1. ABSTRACT :

Alzheimer's disease's, characterization is usually done by reduced levels in the brain of the acetylcholine (ACh) neurotransmitter which is, also one of the common neurodegenerative diseases. Treatment of this disease is tough clinically and is dependent mainly on increasing the cholinergic function by stimulating cholinergic receptors or increasing the availability of Acetylcholine, which is released into the synaptic cleft of nerves with help of agents which improve or restore the levels of ACh.

Acetylcholinesterase is the enzyme which breakdowns acetylcholine and its inhibition is considered for the treatment of Alzheimer's Disease (AD) as a promising strategy.

The following work combines a review of the literature on different species and families of medicinal plants which have been tested for inhibitory activity of Acetylcholinesterase. The listed plant species are potential inhibitors of cholinesterase and thus, may aid scientists in their study of naturally occuring products which may be important in the treatment of Alzheimer's Disease.

Inhibition therapy of cholinesterase serves as an approach for the treatment AD. Lots of acetylcholinesterase inhibitors are used for the indicative treatment of AD. These compounds are reported to have side effects which includes gastrointestinal disturbances.

This study was thus partialy aimed at finding *in vitro* AChEIs in plant medicines traditionally which may be used to treat or cure cognitive disorders, and to find out the role of the plants as potential and likely sources for advancement of freshly potent and safe herbal therapeutic agents of Alzheimer's Disease. AChE's Assay activity has a major role *in vitro* depiction of drugs which also includes potential treatments for Alzheimer's. Maximally and widely used method is based on Ellman's method. The effect on Acetylcholinestearse activity of exactly 50 extracts of 10 different herbal plants were assessed using micro-well plate assay for AChE activity and Ellman's assays.

Keywords: AD, Alzheimer's disease, ACh, micro-well plate AChE activity Assay

2. INTRODUCTION :

The elderly populace around the globe is rising clearly. Corresponding to this rise, however, is the possibility of decline in mental health or can be said as dementia. Alzheimer's disease is known to be the major reason for dementia in aged people. Amidst the continual rise of the aged population, the increase of AD is likely to keep rising. Alzheimer's disease patients show retrogression in mental functions making them confined and helpless to perform daily activities. Aged people are those commonly affected with Alzheimer's disease. However, research shows that it can also affect even people as young as forty years of age. The true nature of the Alzheimer's disease is yet to be found, developing of treatment drugs a complex endeavour, ironically. Lately, the loss or decrease of cholinergic function is the only evidentiary finding that is known responsible for decline in cognition (Sugimoto *et al*, 2002).

Countless medicinal plants are used in Traditional Arabic Palestinian Herbal Medicine (TAPHM). In this, these are used for the treatment and cure of various diseases, including improvement and betterment of memory, Alzheimer's disease and other different diseases (Ali-Shtayeh M, 2006). Nonetheless, the adoption of medicinal plants is typically based on urban tradition and not on the scientific knowledge.

Alzheimer's Disease is one of the most common term related to that of dementia which affects more than thirty five million people across the globe and this count is believed to touch 65.7 million by 2030 (Singhal *et al*, 2012). This is one of the most rampant neurodegenerative disease that leads to progressive loss of cognition and memory, and degradation of all possible intellectual functions (Ferreira *et al*, 2006). This disease has now become the fourth major reason behind death in the aged population (over sixty five years of age) due to different biochemical pathways (Abou-Donia *et al*, 2014). The number of individuals with Alzheimer's is expected to rise substantially in the next years as the pecentage of the population aged sixty five years or more rises sharply (Vinutha B, 2007).

Decrease in acetylcholine is considered to play a pivotal role in the memory & learning decomposition of Alzheimer's patients. Acetylcholine (Ach) is released at nerve endings in the form of neurotransmitter and is organic molecule. It is manufactured with the help of choline acetyltransferase which needs acetyl coenzyme-A and choline as its substrates for the formation of acetylcholine in specific cells also known as cholinergic neurons.

Neurotransmitter disruptions and deficient cholinergic functions are recognised among the disease causing features in central nervous system (CNS) disorders (Greenblatt H, 1999).

Many strategies are devised for improving cholinergic neurotransmission (Orhan I, 2004), despite the one strategy that has been best and successful till now, is the "cholinergic hypothesis", i.e., In this cholinergic receptors are stimulated or availability of Ach is enhanced and is released into the synaptic cleft of nerves by the inhibition of ACh hydrolysis by acetylcholinesterase (AChE) via using AChE inhibitors (Lahiri D, 2002). AChE is usually found in excitable tissues for example synaptic junctions, and is membrane-bound enzyme. The main role of this enzyme is that it terminates transmission of nerve impulse at the cholinergic synapses by rapidly hydrolysing the neurotransmitter Acetylcholine (Howes M, 2003). Therefore, Inhibitors, for example the drugs used in the Alzheimer's therapy, leads to a rise in the duration as well as concentration of action of the synaptic Acetylcholine (Heinrich M, 2004).

Paths to increase cholinergic role in Alzheimer's Disease includes the stimulation of cholinergic receptors or prolonges the Ach availability that is released into the neuronal synaptic cleft by the help of agents which work to restore the level of ACh via inhibiting both AChE as well as BChE. BChE, mainly linked to glial cells and precise neuronal pathways where there is cleaving of ACh in a manner similar to AChE to abort its physiological action. These findings, along with a statistically slower and less decline in the cognitive performance of patients of dementia having specific AChE & BChE polymorphisms that naturally lower activity of these enzymes, have targeted these enzymes as a new way to intercede in the progression of Alzheimer's (Loizzo *et al.*, 2009). Lately, Hodges (2006) showed that the AChE's inhibition holds a major role not only to increase brain's cholinergic transmission but also to decrease the amyloid aggregation and the production of the neurotoxic fibrils in Alzheimer's. Thus, inhibitors of AChE and BChE have become favourable alternatives in treatment of Alzheimer's (Orhan *et al.*, 2004). Current anticholinesterase drugs (example, tacrine, physostigmine, galantamine, donepezil and heptylphysostigmine) for the treatment of cognitive diseases are reported to have several alarming side effects such as hepatotoxicity, low bioavailability a narrow therapeutic window, short duration of biological action, adverse cholinergic side effects in the periphery and low bioavailability (Hung *et al.*, 2008; Sancheti *et al.*, 2009).

In classical medicinal practices, consisting TAPHM, herbal plants have been in use to increase cognitive function and to decrease other symptoms linked to AD (Mukherjee P, 2007). The search for medicinal plant derived AChEI's has paced in terms of the benefits of these drugs in the medication of AD and all other forms of dementia (Erkinjuntti T, 2002; Tang M,2013).

Different synthetic AChEI's drugs take their root from plant-derived compounds and are associated to a diversity of classes of structures and compounds. The bulk of these bioactive substances includes steroidal-, piperidine-, indole- and Amaryllidaceae alkaloids, coumarins, phenylpropanoids, glycosides and terpenoids (Mukherjee P, 2007). Since Alzheimer's is the fourth leading cause of death all over the world, has become a danger to health of public, new medicinal treatment strategies are based on herbal plants have become focused (Mohammed Saleem Ali-Shtayeh, 2014).

The AChE is an alluring target for the drug designing and for the mechanism – based inhibitors discovery because of its contribution in the hydrolytic breakdown of the Acetylcholine neurotransmitter. AChEI's are the most favourable approach to treat the Alzheimer's disease's cognitive symptoms (Kalauni S, 2002; Atta-Ur-Rahman, 2002). The first AChEI's specifically authorised for the treatment of AD was introduced in 1993 as Tacrine (1, 2, 3, 4, - tetrahydro – 9 – aminoacridine) (Whitehouse, 1993). Currently several AChEI's such as Galantamine (Scott et al,2000), Donepezil (Kelly, 1997), Rivastigmine (Gottwald et al, 1999) are accessible for the symptomatically treatment of patients with mild to moderate AD. Although AChEI's were the most widely used medication in AD treatment, some Dhivya et al, 2014 reported that AChEI's have dangerous side effects such as diarrhoea, fatigue, anorexia, nausea, muscle cramps as well as gastrointestinal, genitourinary, cardiorespiratory and sleep disturbances (Chattipakorn S, 2007). This led the researchers in finding new acetylcholinesterase inhibitors with improved bioavailability, higher efficacy and reduced side effects, mainly from natural and herbal sources.

Acetylcholinesterase Inhibitors from Plants: Plants are used for the medication of various of diseases from more than 3000 years. The biologically active natural and herbal substances may have AChEI's activity and thus, may be used as major compounds for the production of new drugs. The bioactive compounds from vegetables, fruits, and medicinal plants mainly includes steroidal-piperidine-alkaloids, furanocoumarins, indole, xanthones, flavonoids and diterpenes derivatives that play a major role in the slowing down many neurodegenerative and pathogenetic disorders such as AD (Houghton P *et al*, 2005; Wszelaki N *et al*, 2010; Rehman E, 2006). Regular consumption of fresh fruits & vegetable reported in prolonging of cognitive decline in later age. Various medicinal plants extracts have been reported to show AChEI activity (Dhivya *et al*, 2014).

3. REVIEW OF LITERATURE :

3.1 Alzheimer's disease (AD) :

Frequency of the Neurodegenerative diseases keeps on increasing with increasing age. Neurodegenerative disease is a common term for various conditions because of chronic breakdown and deterioration of neurons (Houghton and Howes, 2005). Alzheimer's disease is one of the prevalent neurodegenerative disease. Alzheimer's disease was named after its discoverer Alois Alzheimer in 1906 who is a Bavarian neuropsychiatrist (Hostettmann K, 2006).

Ageing is the main risk factor of a large number of neurodegenerative disorders such as Alzheimer`s disease and Parkinson`s disease. Approximately, 5% of people in age 65 years suffer from AD and the pervasiveness of this disease increases with advancing age from 19% to 30% after 75 years of age. Around 95% of AD represents a infrequent form and 5-10% represents known form. Alzheimer`s disease is neurodegenerative disorder which is characterised by cognitive failures, impairment of memory and by dramatic changes in behaviour. Symptoms of AD may include:

• loss of memory,
• difficulty in using the right words or understanding what people are saying,
• difficulty in performing usual routine tasks, and activities,
• problems with the language,
•change in personality and mood.

AD is the most wide-spread progressive neurological disorder in men as they proceed 65 years of age and it becomes a serious all-society problem in consequence of advancing of average age. Although the reasons of Alzheimer's disease are not yet known, most researchers agree that AD, like all other chronic conditions, probably develops because of result of varied factors rather than a single reason. Risk factors for AD are:

• age,
• gender,
• gene polymorphism,
• hypercholesterolemia,
• diabetes mellitus,

• stroke,

• brain injuries,

• education,

• alcohol and smoking.

There is no available effective treatment or a preventive therapy for Alzheimer's today and a definitive identification is yet done post mortem through the histopatological analysis of patient's brain (Hostettmann et al, 2010). The greatest risk factor for Alzheimer's disease is increasing age, but AD is not considered a normal part of ageing Alzheimer's Disease was prevalent in 2006 affecting 26.6 million people worldwide. Alzheimer's disease (AD) is a serious neurodegenerative disease which can not be cured with remedies developed up to date. But, this prevalence is going to quadruple itself by 2050, by which every 85th human will be living with this disease (Dvir *et al*, 2011). This disease primarily affects the elderly people and is a complex, progressive and neurodegenerative disease. AD is estimated to be a cause of dementia in 50-60 percent of people over 65 years of age. However, it is shown evidently that AD can affect individuals as oung as 40 years of age. As said earlier AD affects progressively, that is, the disease onsets with mild symptoms, but with time these symptoms become more severe until the patient loses its capacity to carry on with daily life activities. In AD, there is, progressive loss of neurons in the basal forebrain, which is the major source of cholinergic innervations of the neocortex and hippocampus. These changes include progressive and irreversible impairment of cognitive function, which results in mainly loss of memory, with both neurological and neuropsychiatric disorders (Hostettmann *et al*., 2006).

