

Molecular Docking Study of *Cassia tora*, *Brassica campestris* and *Calotropis procera* as Acetylcholinesterase Inhibitor

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ABSTRACT

Introduction: Acetylcholinesterase inhibitor is the only standard and FDA approved drug therapy for Alzheimer's disease and its associated disorders. Numerous plants and their phytoconstituents are being reported to inhibit acetylcholinesterase. To get insight of the intermolecular interactions, the molecular docking studies are performed at active site of acetylcholinesterase enzyme. **Aim:** In this study, an attempt is made for identification of potential ligands from selected 30 compounds which are reported to be present in *Cassia tora*, *Brassica campestris* and *Calotropis procera*, targeted against acetylcholinesterase using molecular modelling and docking studies. **Results:** The relative binding affinity of the compounds towards AChE was selected on the basis of docking score, GLIDE score and interaction patterns. Several compounds showed strong hydrogen bonding to several important amino acid residues and their hydrophobic interactions could also explain their potency to inhibit acetylcholinesterase. These compounds belong to different classes like flavonoids, vitamin, cardenolides and etc. Some of these compounds have been reported for their beneficial effect on dementia related disorders, while remaining are suggested to be potential acetylcholinesterase inhibitors. **Conclusion:** Hence, this study provides evidence for consideration of valuable ligand molecule as potential acetylcholinesterase inhibitor and further *in vitro* and *in vivo* investigations may prove its therapeutic potential.

Key words: Alzheimer's disease, Molecular Docking, Phytoconstituent, Virtual screening, Cognition enhancer.

INTRODUCTION

Alzheimer's disease is the leading cause of dementia, accounting for about 50% of all cases worldwide.¹ It is characterized by progressive loss of memory and extracellular deposition of amyloid- β peptides and intracellular neurofibrillary tangles.² There has been remarkable improvement in understanding of the pathogenesis of Alzheimer's disease in last few decades. Several independent hypotheses like oxidative stress, dysfunction of cholinergic transmission, inflammatory reaction and Amyloid β metabolism has been put forward but still the exact mechanism remains unclear.³ 'Cholinergic hypothesis' is one of the important hypotheses. Also, inhibition of acetylcholinesterase serves as promising strategy for the treatment of Alzheimer's disease, senile dementia, ataxia, myasthenia

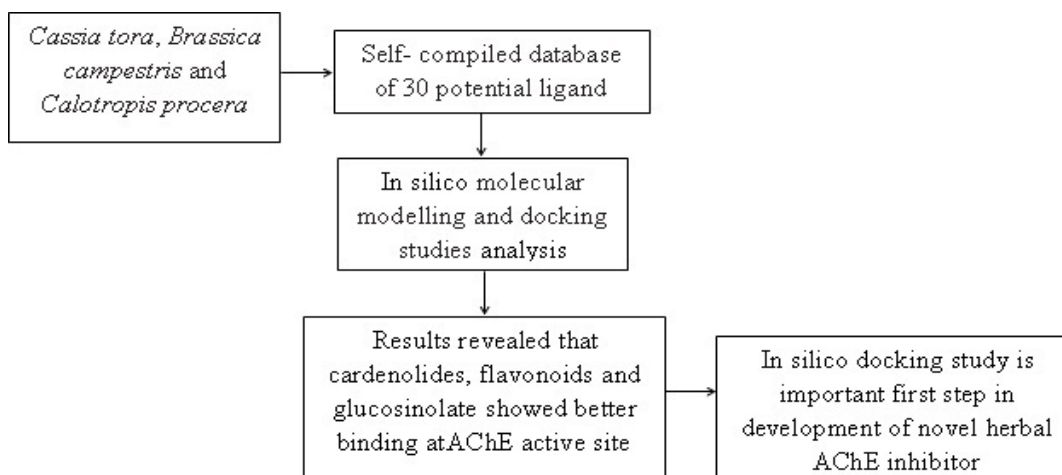
gravis and Parkinson's disease.⁴ Synthetic drugs approved for treating Alzheimer's disease are tacrine and donepezil, the naturally derived drugs are rivastigmine, physostigmine but these compounds have been reported to have adverse effects like gastrointestinal disturbances and problems associated with bioavailability which necessitates the interest in finding better AChE inhibitors from natural resources.⁵ Although, some natural products have shown potential to enhance the cholinergic function in CNS by cholinesterase inhibition, but problem persists with target selection due to chemical diversity of natural products.⁶ To overcome this ambiguity in effect, *in silico* molecular docking approach is adopted, which can be helpful in identifying lead compound with potent inhibitory activity

