brain are two prominent cause of neuronal degeneration in AD patients (Fig. 1). WHO repot support the AD International (London, United Kingdom) statistics of 2014 that nearly 36 million people have Alzheimer's or a related dementia worldwide even when only one in four people with AD have been diagnosed so far [2]. In USA alone, there are an estimated 5.2 million people with AD [3]. It has been observed that women are more susceptible to AD as compared to men and out of the 5.2 million Americans with AD, 3.3 million are women, and remaining 1.9 million are men [4]. A simple reason sometimes given for this is that women tend to live longer than men, but there might be other specific reasons that are still not known.

Aging Demographics and Memory Study (USA) reported 16% of women aged 71 and above have AD and other forms of dementia as compared to 11% of men who suffer from the same in USA [5]. In India, there is no agency that maintains medical records for AD, but recently, number of AD cases reported in India has shown unexpected growth [6]. This incredible increase in number of AD patients worldwide causes a huge economic burden on both the treatment facility for patients and on the immediate caretakers of the patient. Last but not the least, it has been estimated that as of 2013, US spent a total of US$220.2 billion on giving medical aid to patients with AD [3]. This value is half of the Wal-Mart sale and 8 times of MacDonald's revenue of 2012 (Fig. 2). This is one of the leading disorders, which shows a tremendous increase every year.

After the failure of current drugs in the market for the treatment of AD and side effects of synthetic therapeutic compounds, active components of medicinal plants figure prominently in investigations and screening of potential modulator for treatment of AD. The potential of herbal components for the prevention or cure of AD is tremendous. About 25-30% of the plant drugs in market for various diseases have been derived from plants. Initially, the primary enzyme that was being targeted over the years to stop the onset of AD was γ-secretases. A drug that targeted γ-secretases failed during phase 3 of trails motivated researchers to look into α and β-secretase as a target also. Many pharmaceutically manufactured β and γ-secretase inhibitors, monoclonal antibodies have found their way in the AD market but have not proven beneficiary in terms of their effectiveness. A study on AD drugs [7], has shown some drugs have been discontinued for its adverse side effects whereas some are still under trial. There are various species of plants worldwide, which have been investigated and screened for active components that might be helpful in the treatment of Alzheimer's. Various herbal extracts have been prepared by using different methods of extraction in different solvents with different concentrations, and are being used globally as supplements and most of them claim to delay the onset of Alzheimer's. The drug market for AD is US$ 10 billion and this market would tripal by 2022 as described in 2013 [8]. Recently, Dubey *et al.* [9], of Banaras Hindu University, India, was granted a US patent for his formulation of herbal extract for the cure of neurodegenerative disorders.

Uttarakhand, a small state in northern part of India, with most of its geographical coverage in the Himalayan regions and has a treasure trove of medicinal plants. Active components of some species of these medicinal plants have been well investigated, but most of the plants have seldom been screened for active components that might help in the treatment of neurological disorders [10]. As it is always much difficult to take all species and do experiments with them, it is rather logical

