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Evaluating the potency of three plant compounds as HDAC Inhibitors for the treatment of Huntington's disease: An *in silico* study

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Abstract
Transcriptional deregulation is one of the key features of Huntington's disease (HD), which involves the impairment of the general transcription machinery and proteins involved in gene expression. This results in an overall disruption in the transcriptome of cells, namely neurons, leading to defective cellular processes. Histone deacetylases (HDACs) are key enzymes in transcription and are known to have increased activities in HD animal models and patient samples. In this paper, we have used molecular docking studies to evaluate the potential of three plant- derived compounds, Fisetin, Ginkgolide A and Ginsenosides, as HDAC inhibitors. The binding energies of each plant compound was evaluated by docking them to three HDACs known to be involved in HD progression; HDAC3, HDAC4, and HDAC6. The binding energy values of the plant compounds were found to be comparable to that of well-known pharmaceutical inhibitors proving them to be promising therapeutic agents in controlling HD progression.

Keywords: Huntington's disease, histone deacetylases, HDAC inhibitors, phytochemicals, docking

1. Introduction
Huntington's Disease is a late-onset neurodegenerative disease that eventually causes cognitive and movement disorders [1]. Symptoms of this disease include psychiatric problems such as depression, psychosis and obsessive compulsive disorder [1], as well as loss of motor function and self and spatial awareness, depression, dementia and increased anxiety over a period of 10-12 years before death [2]. The disease is caused by a trinucleotide CAG expansion in Htt gene coding for Huntingtin Protein [2], which translates into a defective protein (mutant Huntingtin or mHTT) with an abnormally long poly glutamine (polyQ) tract corresponding to the trinucleotide expansion [3]. These mHTT proteins tend to form aggregates, commonly known as amyloid bodies which disrupt the cellular machinery of neurons leading to nervous system disorders [2]. The various pathways disrupted by amyloid bodies include transcriptional deregulation, altered protein folding, mitochondrial dysfunction and disrupted neuronal machinery [2]. Within the transcriptional deregulation pathway, Histone deacetylases (HDACs) are enzymes known to play a key role in disease progression and are known to have increased activities in several polyQ models [4].
Histone acetylation is a method of chromatin modification that regulates DNA-Histone interactions by adding or removing acetyl groups to and from Histones, which are proteins used to pack DNA within nuclei [5]. Histone acetylation is performed by enzymes known as Histone Acetylases, which add acetyl groups on lysine residues of histone proteins. This process causes an unpacking of DNA, allowing gene transcription. The removal of acetyl groups or deacetylation is done by HDACs, which results in packing of DNA and reduced gene expression. This balance of acetylation and deacetylation was found to be disrupted in HD models as HDAC activity was found to be increased, leading to raised histone deacetylation and lower expression of corresponding genes, which contributes to an overall perturbation of cellular machinery [4]. Among the plethora of HDACs in mammals, HDAC1, HDAC3, HDAC4, HDAC6 have been implicated in HD models [5]. Reduction of these HDACs by the use of molecular inhibitors or genetic knockdown has been shown to improvecellular phenotype and increase lifespan. As a method for treatment, several HDCA inhibitors have been synthesized to slow down disease progression [5]. However, synthetic compounds have side effects on human physiology and thus limits its application as therapeutics agents. This has led to a shift to plant-derived compounds or phytochemicals for controlling neurodegenerative diseases such as HD. In this paper, we have used molecular docking studies to evaluate the potency of three plant-derived compounds, which have been previously

reported to have positive effects on other neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease [6–8], to act as HDAC inhibitors. The three compounds selected for this study are Fisetin (3,3',4',7-tetrahydroxyflavone) found in a variety of fruits and vegetables such as apple, strawberry, grape, onion and cucumber among others [9], Ginkgolide Afound in *Ginkgo biloba* [10] and Ginsenoside Found in the root of *Panax ginseng* [6]. These compounds were tested on three of the HDACs involved in disease progression: HDAC3, HDAC4 and HDAC6. The results were reported based on binding energies obtained from molecular docking experiments. Finally, we propose the possibility of these compounds as a replacement of synthetic drugs as HDAC inhibitors.

