1. INTRODUCTION

1.1. Alzheimer’s disease (AD)
Alzheimer’s disease is a chronic, slowly progressive neurodegenerative disorder and most common cause of dementia in grey population which upon progression leads to deterioration of intellectual capacity in various forms such as learning, language ability, memory, reading, writing, praxis and also interaction with the environment. Even many changes in personality of an individual can also be observed in the early stages such as behavioural disturbances and in the later stages of AD biological functions and also neurological alterations are also developed (Tariot, 1994). AD is a neurodegenerative age related disease observed most commonly among the elderly over the age of 65 (Cummings et al., 2004). Due to its debilitating nature, an enormous social and economic burden is placed on the society. Reports indicated that addition of 4.6 million new cases of AD every year worldwide. According to World Health Organization (WHO) estimation, 71% of 81.1 million dementia cases will be reported by 2040 (Ferri et al., 2005; Kalaria et al., 2008).

1.2. History of AD
AD came into light after a German physician “Alois Alzheimer” practicing in Asylum state at Frankfurt. He investigated the case of Auguste D, a 51 year old lady in 1901 who was suffering from symptoms of cognition, language deficits, delusions, paranoia, aggressive behaviour, auditory hallucinations. After the death of her in 1906 he worked with Emil Kraepelin and performed post-mortem studies of the brain and came with the findings that her brain showed conditions of arteriosclerosis changes, senile plaques and neurofibrillary tangles and these important pathological features were published in the year 1907 (Alzheimer., 1907). After this in the same year Kraepelin coined “Alzheimer’s disease” which is most commonly used for referring senile dementia.

There are several factors which are involved for the progression of AD and any one of these factors leads to the worsening of patient condition (Fig 1.1).
Fig 1.1 Figure representing several causative factors responsible for progression of AD. (Singh et al., 2013)

Fig 1.2. Picture explaining the mechanistic pathway of cholinesterase inhibition at the juncture of pre and post synaptic cholinergic neurons (http://mybiochemology.wordpress.com/page/2/)
1.3. Different hypothesis in AD
The exact reason for the pathogenesis of AD is not well defined but still different hypothesis were put forward on basis of various factors responsible for AD which are as follows

3.1. Amyloid hypothesis
3.2. Tau hypothesis
3.3. Calcium hypothesis
3.4. Isoprenoid change and
3.5. Cholinergic hypothesis

1.3.1. Amyloid hypothesis
The histological observation of a person with AD indicated the presence of plaques. The study on these objects revealed that the building block of amyloidogenic peptide was found to be amyloid beta protein, which is responsible for the formation of amyloid fibrils in the neuritic plaques. This hypothesis considers that due to abnormal processing and functioning of beta amyloid leads to formation of plaques (Glenner and Wong 1984; Hardy and Selkoe 2002). A mis-folded of amyloid beta which is an oligomeric species either in the form of star or toroid deposited in the brain may lead to apoptosis by physically piercing of the cell membrane. Deposition of amyloid plaques or partially aggregated soluble beta amyloid leads to further damage which in-turn starts up with neurotoxic cascade and leads to neuro degeneration resulting in AD (Hardy and Selkoe 2002; Selkoe et al., 2003). Early steps involve oxidative imbalance, oxidative stress, functional changes in beta amyloid (Terry, 1963). Abnormal metabolism of beta amyloids leads to the mutations in APP, ApoE4, presenilin-1, presenilin-2 and SORL 1 for the autosomal dominant onset of early AD (Berkis et al., 2010; Liu et al., 2009). Hence the amyloid cascade hypothesis became popular in 1990’s and became a powerful driving source for directing research.

1.3.2. Tau Hypothesis
The tau hypothesis came into light after the findings of two renowned scientists J.P. Brion and Andre Delacourte who investigated the main component present in the neurofibrillary tangles (Crespo-Biel et al., 2012). Later in 1998, Michel Goedert and co-workers cloned the cDNA of PHF-tau protein. The Tau proteins available in the neurons of CNS stabilize the microtubules and hyperphospholated tau starts to couple
with the other threads of tau protein finally forming neurofibrillary tangles inside the nerve cell bodies (Goedert et al., 1991). The formed neurofibrillary tangles lead to the disintegration of microtubules thereby collapsing the neuron transport chain (Li et al., 2005). This results in malfunctions of biochemical communications and later in death of cells (Chun and Johnson, 2007). This theory is one expected reason for plaques in brain.