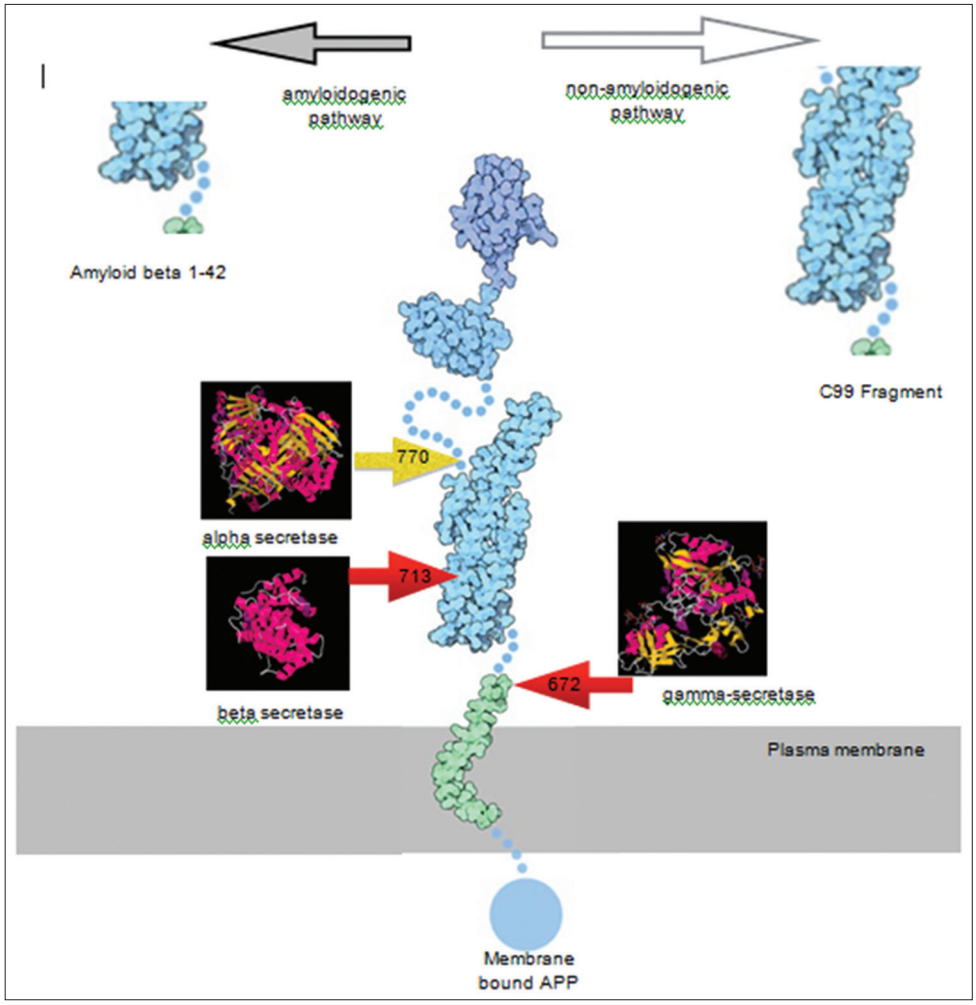

Fig. 1: The cleavage of amyloid-β precursor protein in the neuronal plasma membrane by alpha, beta and gamma secretase enzymes. The amyloidogenic pathway occurs by the cleavage of beta secretase enzyme only by cleaving on amino acid 713

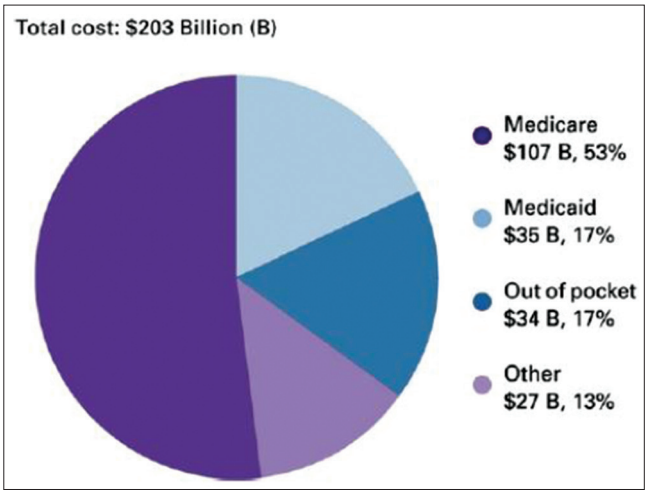

Fig. 2: Estimated cost spent on patients with Alzheimer's disease in the US in 2014. Source: 2013 Alzheimer's disease facts and figures

to do an initial screening using the available bioinformatics tools. With an objective to narrow down a search for an active component from a medicinal plant, we screened about 112 plant species of Uttarakhand using *in-silico* docking models and simulations. Here we report our findings and to the best of our knowledge, this paper is one of the first and few to report the potential active components of medicinal plants of Garhwal Himalayan region of Uttarakhand that has the potential for treatment of AD by targeting the secretase enzyme using *in-silico* approach.