This disease along with declining cognitive functions, also declines non-cognitive functions. Classically, both short and long-term memory is deteriorated while language skills, concentration and attention may get affected. It results in impaired ability to learn as well as retain new skills as well as the loss of existing ones. Non-cognitive functions are used to describe problems such as depression, personality changes, delusions, agitation, and hallucinations. These factors have a significant impact on both, patient behaviour and on the quality of life for both patients and caregivers (Blumberg S, 1978).

Alzheimer's disease is characterised by a remarkable deficiency of cholinergic neuronal transmission which affects cholinergic neurons in the basal forebrain (Price *et al*, 1986; Kasa, 1997).

3.2 ACETYLCHOLINE :

Acetylcholine is first neurotransmitter that was discovered. Acetylcholine is an organic molecule released at the nerve endings as a neurotransmitter. Structure is shown in Figure:1 (made using chemsketch). It is produced by the synthetic enzyme choline acetyltransferase. It uses acetyl coenzyme-A and Choline as substrates for the production of Acetylcholine in specific cells known as cholinergic neurons (Personeni, 2001). Nicotinic ACh receptors (nAChRs) are identified as effector and binding proteins to accelerate chemical neurotransmission at ganglia, interneurons, neurons and the motor endplate. Muscarinic ACh receptors (mAChRs) are identified as effector and binding proteins to accelerate chemical neurotransmission at effector and neuronal organs such as glands, smooth muscle fibres and heart (Wessler, 2008). It is found at :

- all autonomic ganglia,
- at large number of autonomically innervated organs,
- at the neuromuscular junction,
- at a large number of synapses in the central nervous system.
- at preganglionic sympathetic and parasympathetic neurons in autonomic nervous system,
- at the adrenal medulla and serves as the neurotransmitter in all the parasympathetic innervated organs.
- at the sweat glands,
- at the piloerector muscle of the sympathetic autonomic nervous system.
- at the neuromuscular junction between the motor nerve and skeletal muscle in the peripheral nervous system.
- at interneurons in the central nervous system,
- and at a few important long-axon cholinergic pathways. (Mirjana B, 2013)

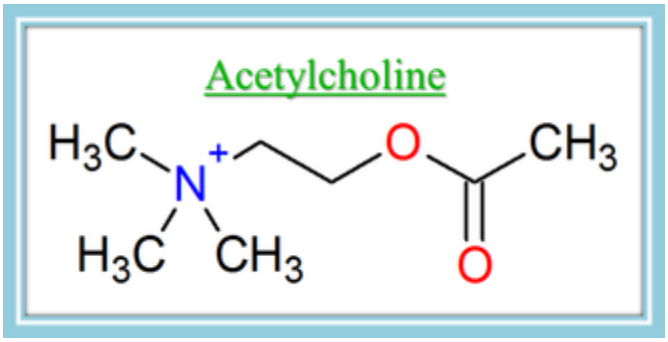


Figure1 : Image showing Acetylcholine structure.

3.3 CHOLINESTERASES :

Cholinesterase belongs to a family of enzymes which catalyzes the hydrolytic breakdown of the neurotransmitter acetylcholine into choline and acetic acid. This reaction is necessary as it permits cholinergic neuron to come back to its resting state after activation. It has following two types:

3.3.1 Acetylcholinesterase (AChE, acetycholine acetylhydrolase, E.C. 3.1.1.7) :

It is found in different kind of conducting tissues : muscle and nerve, peripheral and central tissues, sensory and motor fibers, and cholinergic and non-cholinergic fibers. It is a serine hydrolase and is present at cholinergic brain synaptic junctions AChE has higher activity in motor neurons as compared to sensory neurons (Irizarry and Hyman, 2001). AChE is also present in the red blood cell (RBC) membranes. Its function here is the constitution of the Yt blood group antigen. The enzyme is found in various molecular forms. These forms have similar catalytic properties but are known to differ in their oligomeric assembly as well as in mode of attachment to cell surface. Majority of AChE is present as a tetrameric, G4 form (Adewusi, 2010) in the mammalian brain, along with very less amounts of a monomeric G1 (4S) form Major function of enzyme Acetylcholinesterase (AChE) is neurotransmission. (Kyung *et al*, 2003)

3.3.2 Pseudocholinesterase (BuChE, EC 3.1.1.8) :

It is also called plasma cholinesterase, butyrylcholinesterase, or acylcholine acylhydrolase and is found mainly in the liver. It is different from AChE as BuChE hydrolyzes butyrylcholine more quickly than ACh (Huang *et al*, 2007)

3.4 Acetylcholinesterase :

AChE is a serine hydrolase usually found at cholinergic brain synapses and at neuromuscular junctions. It carries termination of impulse transmission at cholinergic synapses, which is its main biological role. It does so by rapid hydrolysis of the neurotransmitter Acetylcholine to acetate and choline. This enzyme is highly specific for a serine hydrolase mainly. It is discovered that each molecule of AChE degrades about 25000 molecules of ACh per second. (Chacho, 1960). Acetylocholinesterase breaks down Acetylcholine into into Acetic Acid and Choline as shown in figure 2 (Made using Chemsketch).

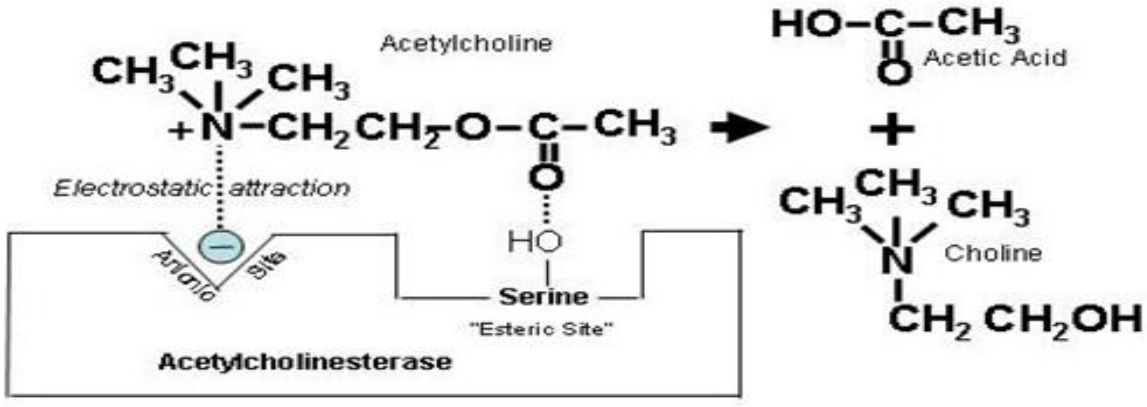


Figure :2 Breakdown of Acetylcholine with Acetylcholinesterase.

The shape of the molecule is ellipsoidal with dimensions ~ forty five Å by sixty Å by sixty five Å. The enzyme monomer contains 12 stranded mixed β sheet which is surrounde by 14 α helices, making it an α/β protein. The structure is deep and narrow gorge, ~ 20 Å long penetrating midway into the enzyme and widens out near its base, which is its outstanding feature (Ordentlich *et al,* 1995). The location of active site of AChE is four Å from the rock bottom of the molecule and includes two subsites - the ″anionic″ subsite and ″esteratic″

subsite correspond to the catalytic machinery and the choline-binding pocket (Malouf et al, 2004). The anionic subsite is uncharged and lipophilic and adheres the positive quartenary amine of choline moiety of Ach and both the quartenary ligands (edrophonium, N-methylacridinium) which act as competitive inhibitors (Pilger *et al*, 2006), and quartenary oximes which reactivate organophosphate-inhibited AChE effectively (Tang *et al*, 2011). The cationic substrates are bound by interaction of 14 aromatic residues that line the gorge which leads to the active site (Pisani *et al*, 2010). All 14 amino acids are conserved in different species (Catto *et al*, 2013). Among all the aromatic amino acids, tryptophan 84 is considered critical and on substituting with alanine, it causes a 3000-fold decrease, in the enzyme activity (Andersen *et al*, 2013) . ACh is hydrolyzed to acetate and choline at the esteratic subsite and contains the catalytic triad of three amino acids: serine 200, histidine 440 and glutamate 327, resembling the catalytical subsites of other serine hydrolases (Pehkonen S, 2002). Figure 3 shows 3D structure of Acetylcholinesterase (Gleenon *et al*, 1999).

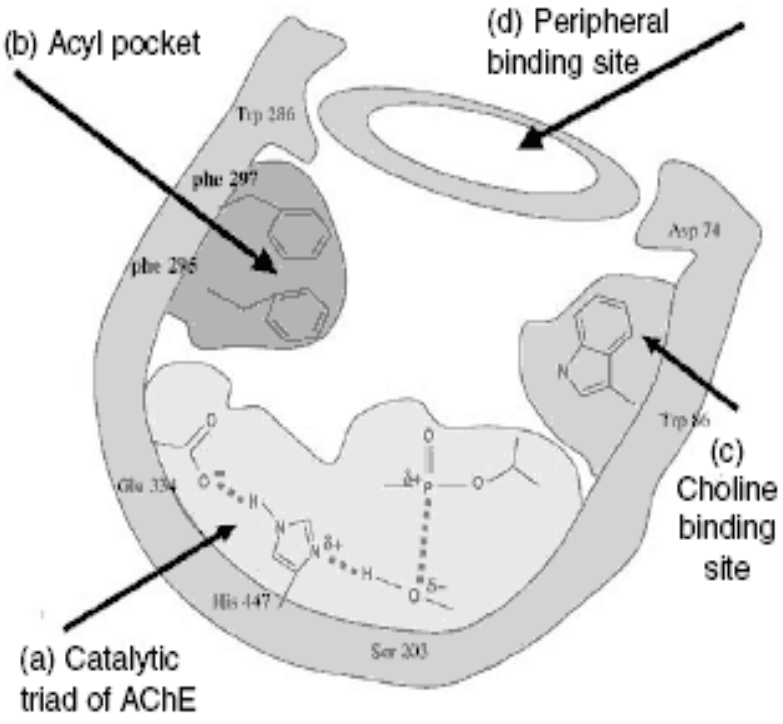


Figure 3:Image showing Acetylcholinesterase 3D Structure.

3.5 AChE & Alzheimer's disease :

As AD is posed by memory loss and cognition and the problem is its unknown etiology. Majority of the cases show isolated late onset. Clinically, Alzheimer's Disease is characterised by following three major events which are its pathological signs: neurofibrillary tangles, beta-amyloid plaques, and synaptic loss which is other name for AChE hypothesis. (Rabinovici et al, 2008).

