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EXTRACTION OF ACETYLCHOLINE ESTERASE INHIBITORS FROM *JASMINUM GRANDIFLORUM* FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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ABSTRACT

Alzheimer`s disease is the most common dementia contributing to 60-70% of the cases. The widely used treatment for the disease is to suppress the activity of the enzyme acetylcholine esterase which breaks down the neurotransmitter acetylcholine thus, resulting in the deceleration of the disease. This paper briefs about the identification and extraction of acetylcholine esterase inhibitors from the methanolic extract of the plant *Jasminum grandiflorum* leaves. Zebrafish is used as the model organism to study the effect of plant leaf extract on activity of acetylcholine esterase in both in-vitro and in-vivo conditions. Titrimetric assay which involves the use of an alkali to neutralize the carboxylic acid released by the enzyme is used to determine the enzyme activity in the presence of the plant extract. It was found that the enzyme activity was decreased by 19.97% in-vitro conditions and in in-vivo conditions of diseased fish the enzyme activity was dropped by 21.39%. The methanolic leaf extract was sent to GC-MS studies to determine the organic compounds present in it and this was followed by in-silico studies which revealed that compound cedrol has minimum energy and maximum binding affinity with the acetylcholine esterase enzyme.

KEYWORDS: Alzheimer`s disease, acetylcholine esterase, enzyme activity, docking.

INTRODUCTION

Alzheimer's disease(AD) is a brain disorder characterizedby a progressive dementia that occurs in middle or late life which is characterized by neural death.[1] [15] Although the exact reasons for the cause of AD is unknown, the neuroimaging of the diseased brain reveals the

critical features of the disease that is the neurofibrillary tangles and senile plaques in the cerebral cortex.[2][3] Neurofibrillary tangles consist of aberrantly phosphorylated fibrillary proteins (tau proteins) aggregated within the neuronal cytoplasm. Their presence signifies the failure of the neuron to properly maintain its Cytoskeleton , which is required to support the extraordinarily complex branching shape of its numerous processes.[3][7][15] Senile plaques are formed due to the imbalance created by the accumulation of amyloid β-proteins which assemble to form insoluble fibres interrupting neurotransmission.[3][7][15] The use of memantine, amyloid precursor protein (APP) and β-secretase and γ-secretase inhibitors, βvaccination, amyloid antiaggregant therapies and other therapies are the recent advancement in the treatment of AD. But the most established treatment is mainly based on the use of Cholinesterase inhibitors.[4] [15][25] Cholinesterase inhibitors mainly function by inhibiting the activity of Acetyl Choline Esterase(AChE). It is a hydrolytic enzyme which cleaves the ester bond in the neurotransmitter acetylcholine(ACh) and breaks it into acetic acid and choline in the synaptic region. The released choline is reused for the synthesis of ACh in the axon terminal and the cycle repeats.

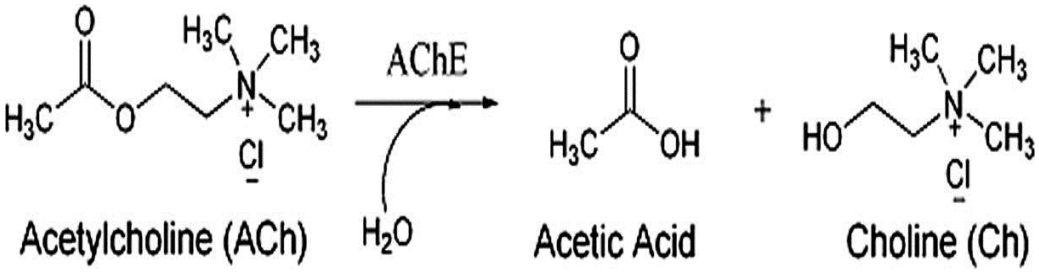


Fig 1: Hydrolytic breakdown of ACh by AChE enzyme.