METHODS

Molecular docking of secretases enzymes and plant active compounds using iGEMDOCK

iGEMDOCK (a docking software developed by Jinn-Moon Yang, a professor of the Institute of Bioinformatics, National Chiao Tung University) generates protein-compound interaction profiles of electrostatic (E), hydrogen bonding (H), and Van der Waal's (V) interactions. Based on these profiles and compound structures, iGEMDOCK infers the pharmacological interactions and clusters the screening compounds for the post-screening analysis. Finally, iGEMDOCK as described by Hsu *et al.*, 2011 [11] and method was adopted from Kapoor *et al.*, 2013 [12], and Semwal *et al.*, 2015 [13] was used to rank or visualize the ligand target docking and energy-based scoring was tabulated for each potential active components form medicinal plants. Ranks or visualizes the screening compounds by combining the pharmacological interactions and energy-based scoring function of iGEMDOCK.

Preparation of binding site

The protein structure of the secretases α and β required for docking analysis was retrieved from Protein Data Bank (PDB) as the only PDB format file can be used for simulations of the binding site in iGEMDOCK. The PDB ID's of both these secretases are;

α-secretase: 1BJB

β-secretase: 1W50

Ligand preparation

The structures of the various plant active components in its mol 2 format were downloaded using the database ZINC AC (University of California Org, San Francisco). The ZINC AC codes and smileys for the various herbal ligands are below:

- Asiatic acid (8221271)
- Asranolaldehyde (drawn using chemsketch)
- Berberine [3779067(1)]
- Rutaecarpine (898237)
- Hypericin
  (smiley: Cc1cc(c2c3c1c4c(cc(c5c4c6c3c7c(c(cc(c7c2=O)O)O)c8c6c(c5=O)c(cc8O)O)O)C)O)
- Hyperforin (27644186)
- Sinapic acid (153654) and
- Taraxerol
  (smiley: C[C@]12CCC(C[C@H]1[C@@]3(CC[C@@H]4[C@]5(CC[C@@H](C([C@@H]5CC[C@]4(C3=CC2)C)(C)C)O)C)(C)C)
- Pepstatin (1115)
- Betulinic (35494088)
- Azadirachtin (71928293)
- Cardifolioside (95346855)
- Plumbagin (58187)
- Kutkin (CAS NO: 25357226)
- Myricetin (14436449)
- Triethyl-2-phosphonobutyrate (17145914).

Docking module

Now, using the PDB format of the secretases and the mol 2 format of the plant active components in store, we checked their mutual interactions with the help of molecular docking software (iGEMDOCK). We then uploaded the mol 2 structural format of the plant along with components named above and docked them with α-secretase and β-secretase, respectively. After the docking between the protein and ligands was complete, the results were obtained as output file (Figs. 3 and 4). All the plant active components also showed a binding energy (H-bond+Van Der Waal's force of attraction + electrostatic force). Binding energies of the protein - ligand interactions are important to describe how fit the ligand binds to its target macromolecule. The result for each binding simulation was tabulated an analyzed.

RESULTS

All selected phyto-components were docked with secretase enzymes, and the best suitable inhibitors of β-secretase and that of α-secretase was selected by comparing the results to the known inhibitor of β-secretase (Triethyl-2-phosphonobutyrate) and a known activator of α-secretase (pepstatin). The known inhibitor of β-secretase (triethyl-2-phosphonobutyrate) has a binding energy of −107.36 and pepstatin the known activator of α-secretase has a binding energy of −73.43. Therefore, the assumed modulators of β-secretase are cardiofolioside (−100.1), kutkin (−121.07), and azadirachtin (−125.12) and the assumed modulator of α-secretase is berberine (−77.83). However, belutinic (−105.1) act as an analog of known inhibitor of β-secretase and asiatic acid (−74.74) act as an analog of known activator of α-secretase.