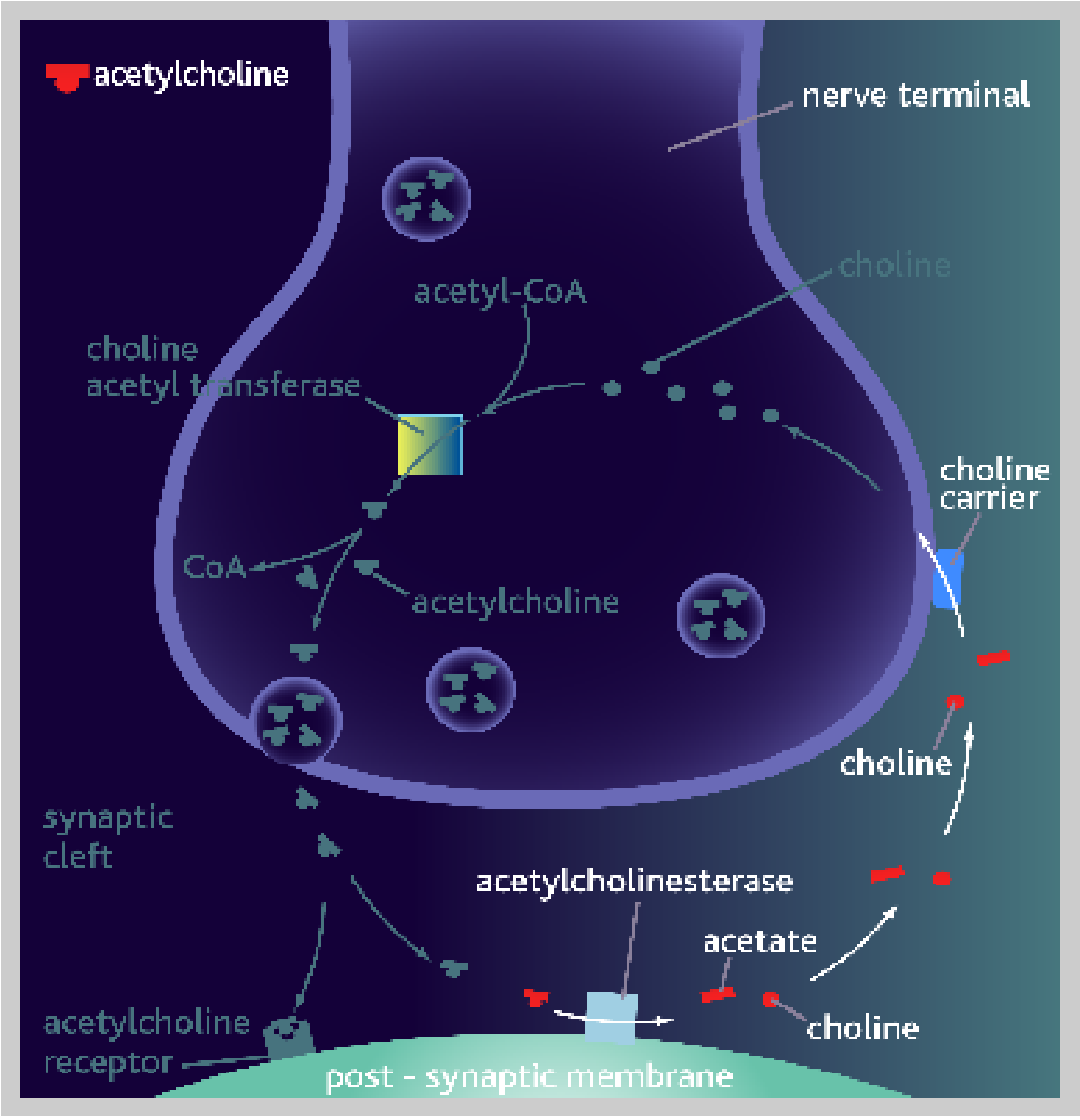


Figure 4:Image showing mechanism of AChE in neurotransmission.

Figure 4 shows the Mechanism of AChE (Dhivya *et al,* 2009) Cholinergic neurotransmission deficiency is known as one of the main reasons behind memory impairments in individuals. (Bennett *et al*, 2005). Cholinergic hypothetical role in memory of humans was conveyed in the early 1970s, by revealing that the scopolamine, which is cholinergic antagonist, impairs learning in human (Jicha *et al*, 2006). On the other hand, physostigmine the AChE inhibitor was demonstrated to elevate the long term memory. Along with that it was also demonstrated that post mortem Alzheimer's Disease brain tissue showed less cholinergic neuronal markers i.e choline acetyltransferase as well as AChE. These conclusions have led to the establishment of a "cholinergic hypothesis" that is Alzheimer's Disease is linked with impaired cholinergic transmission. (Neary, 1990), associating deformities in the cholinergic arrangement to sure pathological and functional changes in AD (Rabinovici *et al,* 2008).
Smith & Cuello established the importance of AChE in Alzheimer's Disease. They advocated that lesions occurring in dfferent cell groups have a common feature, and that feature is AChE. Senile plaques also had cholinesterases (Terry *et al*, 1990) even during the first stage of their production, and also in neurofibrillary tangles . AChE in tangles and plaques have a lower pH optimum was reported by Mesulam and co-workers, along with that they showed less sensitivity to the common inhibitors BW284c51, tacrine, and physostigmine On that basis,it is concluded that AChE has imperative role in the diagnosis, ethiology and therapy of Alzheimer's Disease (Mormino *et al*, 2009).

3.6 Diagnosis of AD :

In 1984, clinically diagnosing Alzheimer's Disease criteria was formulated by National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) along with Alzheimer's Disease and Related Disorders Association (ADRDA) workforce (Chacho *et al*, 1960). This benchmark established were then adopted all over the globe because of its sheer sense and usefulness and even after a quarter of a century it still is able to maintain its position. Last 27 years, however, have been intervening bringing advancement in our conceptualisation and understanding of Alzheimer's Disease, and also our capability has been improved in detection of the pathophysiological procedure of AD, and advancements in approach related to the clinical spectrum of the AD have appeared. During the formulation of criteria by NINCDS-ADRDA, it was assumed that Alzheimer's Disease just like all other

brain diseases will show a correlation between the underlying pathology and the clinical symptoms so as to conclude (i) Alzheimer's Pathology and symptoms proved clinically were similar, and (ii) Patients either were fully disordered with dementia due to complete AD development or they were not at all having this disease not showing any signs of dementia. Different symptoms related to Alzheimer's Disease pathology have been either entirely or bit different from the clinical symptoms. It has been established via clinical-autopsy correlation researches which demonstrate a much stronger correspondence has been established among cognitive impairment than between amyloid pathology and cognitive impairment and neurofibrillary pathology (Ordentlich *et al*, 1995). Neurodegeneration is the major facet of pathology of AD which is most closely linked to cognitive impairment, especially synaptic loss. Those who appear normal cognitively, 30 percent of those show amyloid. This "amyloid positivity" matches with the frequency of test conducted on those individuals with normal cognitivity with both cerebrospinal fluid assays (Malouf *et al*, 2004). Thus, by using these observations, researchers have made some interpretations to develop an ordered sequence for detection of AD positivity and its clinical interpretations(Pilger *et al*, 2001). Instead of the simultaneous development of neurodegenerative pathology, amyloid pathology in form of neurofibrilles and synaptic loss, they occur on different times depending upon the stage. For example, A-β pathology develops very first followed by development of neurofibrillary pathology which accelerates the appearances of the symptoms related to AD (Possamai *et al*, 2007).

Following is the criteria for the clinically diagnosing of AD:

- Establishment of dementia by clinical examination by Mini-Mental Test, or some other similar test and then is approved by neuropsychologist by conducting neuropsychological test. Failing in 2 or more cognitive areas account for the progressive deficits.
- Then the next step of diagnosis of AD is supported by aphasia, apraxia and agnosia which are deterioration of cognitive functions, motor skills and perception respectively. Defective activities of daily life, and changed behavioural patterns. Along with that history of family.
- Laboratory results including change in EEC and CT-scan and other tests that exclude all other possible reasons of dementia other than AD.

- Clinical criteria obtained from autopsy/biopsy should be different from other diseases and subtypes of AD e.g. trisomy-2I presence (Clifford *et al*, 2011)

3.7 Pathogenesis :

The cause for pathogenesis in Alzheimer's is yet to be totally decoded. But, environmental factors and genetic factors are accepted everywhere to be the reason behind triggering the AD Yet again, it is tricky to understand the mechanism of the disease, which may result in the much demanded cure for the AD (Dvir *et al*, 2011). For now, it is hypothesised that abnormal tau protein, beta amyloid protein or may be both of them play major roles in the inception of disease. It is also widely accepted that oxidative damage and a slow inflammatory process and oxidative stress and damage are two possible systems responsible. As for now, no treatment with known disease improving properties is feasible, and all the latest treatments may provide time being and symptomatic relief (Hostettmann *et al*, 2010).The evolution and expansion of acetylcholinesterase (AChE) inhibitors have led to the establishment that in AD, the cerebral cortex and basal forebrain suffer the malfunctioning of cholinergic pathways and this resulting defect in the cholinergic pathway leads to the cognitive impairment AD suffering patients. Other than Acetylcholine, other neurotransmitters are damaged in AD, and their relative importance with respect to clinical data has not been fully decoded.The only group of drugs currently licensed For AD treatment, only AChE inhibitors group of drugs have been licensed. These enzyme function by inhibition of the enzyme acetylcholinesterase (AChE), considered culpable for the breakdown of Acetyl Choline in the neuron synaptic cleft. (McGleenon *et al*, 1990)

3.8 Approved drugs for treatment & their side effects :

Major five anti dementia drugs are recognised by the United States Food and Drug Administration (US-FDA) for Alzheimer's disease (Agronin 2008, Folnegovic-Smalc *et al*. 2002; Mimica &Folnegovic-Smalc 2004). Three among them are acetylcholinesterase inhibitors allowed for the treatment and medication of mild to moderate Alzheimer's and one of them is used in treatment of all the three stages of Alzheimer's disease. The AChEI enhance the levels of ACh in the brain and they may enhance Acetylcholine levels in the peripheral area which causes the dangerous side effects which comprise of the enhanced gastric acid secretion, enhanced bronchial secretions and vagotonic effects on the heart which

can lead to exacerbate bradyarrhythmias, and the potential side effects of succinylcholine in anesthesia (Agronin, 2008). The prevailing gastrointestinal side effects (Müller & Fürstl, 2001) linked to cholinergic mechanisms include vomiting, anorexia, nausea, and diarrhoea (Yaari *et al*, 2008). The beginning of medication with AChEI should be averted in patients with active acute pulmonary disease, unstable bradycardia, peptic ulcer disease or congestive heart failure, and may be convenient when the latter medical conditions have been fixed. Precautions related to acetylcholinesterase inhibitors because of enhanced central and peripheral cholinergic stimulation.

3.9 Side effects of famous major allopathic Anti-Alzheimer's disease:

>Tacrine :

Tacrine is one of the reversible Acetylcholinesterase Inhibitors which was the first one allowed in 1992, for mild to moderate Alzheimer's disease. Figure 4 shows structure of Tacrine (Agronin, 2008). Because of inhibition of periphery of AChE and peripheral inhibition of BChE, tacrine may lead to side effects in gastrointestine. Central inhibition of Acetylcholinesterase can lead to weight loss, sleep disturbances, nausea and vomiting. Nausea, increased gastric acid secretion, dyspepsia diarrhea, vomiting, appetite loss, weight loss, myalgia, rhinitis and rash are eminent side effects. Alarming side effects includes rare seizures and liver toxicity. (Stahl, 2005). Tacrine is no more widely used as it induces elevations of liver transaminase which does not happen with the new generation AChEI (Agronin 2008). Because of the hepatotoxicity, tacrine is now of favor and is thus, no longer used (Yaari *et al*, 2008).