In Alzheimer`s patient the level of ACh is reduced due to the augmented cell death in the brain thus interrupting the transfer of nerve signals causing loss of memory and dementia. Thus, by inhibiting the AChE activity and preventing the breakdown of ACh helps in decelerating the progression of the disease.[4][6][25] AChE inhibitors such as donepezil, rivastigmine, and galantamine are being widely used for the treatment of Alzheimer`s but the use of these therapeutic drugs causes side effects such as facial flushing, dyspepsia, nausea, vomiting, and diarrhoea. Thus, there is a need for an alternate drug with better efficacy and minimum side effects.[4] [5] [25]

Medicinal plants have been used by humans since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants have led to the discovery of novel

drug candidates used against diverse diseases. According to the World Health Organization (WHO), in 2008 more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Reports available on green plants represent a reservoir of effective chemotherapeutants, these are non-phytotoxic, more systemic, and easily biodegradable.[8] [20] The plant material used in this study is *Jasminum grandiflorum* (Family: Oleaceae). The plant is known for its numerous benefits such as antioxidant, antimicrobial, antibacterial and anti-ulcerogenic activity due to presence of flavonoids, terpenes, alkaloids, glycoside, and resin.The leaf of the plant contains ascorbic acid, anthranilic acid and its glucoside, indole oxygenase, alkaloid jasminine and salicylic acid etc, which is been studied in this paper for their effects on the AChE enzyme.

Zebrafish has been established as an excellent model for studying any biological process. This organism possesses many advantages including ease of experimentation, optical clarity, drug administration, amenability to in-vivo manipulation and feasibility of reverse and forward genetic approaches. They are genetically similar to mammals, including humans by sharing 71%of genes with humans. The fish reaches sexual maturity in only 3 to 4 months and adult females are capable of producing 100 to 200 eggs every week. Many fishes can be kept in a single fish facility requiring much less space than mice or other mammals. Therefore, zebrafish is regarded as a cost-effective experimental vertebrate model for large-scale genetic screening. Furthermore, the high degree of homology between the zebrafish genome and that of humans makes such discoveries especially pertinent to human disease and development.[21] Here, the fish is used as the model organism for in-vitro and in-vivo analysis of the plant leaf extracts for inhibiting AChE enzyme for the treatment of Alzheimer's.

MATERIAL AND METHODS

Extraction of organic compounds from *Jasminum grandiflorum* leaves

The leaves were washed thoroughly with distilled water and dried in a hot air oven and were then powdered. Soxhlet extractor was used for the extraction process. 5g of dried leaves were taken in a filter paper and placed in the extraction chamber, 300ml methanol was taken as the solvent in the round bottom flask. The stock solution of 100mg/ml of the leaf extract was prepared for further use and the sample was sent for GC-MS analysis.

GC-MS analysis of plant leaf extract

Gas Chromatography Mass Spectroscopy, a hyphenated system is a very compatible technique and the most commonly used technique for the identification and quantification

purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra. There are at least two significant advantages for using GC-MS in the analysis of herbal medicines. First with the capillary column, GC-MS has in general very good separation ability, which can produce a chemical fingerprint of high quality and secondly with the coupled mass spectral database, quantitative composition information of the herb investigated could be provided by GC-MS, which will be extremely useful for the further research for elucidating the relationship between chemical constituents in the herbal medicine and its pharmacology in further research.[8][20]

The GC-MS analysis of methanolic extract from the plant leaves of *Jasminum grandiflorum* was carried out at "Indian Institute of Science" in Bengaluru, Karnataka, India. The GC-MS running time was 20 minutes and reports revealed the presence of eight constituents. The chromatogram of GC-MS analysis is shown in fig 2. which is been explained in the subsequent table 1.

Docking

The grid maps of all AChE molecules were calculated individually using Auto Grid part of AutoDock tools, focusing on sufficient large to include active site and significant part of surface as well. All the selected drug compounds and experimental ligands were docked into the active sites of AChE using Auto Dock 4.3 program. Automated docking was performed using Auto Dock 4.2 with Lamarckian genetic algorithm (LGA) to model ligand- AChE interaction and binding, in which 100 multiple, independent docking runs were carried out to increase the performance of docking programs. Finally, cluster analysis was carried out on the observed docking values base on the root mean square (RMS, 0.5 Å). Consequently, binding affinity and free energy charge of binding were calculated using Auto Dock Vina and Auto Dock 4.2. From the docking results (shown in table 2.) it was clear that Cedral is the organic molecule which had maximum binding affinity with minimum energy and inhibiting the AChE enzyme activity.