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Graphical Abstract

Table 1: Selected ligands from leaves of <i>Cassia tora</i> , latex of <i>Calotropis procera</i> and seeds of <i>Brassica campestris</i> for docking with AChE			
Plant	Part of Plant	Chemical class	Chemical constituent
<i>Calotropis procera</i> (CP)	Latex	Cardenolides	1. Calotropin
			2. Calotoxin
			3. Calactin
			4. Uscharin
			5. Voruscharin
			6. Uzarigenin
			7. Syriogenin
			8. Proceroside
			9. Calotropagenin
			10. β -amyrin
		Terpenes	11. Lupeol
		Flavanoid	12. Quercetin-3- rutinoside
<i>Brassica Campestris</i> (BC)	Seed	Phytosterol	1. Brassicasterol
			2. Campesterol
			3. Sitosterol
		Glucosinolate	4. Sinigrin
			5. Gluconapine
			6. Glucobrassicinapin
			7. Sinalbin
			8. Glucobrassicin
			9. Neoglucobrassicin
			10. 4-hydroxy glucobrassicin
		Phenolic compound	11. Sinapine
		Vitamin	12. Vitamin A
<i>Cassia tora</i> (CT)	Leaves	Anthraquinone	1. Emodin
			2. Physcion
		Flavonoid	3. Quercitin
			4. Uridine
			5. Stigmasterol
			6. β stigmaterol

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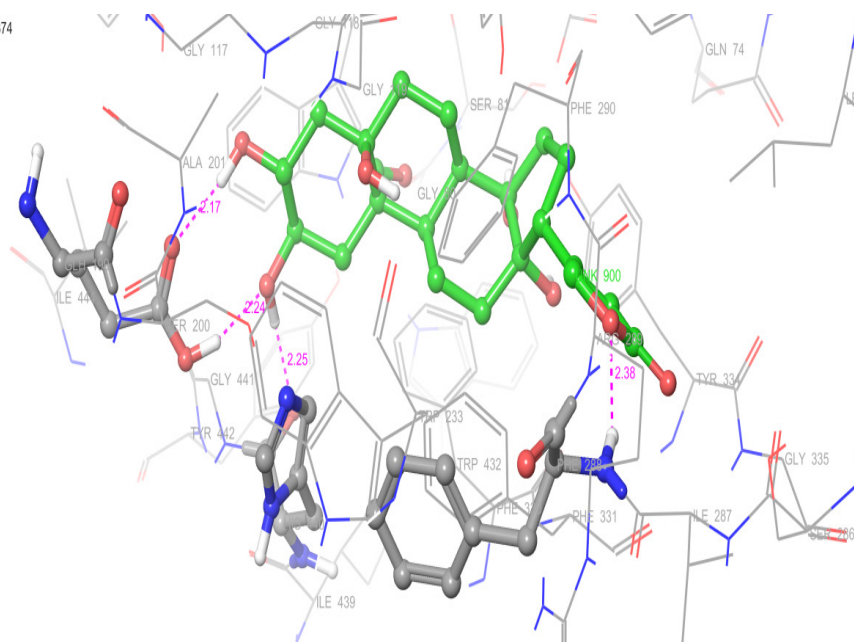


Figure 1A: Orientation of Calotropagenin from *Calotropis procera* in 1EVE active pocket

The O of oxane group of compound forms hydrogen bond with PHE288 (O---NH, distance: 2.38Å), H and O of hydroxyl group of compound forms hydrogen bond with HIS440 (OH---N, distance: 2.25Å) and GLH 199 (O---OH, distance: 2.24Å) respectively, and H of hydroxyl group forms hydrogen bond with GLH199 (H---O, distance: 2.17Å).