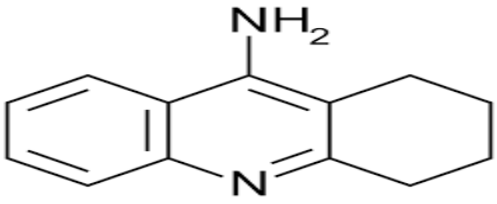


Figure 5:Structure of Tacrine

>Donepezil :

Donepezil is the also a reversible Acetylcholinesterase Inhibitor which is US-FDA accepted for treating mild to moderate and severe Alzheimer's. Figure 6 shows its structure (Agronin 2008). Donepezil is highly specific for AChE as compared to BChE (Jann & Small 2005).

AChE's peripheral inhibition may lead to gastrointestinal side effects (Stahl 2005, Uzun et al. 2005). AChE's central inhibition may cause nausea, weight loss, vomiting and sleep disturbances. Eminent side effects are nausea, , increased gastric acid secretion, weight loss, diarrhea, vomiting, appetite loss insomnia, dizziness, depression, muscle cramps, fatigue and abnormal dreams. Donepezil may lead to more disturbances in sleep than other cholinesterase inhibitors. To decrease insomnia, donepezil is advocated to be used during daytime. Severe side effects are rare syncope and rare seizuures.

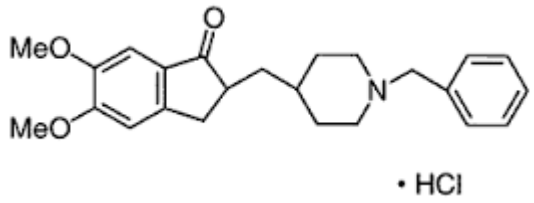


Figure 6:Structure of Donepezil

>**Rivastigmine :**

Rivastigmine is the third common Acetylcholinesterase Inhibitor and is again a reversible AChE and BChE inhibitor accepted from the US-FDA in 2000, for the medication of mild to moderately symptoms realted to AD (Jann & Small 2005, Agronin 2008). It has signalled treatment for dementia linked with Parkinson disease too. Figure 7 shows its structure (Agronin 2008). It is also considered a IInd generation AChEI type which is based on its various pharmacological profile as compared to both tacrine and donepezil. Rivastigmine does not lead to hepatotoxicity. Problems related gastrointestine can appear in a dose-dependent manner. Individuals with gastrointestinal illnesses or peptic ulcer disease should be carefully checked. Adverse effects were generally mild and transient and did not lead to major dropout rate during clinical tests. Major side effects are rare syncope and rare seizures.

Rivastigmine can lead to severe vomiting which may cause esophageal rupture which may appear if rivastigmine therapy is continued without retitrating the drug to full dosing (Stahl 2005).

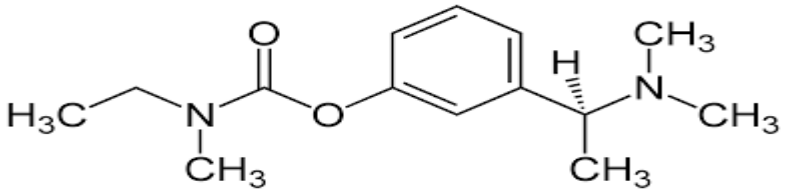


Figure 7:Structure of Rivastigmine

>Galantamine :

It is the again a reversible AChEI and is fourth in the list and figure 8 shows its structure (Agronin 2008). In 2001, it is accepted by the US-FDA for mild to moderate Alzheimer's disease (Jann & Small 2005). Peripheral inhibition of AChE can lead to gastrointestinal adverse effects (Stahl 2005, Uzun et al. 2005). Central inhibition of AChE may leas to vomiting, weight loss, nausea and sleep disturbances. Major side effects are increased gastric acid secretion, nausea, diarrhea, vomiting, appetite loss, weight loss, headache, fatigue, dizziness, depression. Alarming side effects are rare syncope and rare seizures. Negative events were more frequent during the course of treatment and also during the dosage titration from 16-24 mg/day and higher (Yaari et al. 2008).

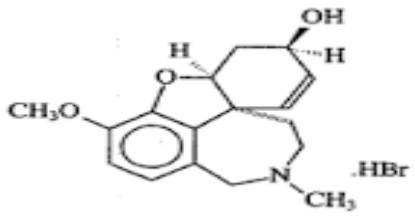


Figure 8:Structure of Galantamine.

>Memantine :

The Us-FDA accepted memantine in 2003, for the treatment and medication of moderate to severe Alzheimer's disease (Jann & Small 2005). Memantine is lately the only FDA - accepted glutamate - or NMDA-receptor antagonist for the medication of Alzheimer's. It is among one of two compounds approved for moderate to severe Alzheimer's. Its structure is given in figure 9 (Agronin 2008). Memantine occurs to be relatively well tolerated among these drugs (Lieberman & Tasman 2006). Major side effects are headache, dizziness and

constipation (Stahl 2005, Uzun et al. 2005). Whereas alarming adverse effects include rare seizures. Memantine is not advised for indivduals with severe renal damage (Jann & Small 2005).

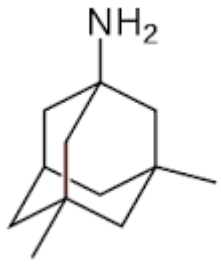


Figure 9:Memantine Structure

As these anti dementives drugs are not well tolerated and not suitable for all types of patients, it is imperative to move on to non allopathic and well tolerated plants. To find the cure for AD, new treatment strategies are focussed on medicinal plants.

3.10 Plants used :

- *Mentha longifolia*
- *Rosmarinus officinalis*
- *Salvia aucheri*
- *Alpinia galanga*
- *Moringa oleifera*
- *Malva silvestris*
- *Acorus calamus*
- *Carthamus tinctorius*
- *Jatropha curcas*
- *Bacopa monnieri*

3.10.1 *Mentha longifolia*

The *Mentha* species are used in food, cosmetic, beverage, confectionary and pharmaceutical industries (Iscan *et al*, 2002). Their essential oils are also used as antipruritic, antimicrobial, astringent, antiseptic and rubefacient. As mint, essential oils have medicinal values, their

biological and compositional activities are checked by many scientists (Gulluce *et al*, 2007). The flowers, leaves and stems of the *Mentha* species have been used as carminative, analgesic, antispasmodic, antiemetic, stimulant and emmenagogue in classical medicine worldwide (Iscan *et al*, 2002). Leaves of this plant have been also used as herbal tea and spice. The phytochemical findings on the *Mentha* species has lead to information that they have flavonoids and their phenolic compounds, triterpenoids, glycosides, steroids, and lignans. Multiple biological studies were also done on these species. Orhan et al. found the mutagenic and antimutagenic activities of luteolin derivatives obtained from *M. longifolia* subsp. *Longifolia* (Orhan, 2012). Linarin used the flower extract of *M. arvensis* produced results of selective dose dependent acetylcholinesterase inhibitory activity. As few of the clinical effects of medicinal and herbal plants are closely linked to their antioxidant activity, antioxidants use in diet and as supplements can decrease the incidence of chronic ailments such as cancer, diabetes mellitus and cardiovascular diseases. Along with that, the antioxidants can be also applicable in slowing down the progression of AD that is frequently seen among elderly population. Because of this, consumers are more interested in natural and herbal antioxidants to protect and improve their health. Otherwise, the defiance building by disease causing microorganisms against the antibiotics is a major health problem, and researches conducting researches on herbal plants have been enhanced to find new antibacterial agents (Oztruk *et al*, 2011). The aim of this study is the chemical compositions relevance with the antioxidant, anticholinesterase activities.

Eight known compounds consisting of two triterpenoids ursolic acid and uvaol (**2**), three steroids stigmast-5-ene-3-yl formate (**3**), stigmast-5-en-3-one (**4**) and sitosterol (**5**), two phenolic compounds bis (2-ethylhexyl) benzene-1,2-dicarboxylate (**6**) and hexacosyl (*E*)-ferulate (**7**), and a flavonoid 5-hydroxy-6,7,3',4'-tetramethoxy flavone (**8**), were isolated from the aerial parts of *M. longifolia* subsp. *noeana* (A.Ertas et al, 2012)

3.10.2 *Rosmarinus officinalis*

Rosmarinus officinalis common name is rosemary which is a perennial herb. In predictive animal models of antidepressant properties, it has produced a selective antidepressant-like effect. Also, the consequence of repeated acute administration of its extract was akin to the action as a result of the traditional antidepressant fluoxetine. In addition to that it was also shown that the antidepressant-like effect of this plant is dependent on its interaction with the

noradrenergic, serotonergic and dopaminergic systems. Few findings suggest that the olfactory properties of rosemary essential oil can lead to objective effects on increment of overall memory quality, cognitive performance and secondary memory factors (Machado *et al,* 2009).

R.officinalis studies which use animal models have also shown that memory production includes a series of biochemical reactions in many areas of the central nervous system, comprising the hippocampus. The biochemical events leading in memory production initially involves the activation of N-methyl-D-aspartate (NMDA) and metabotropic (mGluRs) glutamate receptors and thus the biochemical cascades in the neurons.

Rosmarinus officinalis is one of the accepted and commonly used plants in classical medicine. It is also defined by hepatoprotective as well as antioxidant activities which can be applied to its hydroxyphenolic components, including carnosol, rosmarinic acid and flavonoids. It has also reported that it have neuroprotective effects (Ozarowski *et al*, 2009).

Rosemary which is always known as a spice as well as medicinal herb associates to the Lamiaceae family and is receiving an enhanced attention because of its anti-inflammatory, antimicrobial, and antioxidative constituents.

Rosemary's plant material is of economical interest for its essential oil as well as its antioxidant substances. Carnosic acid and carnosol are proved to be the main phenolic diterpenes in rosemary's leaves. There is an enhanced interest in the phytochemicals as upcoming sources of antimicrobial agents and natural antioxidant. The popular rosemary plant belongs to the Mediterranean region; but, it has been cultured worldwide and approved as one of the spices with best antioxidant activity (Prediger *et al,* 2008).

Rosmarinus officinalis is classically used to enhance memory, linked with Alzheimer's disease and dementia, for familiar symptoms of old age, fatigue and debility. Lately, many studies proved that *R. officinalis* or its main chemicals like carnosic acid (CA), rosmarinic acid (RA) and luteolin (Lut) can be great candidates as alternate nerve growth factor (NGF). Also, *R. officinalis* phenolic substances are enriched with anti Alzheimer's, anti Parkinson's disease and anti amyotrophic lateral sclerosis (Pengelly *et al*, 2012)

3.10.3 *Salvia aucheri*

The genus *Salvia* is the biggest and the major aromatic and medicinal representative of the Lamiaceae family and is shown by more than 900 species throughout the world (Tenore *et al*, 2010). This word Salvia, is derived from the Latin salvare, which means "to heal or to be safe and unharmed" specifying the medicinal value of the plant. Salvia species have long been used in local medicine against colic, cough, flu, diarrhea, colds, stomach problems, tuberculosis, chronic bronchitis, rheumatism, bacterial infections, febrile attacks and sexual debility and in the medication of neuronal and mental conditions (Kamatou *et al* 2005, 2008). Few Salvia species are studied for its different biological and pharmacological properties, including antibacterial, anticholinesterase, antifungal, antioxidant, anti-inflammatory properties in differnt parts of the world. Few representatives of this genus are commercially important because of its use as spices as well as flavoring agents in the perfumery as well as cosmetics (Delamare *et al*, 2007)

Salvia officinalis extract has been indicated to produce major benefits in cognition to the individuals with mild to moderate Alzheimer's after 16 weeks of medication with *S. officinalis.* The studies have shown that the adverse effect linked with *S. officinalis* where similar to those felt with cholinesterase inhibitor. However, frequency of response was higher in placebo group which can prove an added advantage in the management of individual suffering with Alzheimer's disease (Kelen *et al*, 2008).