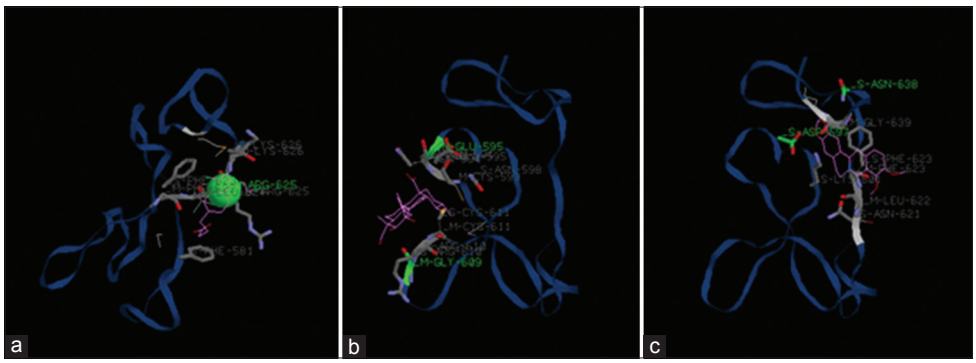

Fig 3: Best interaction between plant ligands and α-secretase protein. These simulations generated by the program indicate: (a) Interaction between α-secretase and pepstatin (known activator of α-secretase), (b) interaction between α-secretase and asiatic acid, (c) interaction between α-secretase and berberine. In all photographs, the blue colored ribbon structure represents the protein α-secretase and the red and green colored ball and stick model of amino acids represents the binding sites of the respective plant ligands on α-secretase

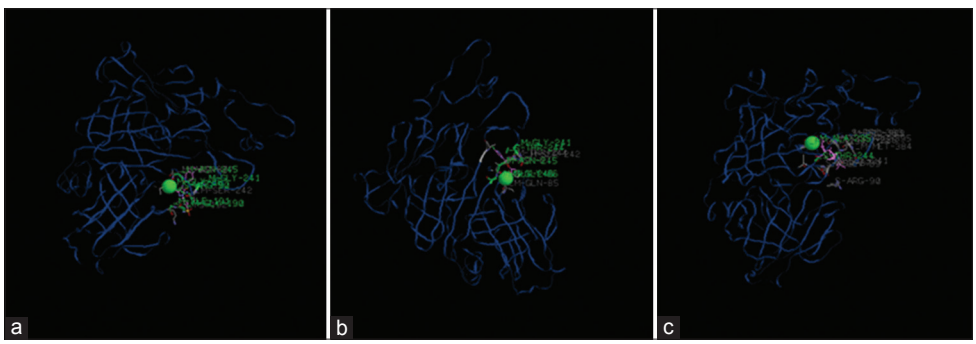

Fig. 4: Best interaction between plant ligands and β-secretase protein. These simulations generated by the program indicate: (a) Interaction between β-secretase and Triethyl-2-phosphonobutyrate (known inhibiter of β-secretase), (b) interaction between β -secretase and cardiofoliosides, (c) interaction between β-secretase and belutinic component. In all photographs, the blue-colored ribbon structure represents the protein β-secretase whereas the red and green colored ball and stick model represents the binding site of the various plant ligands on the protein β-secretase

Table 1: Interaction of α-secretase with different plant ligands showing its binding energy, Van der Waal's force, H-bonding, and its electrostatic force

Ligand	Binding energy (Kcal/mol)	Van Der Waal's	H-Bond	Electrostat
Pepstatin (control)	−73.43	−69.93	−3.5	0
Asiatic acid	−74.7424	65.5367	−9.2057	0
Asranolaldehyde	−57.6847	46.3119	11.3728	0
Berberine	−77.8272	69.8141	8.01308	0
Hyperforin	−81.2443	−77.71	3.53429	0
Hypericin	−95.2982	78.6851	16.6131	0
Rutaecarpine	−69.2502	62.2502	−7	0
Sinapic acid	−66.0999	44.0741	20.8142	1.21163
Taxerol	−61.9602	56.9495	5.01071	0
Plumbagin	−70.11	−57.75	−12.36	0

The data suggests that minimum binding energy showed best binding at the active site of targeted protein

Table 2: Interaction of β-secretase with different plant ligands showing its binding energy, Van der Waal's force, H-bonding, and its electrostatic force

Ligand	Binding energy (Kcal/mol)	Van Der Waal's	H-Bond	Electrostat
Triethyl-2-phosphono butyrate (control)	−107.36	−84.76	−22.6	0
Asiatic acid	−91.0478	−77.2903	−13.6764	−0.08117
Asranolaldehyde	−63.6063	−53.5926	−10.0102	0
Taxerol	−80.6582	−78.9085	−1.77668	0
Hyperforin	−87.75	−84.0874	−3.66256	0
Hypericin	−101.802	−86.0698	−15.7318	0
Rutaecarpine	−82.9917	−68.3859	−14.6058	0
Sinapic acid	−75.1836	−54.7464	−20.4372	0
Belutinic	−105.1	−96.86	−8.4	0.3
Cardifolioside	−100.1	−68.56	−31.53	0
Kutkin	−121.07	−105.64	−15.43	0
Myricetin	−111.37	−76.92	−34.44	0
Azadirachtin	−125.12	−82.25	−42.88	0