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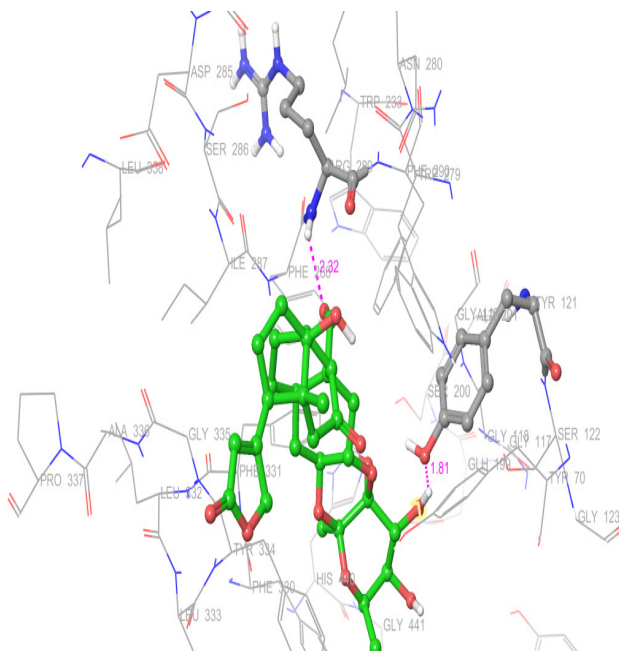


Figure 1B: Orientation of Calotoxin from *Calotropis procera* in 1EVE active pocket

The O of carbonyl group makes hydrogen bond with ARG289 (O---NH, distance: 2.32Å), and H of hydroxyl group forms hydrogen bond with TYR121 (OH---O, distance: 1.81Å)

and also helps to understand the insight of interaction of compounds with target receptor or protein.⁷

In search for natural products with AChE inhibitor activity, this study is focused on *in silico* molecular docking studies to explore the ability of reported compounds from *Cassia tora*, *Brassica campestris* and *Calotropis procera* latex to potentially inhibit AChE. This was achieved through the analysis of information of the reported compounds from available literature sources and other

information databases. The selected three plants have been proved scientifically for the treatment of various diseases including CNS disorders, but interaction of their phytoconstituent with AChE receptor is not well understood, hence *Cassia tora*, *Brassica campestris* and *Calotropis procera* was selected for study. Also, this study attempts to focus on the discovery of novel molecules from *Cassia tora*, *Brassica campestris* and *Calotropis procera* latex that can bind and inhibit AChE enzyme activity.

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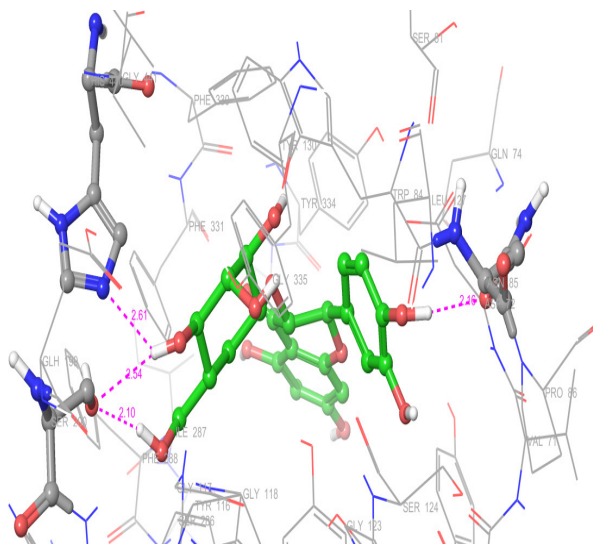


Figure 2A: Orientation of Vitamin A from *Brassica campestris* in 1EVE active pocket

The H of hydroxyl group makes hydrogen bond with HIS440 (OH...N, distance: 2.61Å) and SER200 (OH...O, distance: 2.54Å), and H of hydroxyl group forms hydrogen bond with SER200 (OH...O, distance: 2.10Å).