3.10.4 *Alpinia galanga*

Alpinia galanga is commonly called Greater galangal. *A. galanga* has been continuously searched towards diverse biological activities in majority of the cases in different *Alpinia* species. Lately, chloroform fraction of *A. galanga* is proved as antiamnesic apparently because of the presence of 1′*S*-1′-acetoxyeuginol acetate as lead compound. Its fruit was found to possess the neuroprotective activities and thus many other *Alpinia* species have been reported the same. Protocatechuic acid (PCA) is a major compound of the *A. oxyphylla*, shields against oxidative damage *in vitro* and decreases oxidative stress *in vivo*. It has also been proved that PCA also decreases the hydrogen peroxide or sodium nitroprusside induced apoptosis in PC12 cells in dose-depending manner and thus it offers a promising therapeutic strategy for the treatment of oxidative stress-induced neurodegenerative disease

like Parkinson's disease. Other evidences revealed that *A. katsumadai* seed extract shields neurons from ischaemic damage and the treatment mainly decreased microglia in the hippocampal CA1 region and the activation of astrocytes. Same way, methanolic extract of *A. officinarum* rhizome proved to be protecting against oxidative damage in PC 12 cells (Jaju *et al*, 2009).

3.10.5 *Moringa oleifera*

Moringa oleifera (MO) is a part of family. Its leaves and fruits possess anti-inflammatory and hypotensive effect and are consumed as food. It is found lately that leaf MO extract is not toxic even at increased concentration levels, increases memory via nootropics activity and gives substantial antioxidants like vitamin C and E to fight oxidative stress in Alzheimer's (Ganguly *et al*, 2008). Classified studied showed that monoamines listed in the memory loss are changed by Moringa oleifera leaf extracts . Various lines of proof also tell that colchicines-induced Alzheimer's disease can be ameliorated by ethanolic extract of Moringa oleifera by changing the brain monoamines (norepinephrine, dopamine, and serotonin) and electrical activity in a rat model. Researches on rats showed that the orally dosage of Trasina, a natural formulation, once everyday for 21 days can perfectly ameliorate colchicine induced effects like decreased frontal, cortical and hippocampal acetylcholine (Ach) concentrations, choline acetyltransferase activity, and muscarinic cholinergic receptor binding. Anwala churna is an Ayurvedic preparation which showed ameliorating the scopolamine induced amnesia in young and aged mice an major improvement in memory and brain cholinesterase activity (Mohan *et al*, 2005).*Moringa oleifera* (MO) leaves are great source of antioxidants and vitamins. They also possess fair amount of minerals, vitamin A, proteins, vitamin B complex, essential amino acids and a high content of vitamin E. Studies prove that these substances not only possess antioxidant property but also possess memory facilitating effect. (Ganguly *et al*, 2008).

3.10.6 *Malva sylvestris*

The *Malva sylvestris* is a herbaceous amd both a biennial– perennial plant. A number of local wild plants like *Malva sylvestris* are used in food and medicine both. Classical phytotherapy includes the treatment of inflammatory diseases, cough of mucous using this in Journal of Research in Agricultural Science as well as non specific dermatitis, membranes, stomach

ache, and sore throat (Kültür *et al*., 2007). The aqueous extractions are the most exemplary preparations. This plant is very demulcent and has meager nervine tonic properties (Lust, 1974). Antioxidants protect contrary to free radicals, such as ROS in the human body and are important components. Reactive oxygen species are the major degenerative diseases contributors responsible for aging and are the major factors leading to cancer. Antioxidants can be used as dietary, medicine or food supplements by humans. Currently, both in industry and scientific research in vegetables, fruits, spices and medicinal plants, there is an increasing interest because of their antimicrobial, phytochemicals and antioxidative properties. Above properties are because of many substances, including vitamins, terpenoides, flavonoids, minerals, phytochemicals, etc. (Scherrer *et al*., 2005).

3.10.7 *Acorus calamus*

Acorus calamus also known as Waan-Nam or "sweet flag", is a renowned medicinal plant which is used for hundreds of years in ayurvedic medicine. Its rhizomes are utilized in the Chinese, Indians and American Indians and also by other nativess (Motley, 1994). Roots and rhizomes of this plant are used in many diseases including many mental disorders, such as hysteria, neurasthenia, epilepsy, insanity, insomnia, melancholia diarrhoea and asthma (Mukherjee *et al*, 2007). Different pharmacological activities of this plant such as analgesic (Mukherjee *et al*, 2007), antispasmodic, anti-inflammatory antibacterial, antiulcer, anticonvulsant, antispasmodic, anti-inflammatory antibacterial, antiulcer and cytoprotective activity (Mukherjee *et al*, 2007) tranquilizer, anti-sczhizophrenia , anti-anxiety and CNS depressant activity, neuromodulatory effect in dopaminergic system are reported. Classically, the rhizomes and roots of *Acorus calamus* are in use in the Chinese and India systems of the medicine for many years for their benefitting role in improving learning, and also for their anti-aging effect. In vitro anticholinesterase and antioxidant activity of *A. calamus* showed earlier that with rhizomes & roots methanolic extracts using the rat brain homogenate (Faiyaz *et al*, 2009).Major constituent was BetaAsarone [(Z)-asarone] in the leaves (27.6 to 45.4%), whereas in the rhizomes acorenone was dominant (21.86%) followed by isocalamendiol (11.75%). Besides sequestrine ketones, Monoterpene hydrocarbons (trans- or Alpha) and eugenol Asarone (2,4,5-trimethoxy-1- propenylbenzene), and Beta-asarone (cis-isomer) were

also identified (Kindscher and Kelly, 1992).Sweet flag's rhizomes (Acorus calamus L.) are used widely in many ailments like epilepsy, mental ailments, abdominal tumors, kidney and liver troubles, chronic diarrhea, bronchial catarrh, intermittent fevers and glandular, dysentery, rheumatism, sinusitis and eczema. Also, it is used for an aid to digestion and appetite. This plant is a highly valued plant as it acts as a brain and nervous systems rejuvenator (Sharma *et al*, 2011) and is worldwide employed in modern herbal medicine due to its sedative, diuretic, laxative, and carminative properties. It also possesses insecticidal, anticholinesterase, spasmolytic tranquilizing, antidiarrhoeal, antidyslipidemic, neuroprotective, antifungal, antibacterial, antioxidant, and vascular modulator activities (Shah *et al*, 2010).

3.10.8 *Carthamus tinctorius*

Carthamus tinctorius or safflower are commonly known as Honghua in Chinese and is an biennial or annual herbal plant in the family of Compositae. The tubular flowers are red in color without ovary are generally picked in the summer & when the flowers' color changes from yellow to red, and then is dried in well-ventilated places and shady for the clinical usage (Zhang Y *et al*, 1998). Along with the enhanced extensiveness of studies on the Chinese Material Medica chemical constituents, phytochemical investigations are also conducted on safflower. Lately, more than 104 compounds of this plant are isolated and identified and they include quinochalones, polyacetylene, aromatic glucosides, flavonoids, alkaloids, organic acids, etc (Hiramatsu *et al*, 2009).

Modern pharmacological activities have showed that safflower that with its active substances possesses wide-reaching biological activities, modulating immune system, anticoagulation and antithrombosis, antioxidation, anti-aging, including dilating coronary artery, improving myocardial ischemia, antihypoxia, antifatigue, antitumor, antiinflammation, antihepatic fibrosis, analgesia, etc. Because of its classical use of CTL in the inhibition of cardiovascular disease, this study was thus performed on models of thrombosis in rats and was compared to the standard antiplatelet agent, aspirin (Wang Y *et al*, 2014).

3.10.9 *Jatropha curcas*

Jatropha curcas also known as physic nut is a small tree or drought resistant large shrub, which belongs to the genus Euphorbiaceae and produces oil containing seeds (Jongschaap *et al*, 2007). According to Hartwell, the extracts of this plant are used in folk remedies for cancer. Known to be abortifacient, emetic, hemostat, lactagogue, narcotic, anodyne, antiseptic, diuretic, purgative, stypt, rubefacient and vulnerary; it is a local remedy for alopecia, ascites, burns, convulsions, cough, dropsy, dysentery, dermatitis, diarrhea, dyspepsia, eczema, fever, incontinence, inflammation, gonorrhea, hernia, jaundice, neuralgia, paralysis, parturition, pneumonia, rash, rheumatism, sores, stomachache, syphilis, tetanus, scabies, sciatica, thrush, tumors, ulcers, uterosis, and yellow fever. Latex which is applied topically to wasp and bee stings. Mauritians massage ascitic limbs with the oil. Cameroon locals apply the leaf decoction in arthritis (Henning R, 2004). Colombians use the decoction of leaf for venereal disease. Bahamans drink the decoction of leaf for heartburn. Guatemalans use heated leaves by palcing on the breast as a lactagogue. Cubans use the latex by applying to toothache. Costa Ricans and columbians apply the latex to burns, ringworm, hemorrhoids, and ulcers. Seeds are used also for dropsy, gout, paralysis and skin ailments. Leaves are considered as antiparasitic, rubefacient for paralysis, applied to scabies, rheumatism; also applied to hard tumors. Latex is used for dressing of sores and ulcers and inflamed tongues. Seed is seen as aperient; the seed oil is emetic, purgative and for skin ailments; laxative, decoction of roots as a mouthwash for bleeding gums. ANS-There are reports of salivation, abdominal cramping & sweating occurring in human intoxications of Jatropha macrorhiza root. This suggests cholinergic activity of this plant (Achten *et al*, 2010).

3.10.10 *Bacopa monnieri*

Bacopa is a genus of 70 – 100 aquatic plants belonging to the family Plantaginacea. It is a small creeping succulent herb, whose habitat includes wetlands and muddy shores, so is very common in marshy places throughout India (Bhandari M., 1990) and also in other countries like Nepal, Sri Lanka, China, Taiwan, Vietnam, Florida and other southern states of the USA. *Bacopa Monnieri*, also known as brahmi, is a small succulent herb of Lamiales Order of Plant Kingdom. It usually grows in muddy areas, and hence very common in India. Plant is well known, for its medicinal properties as well as for its use in phytoremediation (Mehta *et al*,

2012). Medicinal nature of plant is due to production of vital secondary metabolites like alkaloids, flavonoids, saponins, terpenoids and steroids. Major metabolites are brahmine, bacosides A, A1 (Jain, 1993), A3 (Rastogi, 1994), B, bacosaponins, phytosterols. It has been observed that plant extract is a well-known braintonic (Stough, 2001), cardio-tonic, diuretic and blood purifier. It has been found to be effective against neurosis, Alzheimer's disease (Dhanasekaran, 2007), diabetes, cancer, etc. Studies have have proved its role in enhancing memory by increasing the retention power. Plant also enhances the antioxidant defences by increasing superoxide dismutase, catalase and glutathione peroxidise activity (Anbarsi, 2006). Recent research has proved its role as immunomodulator by augmenting both Th1 and Th2 cytokine production (Yamada, 2011). Neuroprotective effects of Brahmi appeared to be the results of its antioxidant property to suppress neuronal oxidative stress and the acetyl cholinesterase inhibitory activities, according to the recent study. Therefore, treating patients with brahmi extracts may be an alternative direction for treating neurodegenerative disorders. It is also shown that extract treatment of Brahmi reduces beta-amyloid levels in the brain of an Alzheimer's disease case and acts in inhibition of Acetylcholinesterase Enzyme (Krishnakumar, 2009).