1.3.3. Calcium Hypothesis

The role of activation of amyloidogenic pathway in remodelling of Ca^{2+} signalling pathways leads to cognition explored by calcium hypothesis of AD. The hydrolysis of APP resulted in two products that can influence Ca^{2+} signalling. The amyloid released outside to form oligomers that increase the entry of Ca^{2+} and pumped into endoplasmic reticulum thereby enhancing the sensitivity of ryanodine receptors to enhance the amount of Ca^{2+} release from the internal stores. Secondly the APP intracellular domain alters the expression of key signalling components. This remodelling of Ca^{2+} signalling leads to the deficits in learning and memory that occur in the early stages of AD. Ca^{2+} have the capacity either to increase or decrease the glutamatergic synapses which complicates the learning mechanistic activity coordinated by the neuronal calcium signalling systems (Bojarski et al., 2008; Green and LaFerla 2008). The up regulation of Ca^{2+} may result in progressive decline in memory and increased neuronal cell apoptosis that prevails during AD. Due to the changes in Ca^{2+} signalling in AD it may switch from system of memory storage to memory loss.

1.3.4. Isoprenoid Hypothesis

Isoprenoid levels change in AD compared to healthy brain. In the process of normal aging, human brain shows increase in the levels of dolichol and decrease in levels of ubiquinone but with no further change in cholesterol and dolichyl sulphate concentrations. Whereas in AD the levels of dolichol are decreased and levels of ubiquinone, dolichyl phosphate were increased and the levels of cholesterol was unchanged. Increased sugar carrier, dolichyl phosphate reflects an increased rate of glycosylation in AD brain and increase in endogenous antioxidant ubiquinone protects the brain from oxidative stress such as lipid peroxidation (Edlund et al., 1994).
1.3.5. Cholinesterases Hypothesis

The degeneration of neurons in the CNS leads to loss of memory functions. The finding of Peter Davies in the year 1976 was a landmark for drawing a relation between clinical symptoms of the disease and specific cholinergic deficits in AD brain where the finding opened a new door to neurochemistry (Katzman and Bick 2000). This hypothesis mainly relates to the deficiency of the neurotransmitter, Acetylcholine (ACh) which is caused either due to decreased production of neurotransmitter or increased production of AchE levels in the cerebral cortex of brain (Lahiri et al., 2002; Arce et al., 2009). The decreased levels of neurotransmitter lead to impairment of cholinergic neurotransmission which in turn disrupts the loss of cognitive functions (Fig 1.2). The AchE inhibitors mechanism is to increase the neurotransmitter Ach availability by inhibition of the degradation enzyme AchE. The clinical studies provide sufficient evidence that the AchE inhibitors provide improvement in cognition and global measurers relevant to dementia (Rosler et al., 1999; Persson et al., 2009; Winblad et al., 2001; Almkvist et al., 2004; Wilcock et al., 2000; Raskind et al., 2004). This hypothesis describes that the cholinergic augmentation leads to improved cognitive functions in AD. The therapeutic approach for treating cognitive loss in AD is to enhance cholinergic system using nicotinic, muscarinic receptor ligands or acetylcholinesterase (AchE)/ butyrylcholinesterase (BuchE) inhibitors (Terry et al., 2003). In a healthy human brain AchE plays an important role in regulation of acetylcholine enzyme though BuchE plays a minor role it has significant activity hence BuchE is also equally important.

1.3.5.1. Advantages of cholinesterase hypothesis

Cholinesterase inhibitors were well known for improving the cognition, behaviour, activities of daily living and global functioning in mid to moderate AD. They were also beneficial for care givers in decreasing their stress and nursing time. Meta-analysis of cholinesterase inhibitors indicated that inspite of high drop-outs and side effects than placebo they confer modest but significant therapeuic benefits to patients of AD and there is increasing evidence to support the therapeutic benefits in treating moderate to severe AD (Thompson et al., 2004).
1.4. Therapeutic options for AD
Currently the only treatment option approved by US Food and Drug Administration (FDA) for AD was cholinesterases inhibitors. Out of all the approaches, enhancing the cholinergic functions by using drugs that inhibit cholinesterases activity is the first therapeutic option in AD treatment particularly in mild to moderate stages (Ho et al., 2010). The cholinesterase inhibitors such as donepezil, Rivastigmine, tacrine and galantamine are currently licensed for treatment of AD. These agents are also used in other types of dementia mostly in vascular dementia and lewy body dementia. All the drugs mentioned above inhibit AchE and few drugs even inhibit BuchE (rivastigmine) (Howes and Perry, 2011).