2. Materials and Methods

Molecular docking studies were done using downloaded protein and ligand structures available on different databases. The structure of Fisetin ($C_{15}H_{10}O_6$), Ginkgolide A ($C_{20}H_{24}O_9$) and Ginsenosides ($C_{30}H_{52}O_2$) were downloaded from Drug Bank database (https://www.drugbank.ca) (Accession Numbers:DB07795, DB06743 and DB14152 respectively) and the structures of the HDAC enzymes were downloaded from Protein Data Bank (https://www.rcsb.org/). The specific HDACs used were *Homo sapiens* Histone Deacetylase 3(PDB ID: 4A69), *Homo sapiens* Histone Deacetylase 4 (PDB ID: 4CBT) and *Danio rerio* HISTONE Deacetylase 6 (PDB ID: 5EEI). The ligands and proteins used for docking were prepared using Auto Dock Tools 1.5.6. Water molecules were removed and hydrogens were added to the proteins. Gasteiger charges were computed to both the ligands and proteins. The charges were equally distributed over the entire protein structure. Based on Lig Plot data from PDB sum (http://www.ebi.ac.uk/thornton-srv/databases/cgibin/pdbsum/GetPage.pl?pdbcode=index.html), the interacting amino acids of each protein were known and used to determine the grids for the docking experiments. The details of each of the HDACs are as follows:

2.1 HDAC3

The X, Y and Z centers of the grid were set as 37.98, 55.675, 24.961 respectively. The size of the grid (X x Y x Z) was 60x34x44. Energy range and exhaustiveness were set as 4 and 8 respectively. The interacting amino acids are Gly 143, Cys 145, Asp 175, His 134, His 172, Asp 168, Gly 296, Asp 170, Tyr 298, and His 135, all of which are in chain A of the protein.

2.2 HDAC4

The X, Y and Z centers of the grid were set as 9.581, 10.248, 139.229 respectively. The size of the grid (X x Y x Z) was 42x54x44. Energy range and exhaustiveness were set as 4 and 8 respectively. The interacting amino acids are His 842, Asp 934, Asp 840, Gly 975, His 802, Gly 974, Asp 838, Leu 943, His 803, Phe 812, Gly 811, and Phe 871, all of which are in chain A of the protein.

2.3 HDAC6

The X, Y and Z centers of the grid were set as 5.4, -7.464, 0.356 respectively. The size of the grid (X x Y x Z) was 44x46x44. Energy range and exhaustiveness were set as 4 and 8 respectively. The interacting amino acids are Ser 531, Asp 612, His 573, Phe 583, Gly 582, Gly 743, Tyr 745, Leu 712, Pro 464, His 574, Phe 643, and His 614, all of which are in chain A of the protein.

Two known HDAC inhibitor Valproate and Vorinostat [5], the structures of which were also downloaded from DrugBank (Accession No.: DB00313 and DB02546) were used as standards. The docking and binding energy evaluation of each protein with the three plant compounds and the two standards were performed using Autodock Vina [11]. The binding energies of Fisetin, Ginsenosides and Ginkgolide A were compared to that of Valproate and Vorinostat to evaluate their abilities to bind effectively and inhibit the various HDACs. The docked ligand and protein was visualized using PyMOL molecular viewer.