In-vitro analysis

Titrimetric assay technique was employed in in-vitro analysis. As AChE breaks down ACh into choline and acetic acid, the activity of AChE enzyme was determined by titrating the incubated solution of AChE and ACh which would eventually contain acetic acid upon the

activity of AChE, against NaOH. Thus, by calculating the amount of acetic acid in the solution would help in determining the AChE enzyme activity.[9]

- **Enzyme source**

Five fully grown zebra fish were taken and their brain was dissected. The brains were homogenized using pre-chilled mortar and pestle with 0.1M phosphate buffer of pH 8. The solution was centrifuged at 5000 rpm for 5 mins at 4℃. The supernatant was collected and diluted with distilled water at dilution factor of 2.

1ml of enzyme source, 3.5ml of 0.1M phosphate buffer at pH 7, 0.5ml of substrate (acetylcholine) was mixed gently in a beaker and incubated at room temperature for 30 mins. The enzyme activity was ceased by placing it into a hot water bath for 2-3 mins.

0.1ml, 0.4ml and 0.8ml of leaf extract solution of concentration 100µg/ml was taken in three different beakers. 1ml of enzyme source was added to each and incubated at room temperature for 30 minutes. Then for each beaker 3.5ml of 0.1M phosphate buffer of pH 7 and 0.5ml of substrate (acetylcholine) was added and incubated at room temperature for 30 minutes. The enzyme activity was ceased by placing it into a hot water bath for 2-3 minutes.(2 such replicas were made).

3 drops of phenolphthalein was added to above solutions and were titrated against 0.5M NaOH solution till a pale pink coloration was observed.

From the titrant values the amount of acetic acid released by the activity of the AChE enzyme on the substrate acetylcholine was calculated which was used in the determination of the enzyme activity.

Amount of acetic acid liberated by the action of AChE enzyme was found using the formula:
$N_{CH3COOH}\ V_{CH3COOH}=N_{NaOH}V_{NaOH}$.

The enzyme activity was calculated using the formula.

$$enzyme\ activity = \frac{\{(amount\ of\ product\ formed) * 2 * df\}}{\{(molecular\ weight\ of\ substrate) * T\}}$$

df - Dilution factor

T - Time of assay in minutes

The titration results show that (depicted in graph 1.) the enzyme activity was reduced by 19.97% compared to the control by adding 0.4 ml/0.8ml of plant extract.

In-vivo analysis

Alzheimer`s was induced into the zebra fish by using scopolamine which blocks the neurotransmitter and mimics a condition in zebra fish which is similar to Alzheimer`s disease. Two tanks containing 1L of dechlorinated water was taken and each tank was aboded by two fishes and were labelled as A and B. For both the tanks scopolamine was added to induce Alzheimer`s in them. The fishes were monitored in same condition for 2 days. Later the fishes were transferred to fresh chlorinated water and from the next day the fishes in tank B was treated with the plant extract by injecting it into the water. The fishes were maintained in this state for couple of days. Thus, the fishes in both tanks were infected by Alzheimer`s disease but only tank B fishes were treated with plant extract and tank A fishes were referred as control. The brains of the fishes of each tank was dissected and subjected to titrimetric assay separately by following the same steps as that of in-vitro assay. The titration results show that (depicted in graph 2.) the enzyme activity was reduced by 21.39% compared to the control by addition of plant extract.