4. METHODOLOGY :

4.1 Reagents & Chemicals :

- Dithiobis nitrobenzoic acid (DTNB)
- Acetylthiocholine iodide (ATCI)
- Tris–HCl with pH 8.0 was used as buffer.
- AChE enzyme is used in lyophilised form and was prepared in the buffer (Purchased from ThermoFisher life Sciences)

4.2 Plant Materials :

Dried form of the following plants is used for the inhibitory assay :

- *Mentha spicata*
- *Rosmarinus officinalis*
- *Salvia aucheri*
- *Alpinia galangal*
- *Moringa oleifera*
- *Malva silvestris*
- *Acorus calamus*
- *Carthamus tinctorius*
- *Jatropha curcas*
- *Bacopa monnieri*

Out of these *Jatropha curcas & Bacopa monnieri* were taken from Delhi Technological University and others were taken from Botanical garden of Jamia Hamdard University, Delhi.

4.3 Evaluation of AChE Inhibitory Activity Using Ellman's Method :

AChE activity's inhibition was measured with the help of a 96-well microplate reader which is based on Ellman's method. Mechanism is shown in Figure 10 (Ellman, 1961). The chemical reaction's principle is illustrated in following figure. This enzyme carries hydrolytic breakdown of the substrate ATCI to thiocholine and acetic acid. Thiocholine is then left to

react with DTNB, and this reaction leads to the development of a yellow color. The intensity of color of the product is measured at 405 nm, and it is directly proportional to the activity of enzyme.

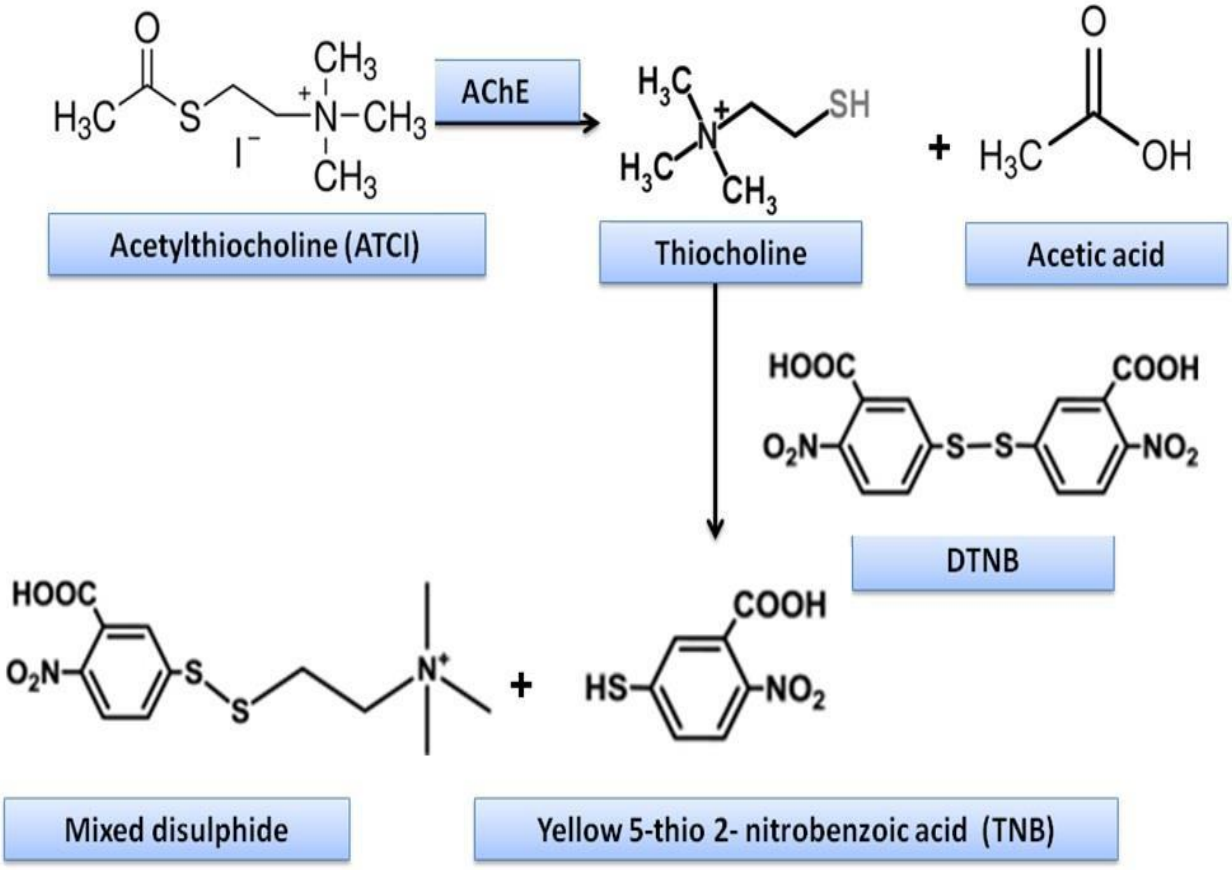


Figure10 :Chemical mechanism of Ellman's method

4.4 Sample preparation for AChE inhibitory activity assay :

Following steps were followed for sample preparation :-

- From listed plants, different plant parts were excised depending upon which part showed best anti-alzheimer's activity.
- After excision of the desired part, they were cut into small-sized pieces which is followed by thoroughly washing the part with sterilised double distilled water.

- These excised parts were then dried in an oven at 32 °C.
- The dried pieces of the plants were finely ground to a fine powder using mixer grinder.
- Exactly 15 g of dried powder of every sample was macerated separately, with HPLC (high performance liquid chromatography) grade methanol (75 ml) at room temperature for time perod of 24 hours.
- The extracts thus formed were obtained using filtration using Whatman No. 1 filter paper and were concentrated to dryness.
- Then, desired concentrations of methanolic extracts of each of the ten samples were prepared by dissolving completely the dried extract in methanol in desired ratio to have stock solution of strength 'x' mg/ml (Adewusi, 2010).

4.5 AChE inhibitory activity assay :

Procedure for the assay can be given as :-

- For each of the eight samples, five dilutions of 20, 40, 60, 80 and 100 µg/ml concentration were prepared.
- The assay for measuring Acetylcholinesterase inhibitory activity was improvised from the method described by Ellman *et al* (1961) and Ingkaninan *et al* (2000).
- Briefly, 4 µl of 3 mM dithiobis nitrobenzoic acid (DTNB), 20 µl of 15 mM acetylthiocholine iodide (ATCI), 130 µl Tris–HCl and 20 µl of each diluted sample was taken and added to the wells of microplate, followed by 50 µl of 0.28 U/ml AChE enzyme.
- The loaded microplate was then read at 405 nm every 5 s for 2 min by a CERES micoplate reader.
- Mean absorbance per minute (*A*) was calculated for every dilution of different samples. Each plate had one blank well and one control well also. Percentage of AChEI was given by concentration of sample and was calculated by using the formula:

$$\text{Per cent inhibition} = \left[\left(A_{\text{control}} - A_{\text{extract}}\right) / A_{\text{control}}\right] \times 100$$

- The percentage of inhibition obtained above was plotted on x-axis vs. corresponding concentration of the extract of each sample on y-axis.

- In total, there were three replicates for every diluted concentration. Also, the experiment was repeated for two more times.

5. RESULTS:

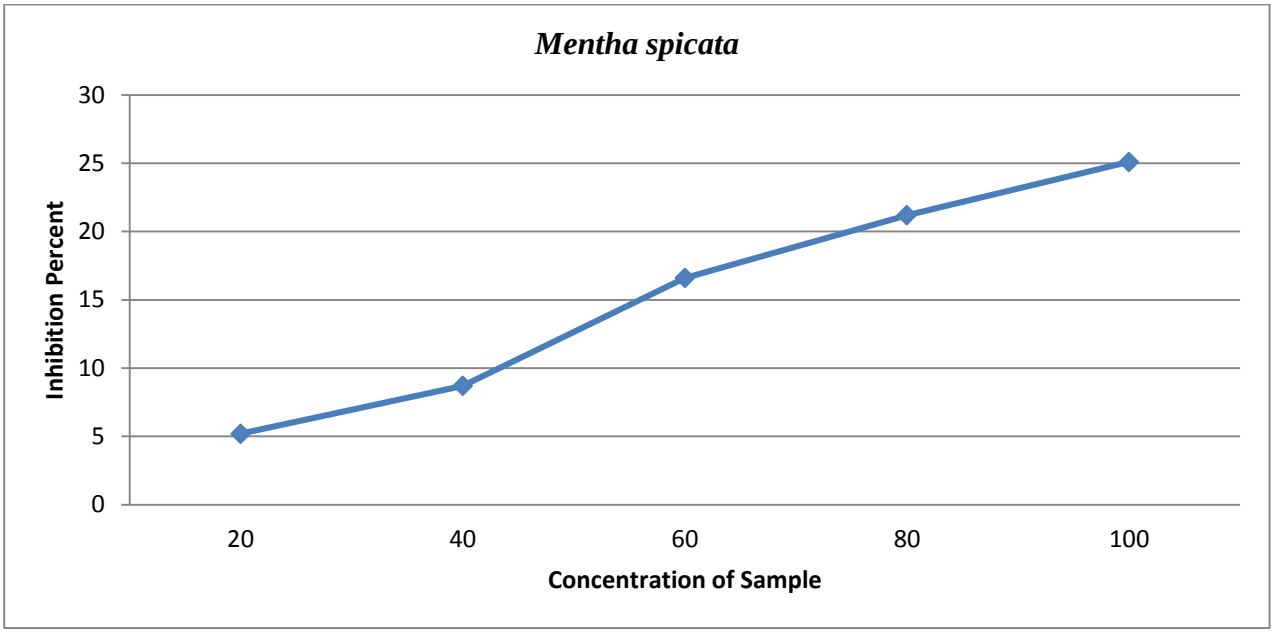
Ten plant species were selected based on their uses as remedies for the central nervous system diseases for human or to improve memory and cognitive function. The inhibition effect of the 50 different extracts on AChE activity was screened using the Ellman's method.
Different extract concentration is used of the selected ten samples which inhibited the AChE enzyme by different percentage, as is acknowledged by AChE inhibitory assay.
The inhibitions that are obtained with different samples can be categorised in following three groups: low (5–20 %), moderate (20–40%) and good (40–100 %) inhibition (Adsersen *et al*, 2006). Extracts of various medicinal plants are reported to show AChE inhibitory activity. AChE's Inhibition –it is the main enzyme in the breakdown of acetylcholine and is studied as one of the medication strategies against various neurological disorders including Alzheimer's disease. Various herbal plants have been tested have shown to have an inhibitory effect on Acetylcholinesterase.
As most of the AChE inhibitors are said to contain nitrogen, the heavy activity of plants which belong to these families can be because of their rich alkaloid content (Orhan et al., 2004). Extracts of plants which have activities where percentage inhibition of the enzyme is either 60% or more are thought to have strong inhibitory activity, while moderate activity refers to percentage inhibition between 20 to 40% (Adsersen *et al*, 2006) and extracts of plants which have percentage inhibition less than 20% do not exhibit any major inhibition of the enzyme.