Whereas Memantine, a glutamate NMDA receptor antagonist is the only non-cholinesterase inhibitor approved for treating AD. All the drugs listed out are known to show clinical improvement in placebo however these drugs are only able to hinder the disease progression but with evidence that cholinesterase inhibitors are beneficial in treatment level but there are some case where based on the individual response the beneficial effects (Muñoz-Torrero., 2008) or cholinergic dysfunction (Ballard et al., 2007) at an early stage of dementia which implies the importance of early treatment with cholinesterase inhibitors. There are some common side-effects reported for these drugs which include gastrointestinal discomfort (Diniz et al., 2009). However there are still several drugs in research from natural sources which can act as cholinesterases inhibitors.

1.5. Cholinesterases Inhibition
1.5.1. Cholinesterases in normal brain
In a healthy human brain AchE plays an important role in regulation of acetylcholine enzyme though BuchE plays a minor role it has significant activity. Butyrylcholine is not a physiological substrate in brain but in contrast it is a synthetic compound and used as a substrate to differentiate in between two types of cholinesterases. This is the key reason why BuchE cannot be distinguished from AchE and interpreted in brain. In a healthy human brain both AchE and BuchE were found in glia, neurons as well as plaques and in tangles of diseased brain (Wright et al., 1993).
1.5.2. Cholinesterases in diseased brain

The role of BuchE actually comes into play when AchE levels decline and the ratio increases from 0.6 (healthy brain) to 11 (diseased brain) in the cortical areas of the brain. Generally in the diseased brain the activity of AchE decreases slowly in mild to severe stages of AD and almost and resulting in only 10-15% of normal values in certain parts of brain. At a closer view at the glial cell protoplasm and synaptic gap, the extracellular diffusion of Ach come into contact with the glial BuchE and effectively hydrolyses by means of intracerebral micro dialysis in rodent’s cortex (Cuadra et al., 1994; Giacobini et al., 1996). BuchE activity is not altered and in contrast to AchE the levels were increased to about 120% (Perry et al., 1978).

Pharmacological treatment of AD is based on the use of AchE inhibitors (currently approved by US-FDA for AD treatment) which have beneficial effects on cognitive, functional and behavioural symptoms of the disease. Hence AchE and BuchE therapeutic targets can be used for improving cholinergic deficit (Greig at al., 2002).

1.6. Significance of BuchE inhibition in AD patients

Most of the BuchE is present in the neuritic plaques along with AchE. A drug which inhibits BuchE theoretically will also reduce the formation of beta amyloid plaques in AD on basis of assumption that both AchE and BuchE binding sites coincide (Inestrosa et al., 2000). First clinical trial attempt was made on the BuchE inhibitor, ethopropazine which supports the new therapeutic approach (Darvesh et al., 2001). Later development of selective inhibitors for BuchE such as cymserine (15 fold) bisnorcymserine (110 fold) and phenenthylcymserine (5000 fold) (Giacobini et al., 1996; Lahiri et al., 2002) research exhibited the increased levels (represented above in parenthesis) of AchE in rodents brain when measured by means of in vivo micro dialysis method. However with the hypothesis of micro dialysis experiment in rats (Giacobini et al., 1996) BuchE represents complementary enzymatic pool to AchE and a mechanistic pathway for regulation of Ach levels in the cholinergic synapses of CNS within particular conditions such as in transgenic mice nullizygote for AChE (Mesulam et al., 2002) or in AD (Mesulam and Geula 1994). These hypothesis were supported by the findings of Giacobini et al., (1996) describing the selective effects of BuchE inhibition in cortical levels of Ach and correlation between the BuchE inhibition and the CSF cognitive functions of AD patients (Giacobini, 2002). A strong argument in favour of a synaptic role of BuchE is the finding of the hydrolysis of
ACh in the brain of transgenic animals lacking AchE (Mesulam and Geula 1994). A consequence of such findings is the possibility of utilizing dual action cholinesterases inhibitors representing a new and attractive alternative in AD therapy.

1.7. Siddha system of medicine
Plants used in Siddha system of medicine (SSM) are having potent efficacy for CNS related disorders which were not revealed due to the lack of scripts (in a language other than Tamil) and thorough exploration, due to which the system lacks scientific evidence for herbs having potent activities. In contrast, great extent of studies was done on various herbs and formulations of Ayurveda (Vasudevan et al., 2007; Bhattacharya and Kumar, 1995). Several herbs from different alternative systems of medicine world-wide were screened for the identification of better lead molecules for AD (Ferreira et al., 2006; Oh et al., 2004; Gholamhoseinian et al., 2009). Hence in the current research herbs used in SSM for treating CNS related disorders were selected for the study.