RESULTS

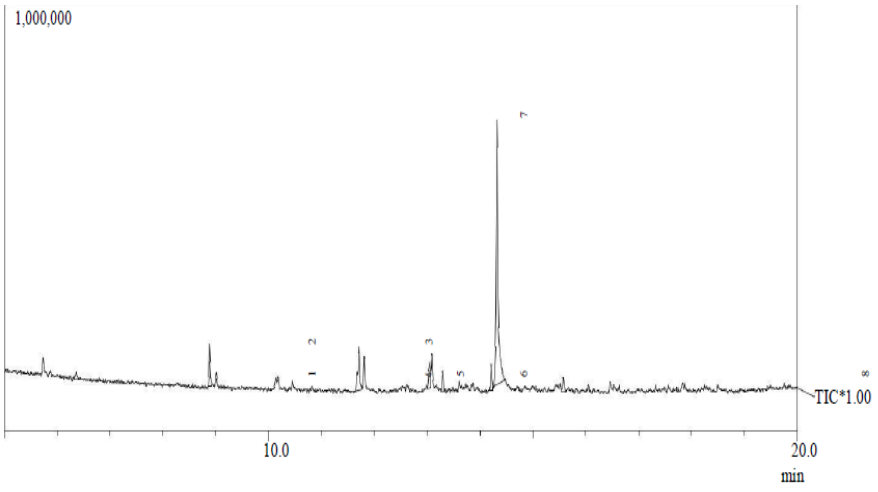


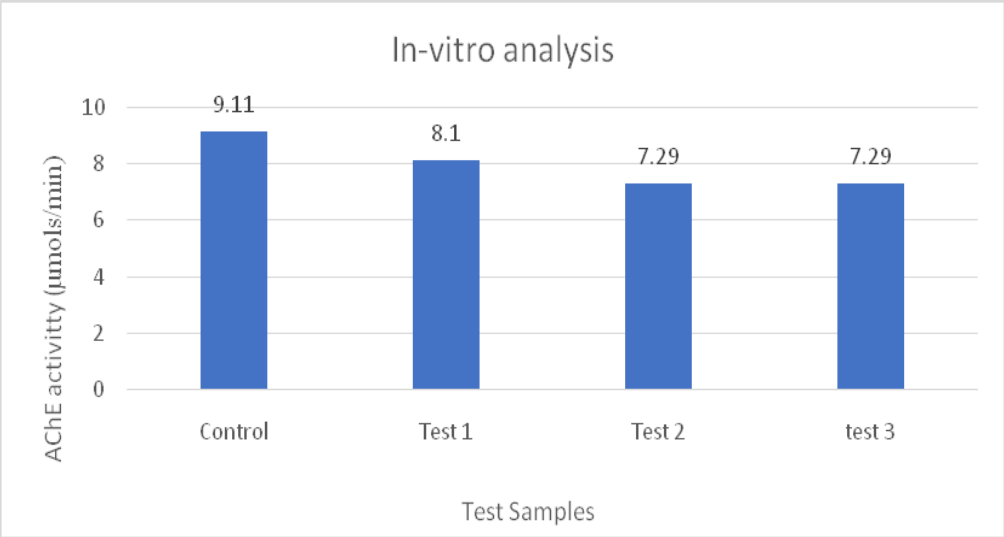
Fig 2: Chromatogram of methanolic extract of *Jasminum grandiflorum* leaves.

Table 1: Peak report of the organic compounds present in the methanolic extract of *Jasminum grandiflorum* leaves.

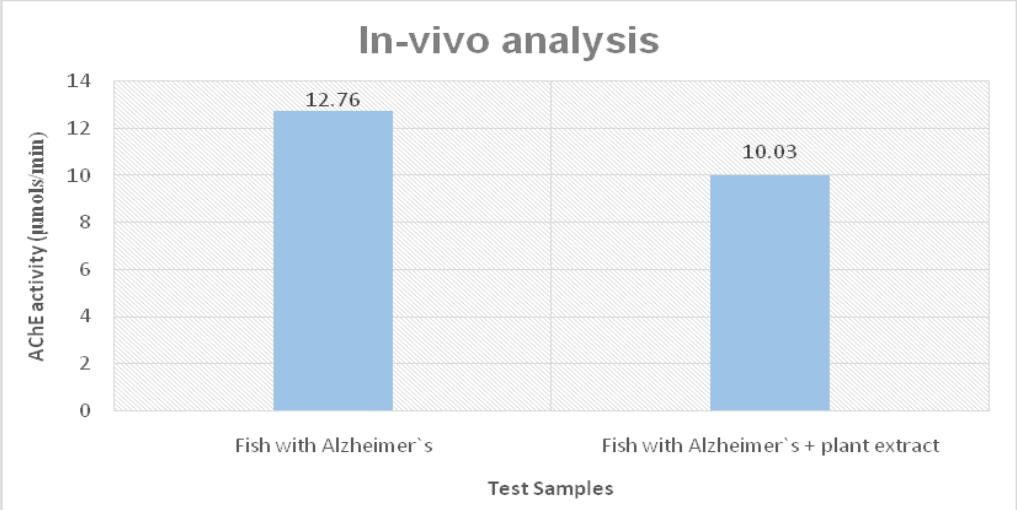
Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	6.356	6.317	6.408	45976	1.82	Benzyl acetate
2	8.882	8.858	8.925	160364	6.36	Nerolidol
3	11.707	11.642	11.767	277541	11.00	Cedrol
4	13.047	13.000	13.067	162435	6.44	Nonadecane
5	13.290	13.258	13.325	82868	3.28	Phenol,2,4-bis (1,1dimethylelthyl) -(CAS)2,4-Di-tert-butylphenol
6	14.210	14.167	14.242	110018	4.36	3-Hexadecene, (Z)
7	14.319	14.242	14.458	1612785	63.91	Hexadecanoic acid
8	15.571	15.542	15.625	71344	2.83	Tricosane