The data suggests that minimum binding energy showed best binding at the active site of targeted protein

DISCUSSION

In this study, 112 species of medicinal plants from the state of Uttarakhand, India were carefully screened for novel active components. The potential candidates were screened on the basis of their ability to modulate the activity of secretase enzymes using bioinformatics tools. A possible candidate to cure AD, would either be an inhibitor of β-secretase or an activator of α-secretase. The screening was done using bioinformatics tools to screen out plant active components which have a high binding efficiency by carrying out its molecular binding interactions. The concept of binding efficiency was formulated by iGEMDOCK minimum binding energy concept and in general states that maximum binding efficiency with targeted protein. Bioinformatics based screening of active components of medicinal plant using *in-silico* models has helped us extremely, not just by saving our time and money in discovering these active herbal components, but by also providing us data that helps us understand and calculate the binding efficiencies of active herbal components to various receptor or sites. These interactions are very specific because they are based on virtual screening of known receptors or a particular binding site. Balavignesh *et al*., 2013 [14], reported that the compound which gives the lowest binding energy is considered to be the best inhibitor. However, specific property of a compound as an inhibitor or activator need to be further verified with actual wet lab experiments.

In the present paper, we have exploited molecular docking software iGEMDOCK to screen herbal components from the medicinal plants of Uttarakhand which can be very useful in the long run to help patients with AD by modulating the activities of α and β-secretase enzymes. The results obtained after docking α and β-secretase with the various active components of selected plants has been described in Tables 1 and 2, respectively with its generated docking poses in Figs. 3 and 4 respectively. In addition to the phytochemicals named in this study, we have also studied the *in-silico* interactions of various other chemical compounds extracted from the plants grown in Uttarakhand and whose potential to modulate α and β-secretases have been already reported. These chemical compounds include myricetin (−111.37*) reported by Shimmyo *et al*. 2008 [15], and Chakraborty *et al*. 2011 [16], asiatic acid (−74.73) reported by Patil, 2010 [17], plumbagin (−70.11) reported by Son and Camondela 2010 [18], and hypericin (−95.30, −101.81*) reported by Bramanti *et al*., 2010 [19]. The values with an asterisk (*) sign represent the iGEMDOCK values for inhibition of β-secretase while the ones without an asterisk represent the values for activation of α-secretase.

Future direction

The present drug search for AD is constrained by a number of issues. As the number of dementia patients is fast growing, the need of the hour is to identify an active molecule that could help both the already existing patients as well as prevent the onset of AD in healthy individuals. The present research is ongoing with actual wet lab experiments with a number of possible screened compounds from medicinal plants. The aim is to develop an effective herbal supplement/product for delaying or completely stopping the occurrence of AD. Bioinformatics based tools have comes to rescue and saves both - our time and money when we compare it to the method of manually discovering novel compounds. With the advent in bioinformatics, it has become much easier to screen compounds of interest through *in-silico* methods, which gives us a very interesting insight into the interactions between our desired molecular compounds. As far as this study is concerned, it reveals the ability of certain herbal components to modulate the activities of α and β-secretase after comparing its molecular docking results with a known modulator set as control, generated through a protein-ligand docking software; iGEMDOCK.

CONCLUSION

Based on our bioinformatics based tool iGEMDOCK analysis of our binding data suggests that cardiofolioside, azadirachtin, berberine, and kutkin can be best possible candidates for modulation of secretase enzyme, and thus hold potential to help patients with AD. The phytochemicals screened in this study might be exploited for their potential to treat AD through the modulation of α and β-secretase. As we have seen the failure of numerous drugs to treat AD targeting gamma secretase specifically, this new approach to modulate alpha and beta secretase with these screened phytochemicals might provide a key clue to solving the problems associated with AD.

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