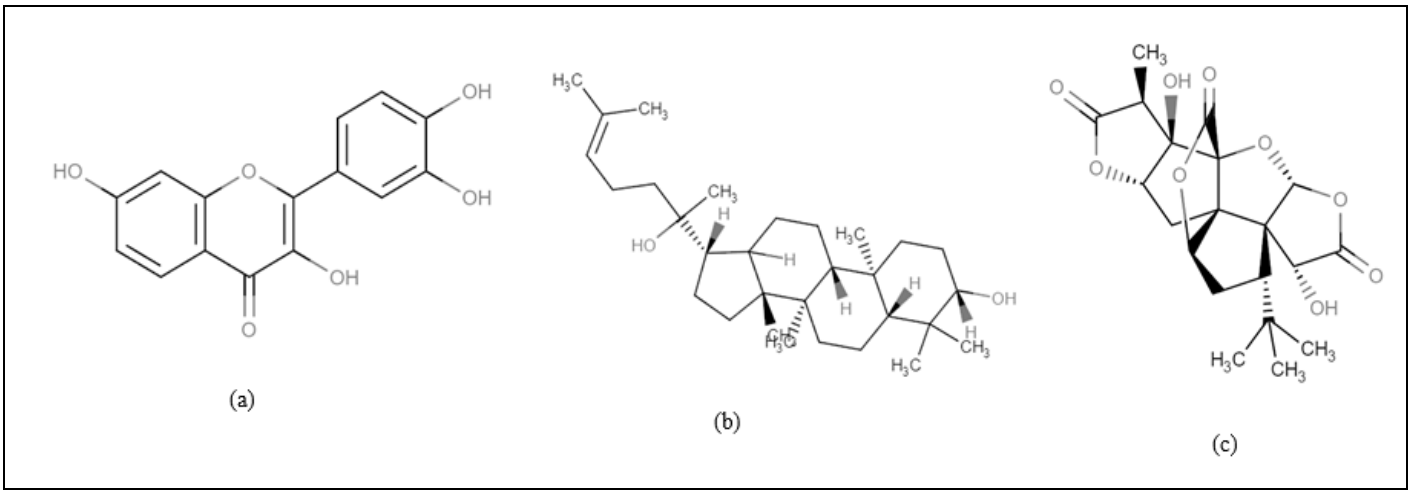

Fig 1: Molecular structures of (a) Fisetin, (b)Ginsenosides and (c) Ginkgolide A

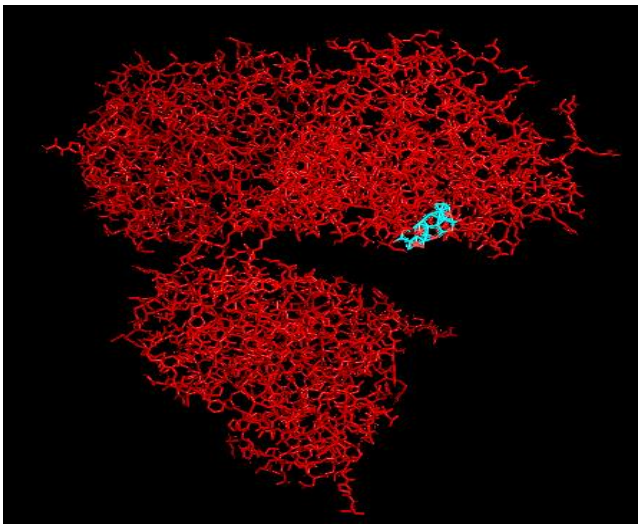

Fig 2: Structure of HDAC4 (red) with Ginkgolide A (blue) docked in the active site.

3. Results and Discussion

The discovery of HDAC inhibitors has been vigorously pursued as it was shown that inhibiting the activities of HDACs has proven to be beneficial in HD treatment [5]. For instance in a previously conducted study, knockdown of HDAC3 has been shown to reduce mHTT neurotoxicity in HD mouse models [12]. Experiments involving knocking down of HDAC4 in mouse models has shown that cytoplasmic mHTT aggregation can be delayed and neuronal function can be rescued [13]. Inhibition of HDAC6 was shown to improve tubulin acetylation and intracellular vesicle transport, both of which aredisturbed in HD [14]. These studies have inspired researchers to develop or discover inhibitors of HDACs for HD treatment. In our study, we have shown that three phytochemicals, Fisetin, Ginsenosides and Ginkgolide A have the potential to replace synthetic drugs as efficient HDAC inhibitors. Fisetin has previously been reported to be useful in the treatment of age-related neurodegenerative diseases involving cognitive impairments such as Alzheimer's Disease (AD) [8]. Ginkgolides such as Ginkgolide A extracted from *Ginkgo biloba* has shown to be effective in the treatment and prevention of neural diseases such as AD and has no known side effect [10]. The roots of *Panax ginseng*, with Ginsenosides being the bioactive component, have long been used in countries such as Japan, Korea and China as herbal medicine against cancers, cardiovascular diseases, immune deficiencies, hepatotoxicities and central nervous system disorders such as Parkinson's disease [6]. In our study using molecular docking, we have shown that these phytochemicals can bind strongly to HDACs 3, 4 and 6. The docking results are presented in table 1. As shown in the table, the three plant compounds have higher binding affinities than the controls with all three HDACs tested. Ginkgolide A has the highest binding affinity and may have the highest potency as a HDAC inhibitor followed by Ginsenosides and Fisetin.

Table 1: Binding affinities of the docked ligands and proteins in kcal/mol

Ligands	Proteins		
	HDAC3	HDAC4	HDAC6
Valproate	-4.9 kcal/mol	-5.5 kcal/mol	-4.9 kcal/mol
Vorinostat	-7.1 kcal/mol	-5.4 kcal/mol	-5.8 kcal/mol
Fisetin	-8.1 kcal/mol	-7.0 kcal/mol	-8.2 kcal/mol
Ginkgolide A	-10.1 kcal/mol	-8.9 kcal/mol	-9.6 kcal/mol
Ginsenosides	-7.5 kcal/mol	-8.0 kcal/mol	-8.9 kcal/mol

4. Conclusion

Based on our *in silico* study, we conclude that the plant derived compounds Fisetin, Ginsenosides and Ginkgolide A are effective inhibitors of HDAC3, HDAC 4 and HDAC6 and can potentially replace synthetic HDAC inhibitors such as Valproate and Vorinostat, in the treatment of Huntington's disease, thus providing non-toxic methods with no or minimal side effects.

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6. References

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