Table 2: Binding energy of the organic molecules obtained from the plant leaf extract with AChE enzyme.

Compound name	Affinity (kcal/mol)
3-Hexadecene	-4.1
Benzyl acetate	-5.7
Cederol	-8.1
Hexadeconic acid	-4.8
Nerolidol	-6.3
Phenol, 2,4-bis(1,1-dimethylethyl)-(CAS) 2,4-Di-tert-butylphenol	-7.3
Nonadecane	-5.5
Tricosane	-3.6



Graph 1: In-vitro analysis results: AChE enzyme activity has been decreased by 19.97% by addition of 0.4ml of plant extract with respect to the control.



Graph 2: In-vivo analysis: AChE enzyme has been decreased by 21.39% by treating the fish with Alzheimer's disease with plant extract with respect to the untreated fish.

CONCLUSION

The leaves of plant *Jasminum grandiflorum* contains the inhibitors for the enzyme acetylcholine esterase and from the GC-MS analysis and in-silico studies it is found that cedrol is the organic compound with minimum energy and maximum binding affinity. This was supported by the results from the in-vitro and in-vivo analysis done by using the zebra fish brains. Thus, the plant can be used for the extraction of cedrol as the potential inhibitor of AChE enzyme and may be used for the treatment of Alzheimer`s and other forms of dementia such as Parkinson's disease, Huntington's disease, vascular dementia etc. Further studies are required for the purification of cedrol and the quantification of dosage for the treatment in higher organisms.

REFERENCES

1. Guy McKhann, David Drachman, Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease, Neurology, 1984.
2. Ana M Garcı´a, Antonio Sisternas and Santiago Perez Hoyos. Occupational exposure to extremely low frequency electric and magnetic fields and Alzheimer disease: a meta-analysis, International Journal of Epidemiology, 2008; 37: 329–340.
3. David G. Munoz, Howard Feldman, et al. Causes of Alzheimer's disease, CMAJ, 2000; 162(1): 65–72.
4. Judith Neugroschl, Mary Sano, Current Treatment and Recent Clinical Research in Alzheimer's Disease, Mount Sinai Journal of Medicine, 2010; 77: 3–16.