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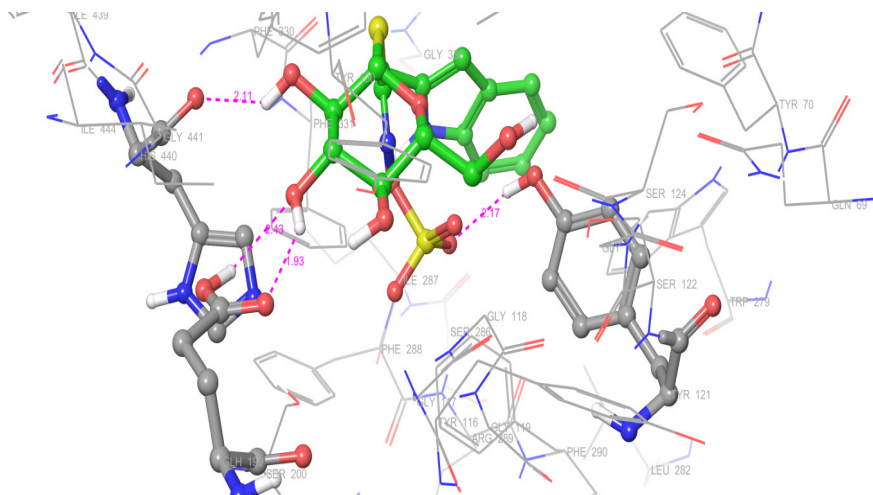


Figure 2B: Orientation of Glucobrassicin from *Brassica campestris* in 1EVE active pocket

The H of hydroxyl group forms hydrogen bond with HIS 440 (OH...O, distance: 2.11Å), H and O of hydroxyl group makes hydrogen bond with GLH 199 (OH...O, O...OH; distance: 1.93Å and 2.43Å respectively), and H of thio group forms hydrogen bond with TYR 121 (SH...OH, distance: 2.17Å).

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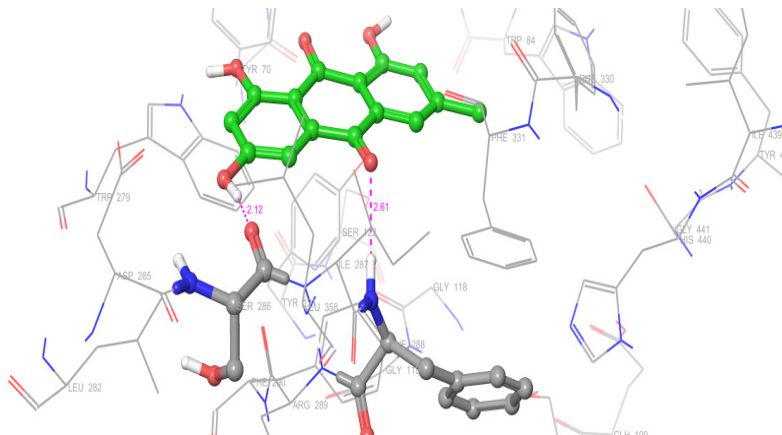


Figure 3: Orientation of physocin from *Cassia tora* in 1EVE active pocket

The H of hydroxyl group of our compound makes hydrogen bond with SER286 (H...O, distance: 2.12Å), and carbonyl oxygen forms hydrogen bond with PHE288 (O...NH, distance: 2.62Å).