1.8. Drugs from natural sources
Drugs in the current market are either directly or indirectly derived from herbal sources were recognised as an outstanding source for cholinesterase inhibitors such as huperzine, galantamine, tacrine, rivastigmine, donepezil etc. But these drugs exhibit limitations such as low bioavailability, shorter half-life, pharmacokinetic parameters, permeability through BBB and hepatotoxicity (Mukherjee et al., 2007). Some of the drugs derived from natural source are given in table 1.1 as shown below which gives the glimpse of the molecule along with structure of the compound and its derivatives.

1.8.1. Side-effects of current therapy
AchE inhibitors such as donepezil and galantamine have drawbacks of severe peripheral and central side effects such as gastrointestinal disturbances, cardiorespiratory, extrapyramidal, sleep disturbances, genitourinary and musculoskeletal problems (Thompson et al., 2004).

Due to the side effects caused by licensed drugs it is necessary to investigate safer AchE and BuchE inhibitors from natural sources. Due to these limitations search for new drug candidates for AD from herbal sources was still in demand because they
were not only effective for treating AD but also effective in the treatment of other forms of dementia like vascular dementia, lewy body dementia and Down’s syndrome etc (Erkinjuntti et al., 2002).

Therefore ethnopharmacological screening of plants having traditional claim may provide useful insights in the discovery process of new drugs for AD treatment i.e. by inhibition of AchE and BuchE activity thereby enhancing neuronal protection and delaying progress of AD.
### Table 1.1. List of compounds derived from natural sources for the treatment of AD and related CNS disorders.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Nature of Compound</th>
<th>Source</th>
<th>Indications</th>
<th>Structure of compound and its derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galantamine</td>
<td>Alkaloid</td>
<td><em>Galanthus woronowii</em> (Amaryllidaceae)</td>
<td>Mild to moderate dementia in AD (Ellis <em>et al.</em>, 2009)</td>
<td><img src="image1" alt="Galantamine" /> <img src="image2" alt="Ungereimine" /> <img src="image3" alt="Lycorine" /></td>
</tr>
<tr>
<td>Physostigmine</td>
<td>Pyrrolidine alkaloid</td>
<td><em>Physostigma venenosum</em></td>
<td>Reverses scopolamine induced memory impairment (Houghton <em>et al.</em>, 2006).</td>
<td><img src="image4" alt="Physostigmine" /> <img src="image5" alt="Rivastigmine" /></td>
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<tr>
<td>Huperizines</td>
<td>Quinolizidine alkaloid</td>
<td><em>Huperzia serrata</em></td>
<td>Memory impairment (Ma <em>et al.</em>, 2007)</td>
<td><img src="image6" alt="Huperizine A" /> <img src="image7" alt="Huperizine B" /> <img src="image8" alt="Huperine Z" /></td>
</tr>
<tr>
<td><strong>Vinca derived alkaloids</strong></td>
<td><strong>Indole alkaloids</strong></td>
<td><strong>Vinca minor</strong></td>
<td>Neuroprotective against Aβ and decreases cytokine secretion by microglia <em>in vitro</em> (Romero <em>et al</em>., 2009).</td>
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<tr>
<td><strong>Phenolics</strong></td>
<td><strong>Phenolics</strong></td>
<td><strong>Cannabis sativa</strong></td>
<td>Inhibits hyper phosphorylation of tau in Aβ-stimulated neuronal cells (Esposito <em>et al</em>., 2006) and it acts as antipsychotic and anxiolytic (Janero <em>et al</em>., 2009).</td>
<td></td>
</tr>
<tr>
<td><strong>Polyphenols</strong></td>
<td><strong>Wine</strong></td>
<td></td>
<td>Oxidative stress and cognitive impairments (Kim <em>et al</em>., 2010; Kumar <em>et al</em>., 2007), improved memory in rats infused with human Aβ in the cerebral ventricles (Kim <em>et al</em>., 2010).</td>
<td></td>
</tr>
</tbody>
</table>
al., 2010) and reduced plaque formation in an AD transgenic mouse model (Karuppagounder et al., 2008).

*Curcuma longa* Neuroprotection from Aβ (Park et al., 2002; Kim et al., 2001) as a result of anti-oxidant effects. Reduces metal induced amyloid beta toxicity (Baum and Ng, 2004).