A summary of inhibitory screening test studies of these plants is given in following Table 1,along with their family, contains with their scientific names, solvent extract, plant part, percentage inhibition and the concentration at which the enzyme is inhibited.

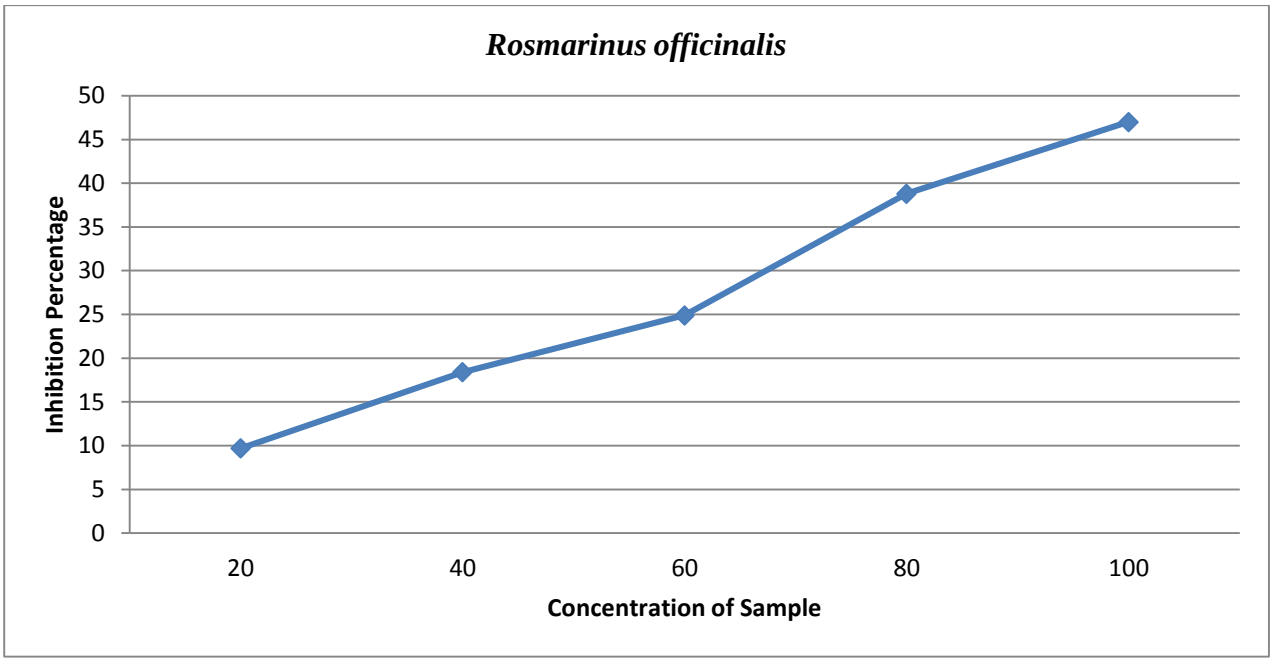
Botanical Name	**Parts used**	**Activity (%inhibition±SD)**	**Family**
Mentha spicata	Whole	25.0±0.00 (1mg/ml)	Lamiaceae
Rosmarinus officinalis	Whole	47.00±0.00 (0.1mg/ml)	Lamiaceae
Salvia aucheri	Whole	57.6±0.00 (1mg/ml)	Lamiaceae
Alpinia galanga	Rhizomes	36.98±0.37 (100µg/ml)	Zingiberaceae
Moringa oleifera	Bark	14.99±2.74 (100µg/ml)	Moringaceae
Malva sylvestris	Aerial parts	28.10±5.70 (5mg/ml)	Malvaceae
Acorus calamus	Whole	43.79±7.2 (0.1mg/ml)	Araceae
Carthamus tinctorius	Flower	30.33±9.22 (0.1mg/ml)	Asteraceae
Jatropha curcas	Leaves	23.86±0.96 (42.5µg/ml)	Euphorbiaceae
Bacopa monnieri	Whole	67.61±1.5 (1mg/ml)	Scrophulariaceae

Table 1

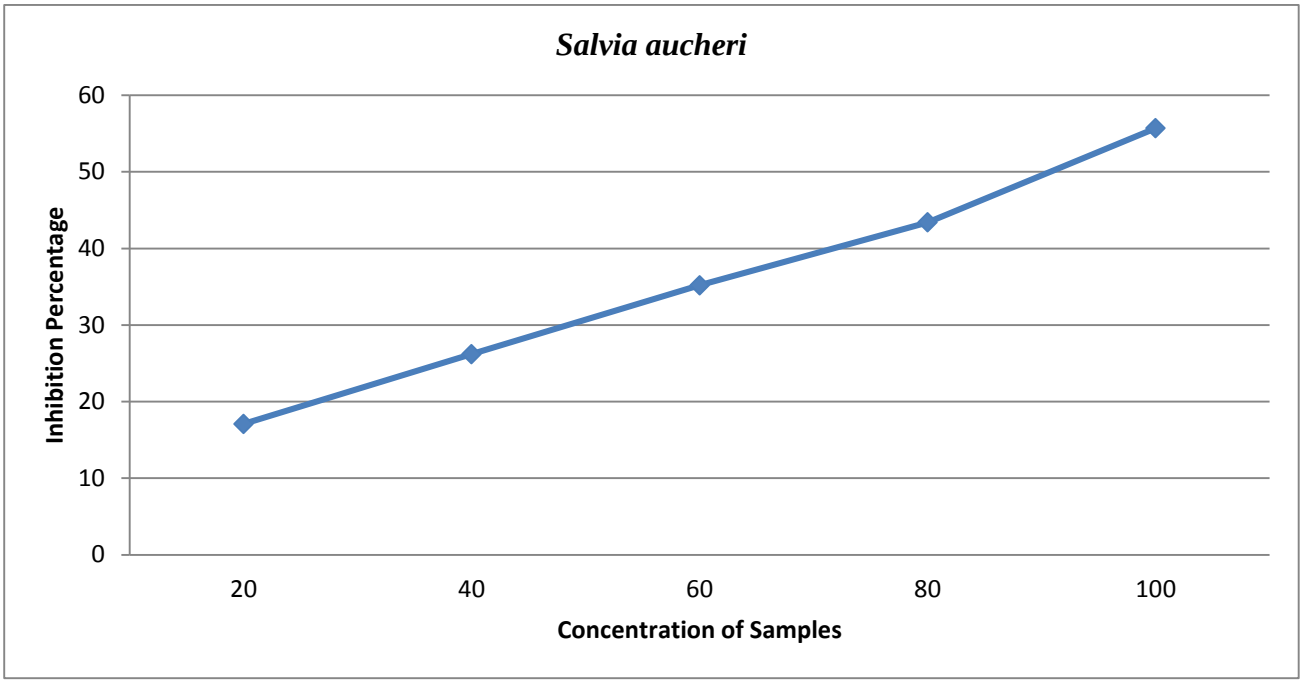
Following are the Graphs containing the inhibition percentages. These graphs show the plants with their Acetylcholinesterase inhibiting capacity :



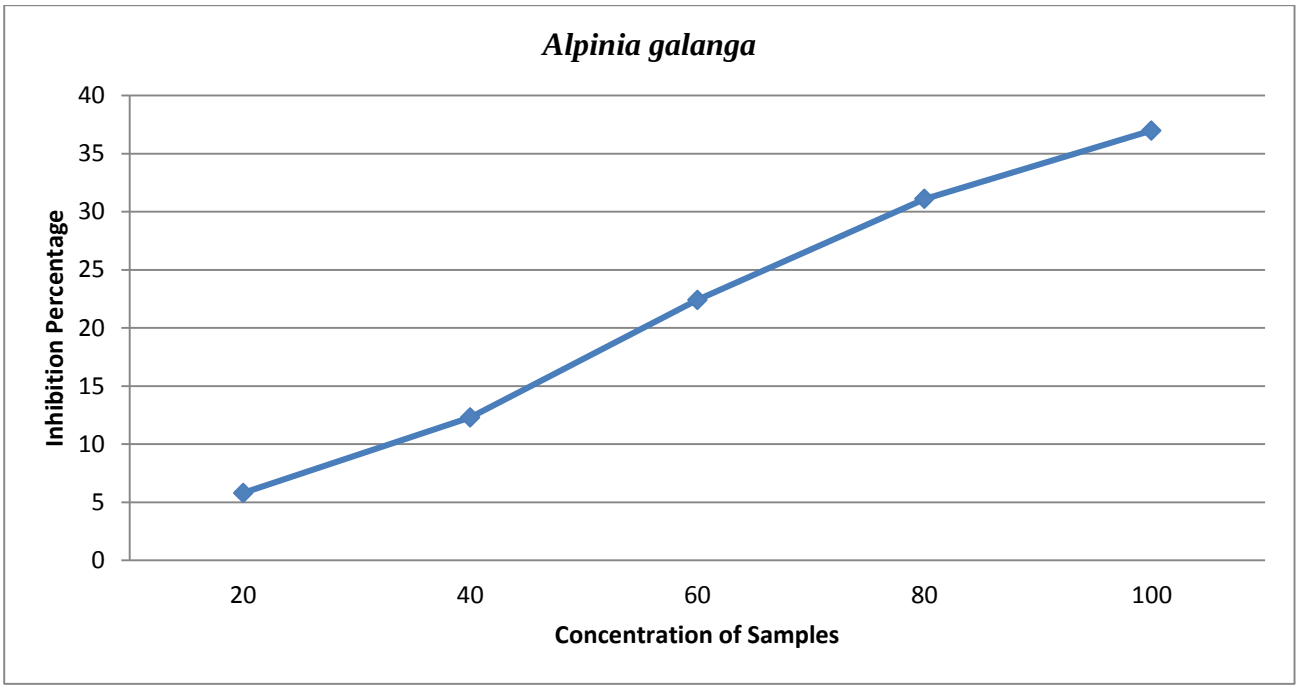
M.spicata showed low inhibitory activity as shown in the graph.