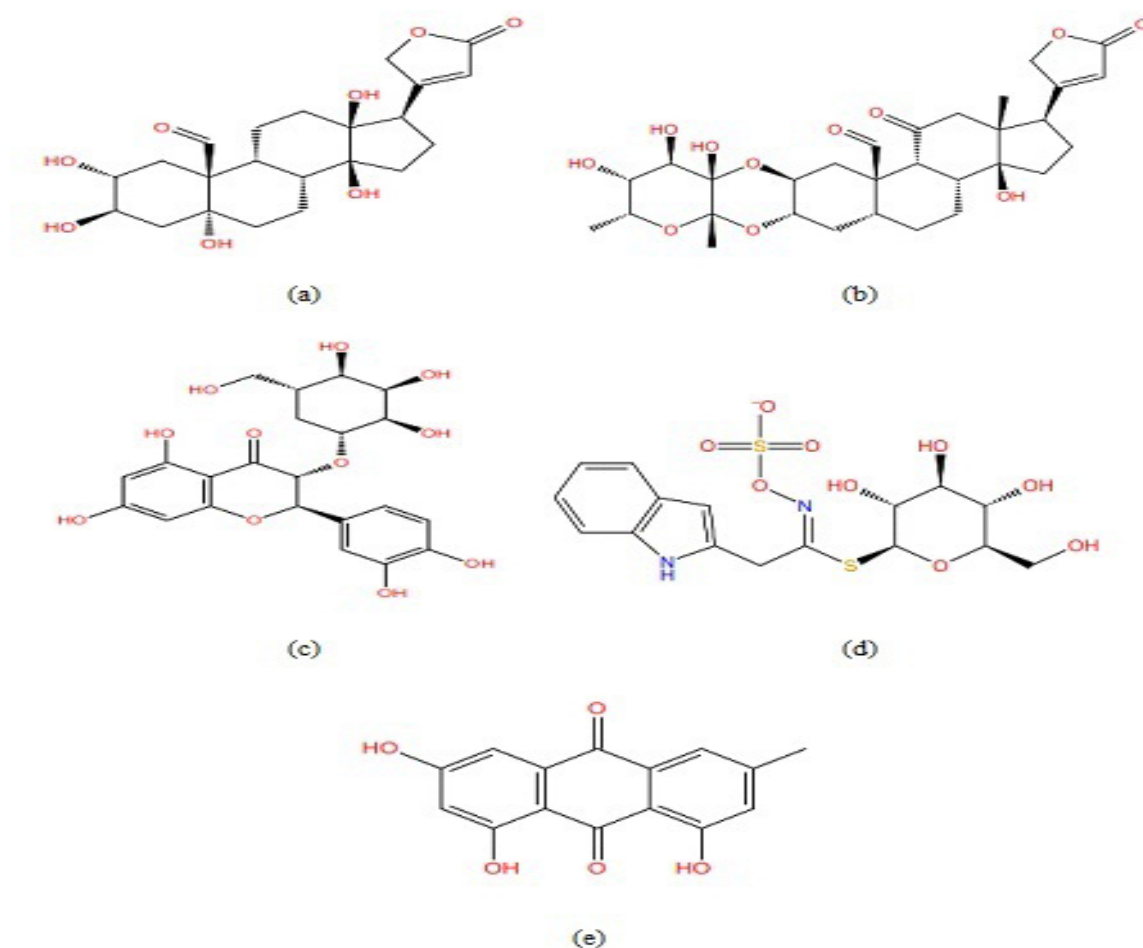


Figure 4: Structure of most active compounds from *Cassia tora*, *Calotropis procera* and *Brassica campestris*

(a-Calotropagenin, b-Calotoxin, c-Vitamin A, d-Glucobrassicin, e-Physocin).

MATERIALS AND METHODS

Protein structure preparation

3D structure of acetylcholinesterase complexed with Aricept (PDB ID: 1EVE) was retrieved from RCSB Protein Data Bank (PDB). The major form of *Torpedo californica* (Tc) AChE is a homodimer, covalently attached to plasma membrane through phosphatidylinositol group. It has ellipsoidal shape and belongs to the class of α/β proteins and consists of a 12 stranded central mixed β sheet surrounded by 14 α helices, it also contains two active subsites, esteratic and anionic.⁸ The E2020 has a unique orientation, extending from the anionic subsite of the active site to the peripheral anionic site. The E2020 makes principal interactions along the active subsites of the enzyme through the benzyl moiety, the piperidine nitrogen, and the dimethoxyindanone moiety.⁹ The obtained protein structure was prepared for molecular docking by removal of water molecules from its 3D structure. To depict the *in vivo* interaction, the energy of

the target protein was minimized before performing the docking simulations.

Ligand structure preparation

In this study, overall 30 compounds reported to be present in leaves of *Cassia tora*¹⁰, latex of *Calotropis procera*¹¹ and seeds of *Brassica campestris*¹² which are of medicinal importance were selected from the literature survey (Table 1). These comprise diverse series of compounds including flavonoids, cardenoloids, steroids, terpenes, vitamins, phenols etc. For the development of pharmacophore, the 2D structures of the compounds identified were imported into Discovery Studio (DS) version 2.1 (Discovery Studio, 2012) and then converted into 3D structures, so that molecules can align in energetically favourable conformation to bind to a receptor site.

Docking protocol

To study the molecular basis of interaction and affinity for binding of selected compounds to AChE protein, all the ligands were docked into the active site of AChE.

The structural binding pockets and active sites in the protein were determined by literature search.