5. Hanna Kaduszkiewicz, Thomas Zimmermann, et al. Cholinesterase inhibitors for patients with Alzheimer's disease: systematic review of randomised clinical trials. Department of Primary Medical Care, Center of Psychosocial Medicine, University Medical Center Hamburg-Eppendorf, Martinistraße 52, D-20246 Hamburg, Germany.
6. M-M. Mesulam, E. J. Mufson, Atlas of Cholinergic Neurons in the forebrain and upper brainstem of the macaque based on monoclonal choline acetyltransferase immunohistochemistry and acetylcholinesterase histochemistry, neuroscience, 1984; 12(3): 669-686.
7. Patrick Cras, Mitsuru Kawai, et al. Senile plaque neurites in Alzheimer disease accumulate amyloid precursor protein, Proc. Nadl. Acad. Sci, 1991; USA, 88: 7552-7556.
8. Sermakkani M, V. Thangapandian, GC-MS Analysis of Cassia Italica Leaf Methanol Extract, Asian Journal of Pharmaceutical and Clinical Research, 2012; 5(2).
9. Jayanth A, Guruprasad R, Assay of Acetylcholine esterase enzyme activity by titrimetric method,Int J Pharm Bio Sci, 2014 Oct; 5(4): (B) 643–647.
10. Sussman, Joel L;Harel, Michal; et al. Atomic Structure of Acetylcholinesterase from Torpedo californica: A prototype of acetylcholine-binding protein Aug, 1991; 23: 253, 5022; Agricultural & Environmental Science Database, 872.
11. Hermona Soreq, Shlomo Seidman. Acetyl cholinesterase — new roles for an old actor.
12. Shreyas Meda, Praveen A, Suvratha J, Anmol Kulkarni, Guruprasad R. Extraction of Acetylcholine Esterase Inhibitors from JACARANDA ACUTIFOLIA and screening of its activity on zebrafish brain. World journal of pharmacy and pharmaceutical sciences, 2014; 3(10): 1087-1093.
13. Agneta Nordberg, Anne-Lie Svensson, Cholinesterase Inhibitors in theTreatment of Alzheimer's Disease, Drug Safety, 1998.
14. Guruprasad R, Deepikaa VGS, Devika M, Lavanya K, DivyA M, Zebrafish as a model for hyperpigmentstion using Mangniferaindica, World journal of pharmacy and pharmaceutical sciences, 2015; 5(1): 707-718.
15. Elio Scarpini, Philip Scheltens, and Howard Feldman, Treatment of Alzheimer's disease: current status and new perspectives, THE LANCET Neurology, 2003; 2.
16. A.J.M. Difran, Abhinaya H.V., Sanchi Verma, Sanju Sampath, Umme Umaima, Swetha B.N. and Guruprasad R. Extraction of tyrosine ammonia lyase(TAL) from murrayakoenigii plant for the treatment for the hyperpigmentation- involves in-vitro analysis on zebrafish embryos and molecular docking. World journal of pharmacy and pharmaceutical sciences, 2016; 5(4): 1769-1780.

17. W. W. Chanaka Prasad, M. P. ManujaViraj, Roja Philip, Jyothi Rani, Swetha. BN. Guru Prasad R. Extraction of Acetylcholine Esterase Inhibitors from Plumeria Pudica and analysing its activity on zebrafish brain, World journal of pharmacy and pharmaceutical sciences, 2016; 5(4): 1781-1791.
18. Neda Adibpour, Fakher Rahim, SaeidRezaeei, Ali Khalaj and Amar Ebrahimi,In silico designing selective inhibitor of drugs, medicinal plants compounds and experimental ligands for pteridinereductase targeting visceral leishmaniasis, African Journal of Microbiology Research, 2012; 6(5): 917-926.
19. Carmen W. Huie. A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants. Anal Bioanal Chem, 2002; 373: 23–30.
20. P. Yamuna, P. Abirami, M. Sharmila, P. Vijayashalini, GC-MS analysis of bioactive compounds in the entire parts of ethanolic extract of Gomphrena globosa Linn. International Journal of Research in Pharmacy and Pharmaceutical Sciences, 2017; 2(4): 57-64.
21. Francisca Alcaraz-Pérez, VictorianoMulero and María L Cayuela, Application of the dual-luciferase reporter assay to the analysis of promoter activity in Zebrafish embryos, BMC Biotechnology, 2008; 8: 81.
22. Pyati UJ, Look AT, Hammerschmidt M: Zebrafish as a powerfulvertebrate model system for in vivo studies of cell death. *Sem Cancer Biol*, 2007; 17: 154-165.
23. Nigel J. Cairnsh, i, Robert C. Green,et al. The Alzheimer's Disease Neuroimaging Initiative: A review of papers published since its inception, Alzheimer's & Dementia, 2013; 9: e111–e194.
24. Mary Sano, Christopher Ernesto, et al. A controlled trial of Selegiline, Alpha – Tocopherol, or both as a treatment for Alzheimers's disease, The New England Journal of Medicine, 1997.
25. Reena S. Shah, Hyoung-Gon Lee, et al. Current approaches in the treatment of Alzheimer's disease, Biomedicine & Pharmacotherapy, 2008; 62: 199e207.
26. William E. Klunk, Henry Engler, Imaging Brain Amyloid in Alzheimer's Disease with Pittsburgh Compound-B, Annals of Neurology, 2004; 55(3).