The molecular docking studies were performed using Maestro, Schrodinger suite. The best docked structure was chosen using a GLIDE score (Dock Score) function and Hydrogen bonding. GLIDE score incorporates the coulomb and vdW interaction energies between the ligand and the receptor and also introduces a solvation model. It has negative regression coefficient towards the binding affinity. GLIDE module was set to extra precision (XP) mode so that Hydrogen bond could be visualized in the XP Visualizer.¹³

RESULT AND DISCUSSION

The main objective of this study is to identify novel acetylcholinesterase inhibitors based on their interaction with AChE. The protocol was validated by reproducing conformation of docked ligand in crystal structure of AChE and the docked Aricept was removed from active site and docked again. The top three conformations of Aricept were taken into consideration. The acceptable result denotes that the docked ligand is having similar binding position and orientation with that of Aricept.

In the present study, preliminary screening of 30 chemically diverse compounds was carried out for their inhibitory effect on AChE. Calotropagenin (cardenolides) present in latex of *Calotropis procera* showed significant GLIDE score of -13.02 and bond strength of -4.23 Å (Figure 1A and 4) as compared to GLIDE score of -11.78 and bond strength of -1.27 Å for Aricept-AChE complex. While, Calotoxin has GLIDE score of -11.23 and bond strength of -2.51 Å (Figure 1B and 4), also Quercetin-3-rutinoside has GLIDE score of -11.21 and bond strength of -0.66 Å showed interaction comparable with Aricept-AChE complex. This binding study may predict mode of action and active chemical moiety from *Calotropis procera* latex responsible for AChE inhibition and its claim to possesses anti-amnesic¹⁴, anti-convulsant¹⁵ and neuroprotective effects.¹⁶

Among the selected compounds from seeds of *Brassica campestris*, Vitamin A showed GLIDE score -11.02 and bond strength -5.54 Å (Figure 2A and 4) while Glucobrassicin (Glucosinolate) has GLIDE score of -10.85 and bond strength of -4.0 Å (Figure 2B and 4) which denotes tight binding and high energies when compared with Aricept-AChE complex. This result indicate the beneficial effect of Vitamin A in cognition enhancing effect

and is currently being studied for its beneficial effect on Alzheimer's disease.¹⁷ Also, the presence of Glucosinolate in higher concentration in *Brassica campestris* species can be responsible for its cognition enhancement activity.¹⁸

While compounds like physcion from *Cassia tora* leaves has GLIDE score of -10.2 and bond strength of -2.22 Å (Figure 3 and 4) showed tight binding between ligand and AChE, the flavonoids like quercetin, stigmasterol, β stigmasterol showed comparable GLIDE score but weak bonding with AChE proteins when compared with Aricept-AChE complex. These results are in close proximity with earlier studies reporting beneficial effect of flavonoid in CNS disorders¹⁹, also proves the use of *Cassia tora* as nervine tonic and neuroprotective agent.²⁰

CONCLUSION

A structure-based pharmacophore model was generated and validated to obtain active AChE inhibitors from our self-compiled database of known compounds reported from leaves of *Cassia tora*, latex of *Calotropis procera* and seeds of *Brassica campestris*. Also an attempt was made to co-relate their reported biological activity along with marketed AChE inhibitors with their docking scores.

The docking study revealed that cardenolides, flavonoids and glucosinolate showed better alignment at active site by interacting with all crucial amino acid residues. Thus, the *in silico* method adopted in the present study helped in identifying the lead compounds and also may partly explain their beneficial effect in *in vivo* study. Overall, this could be an important first step in development of novel AChE inhibitor. Studies have been undertaken to isolate the compounds and to test their effect in *in vitro* assays and in *in vivo* models.

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ABBREVIATION

AChE : Acetylcholinesterase

GLIDE: Grid based Ligand Docking with Energetics

AD : Alzheimer's disease

CNS : Central nervous system

FDA : Food and drug Administration

SUMMARY

- 30 compounds which are of medicinal importance and are reported to be present in leaves of *Cassia tora*, latex of *Calotropis procera* and seeds of *Brassica campestris* were selected from the literature survey.
- These compound was docked with 3D structure of acetylcholinesterase complexed with Aricept (PDB ID: 1EVE) retrieved from RCSB Protein Data Bank.
- The docking study results revealed that cardenolides, flavonoids and glucosinolate showed better alignment at active site by interacting with all crucial amino acid residues.

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