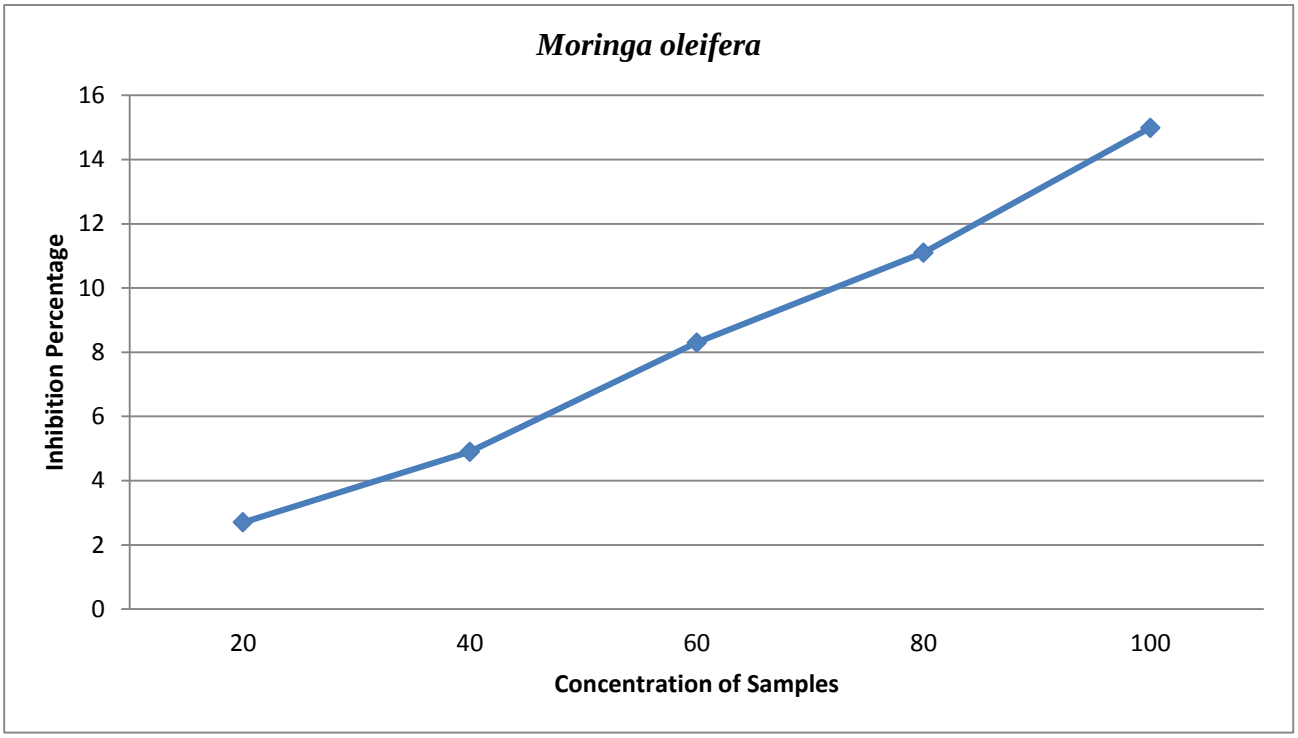
R.offcinalis showed good inhibitory activity as shown in the graph.



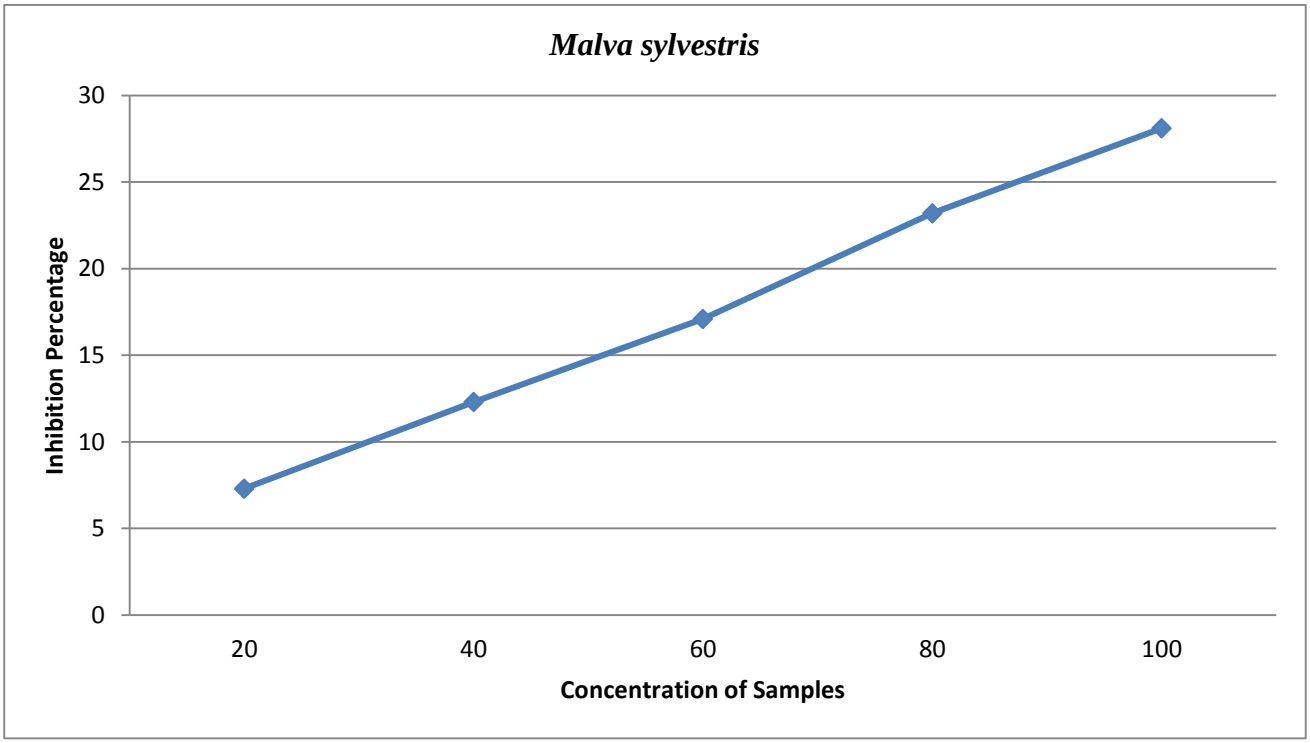
S.aucheri showed good inhibitory activity as shown in the graph.



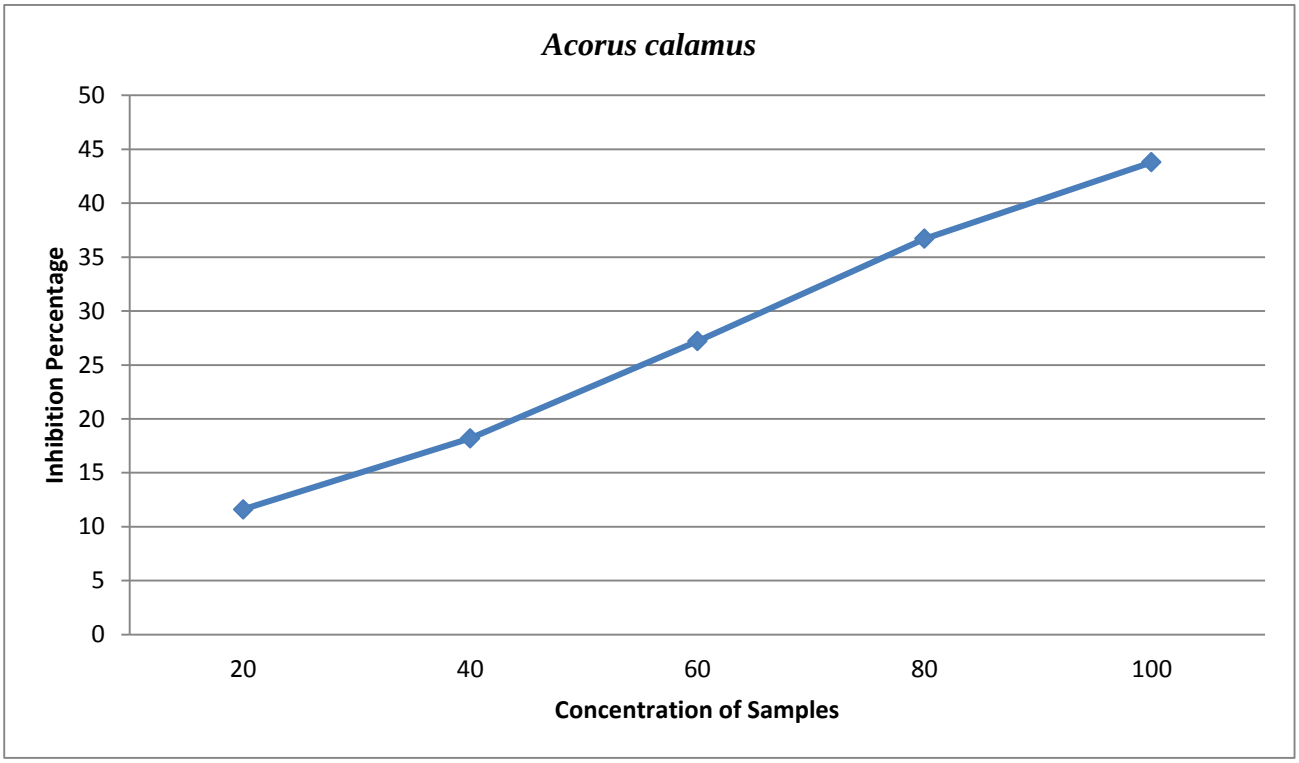
A.galanga showed moderate inhibitory activity as shown in the graph.



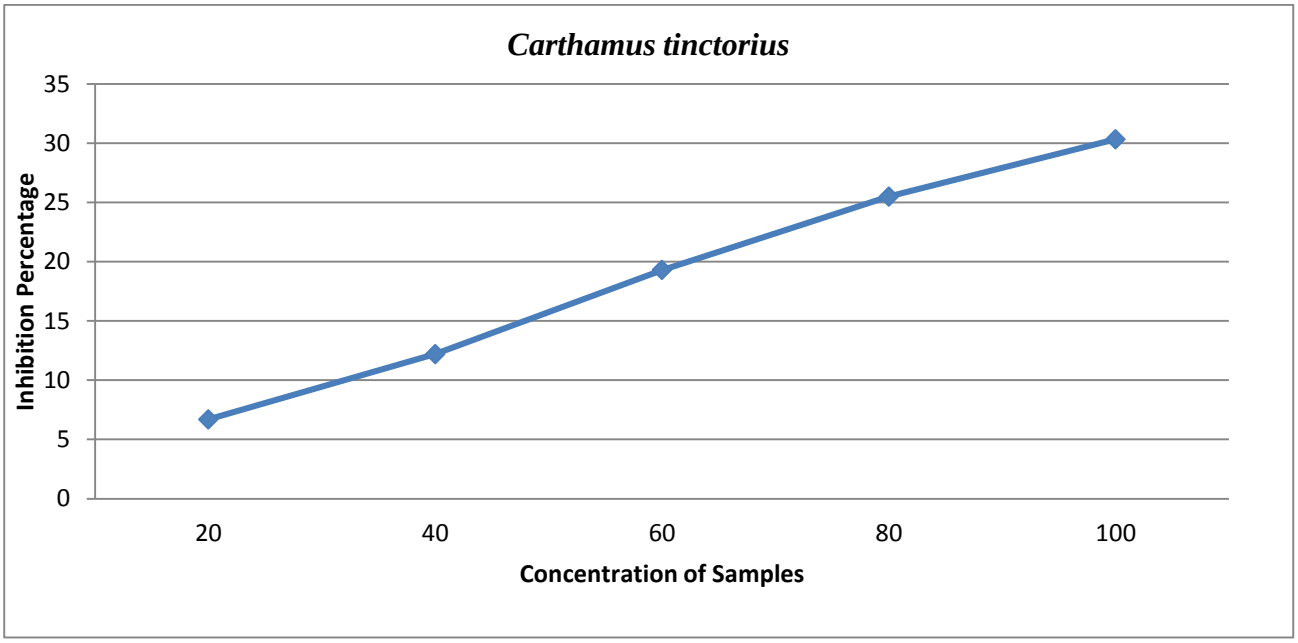
M.oleifera showed low inhibitory activity as shown in the graph.



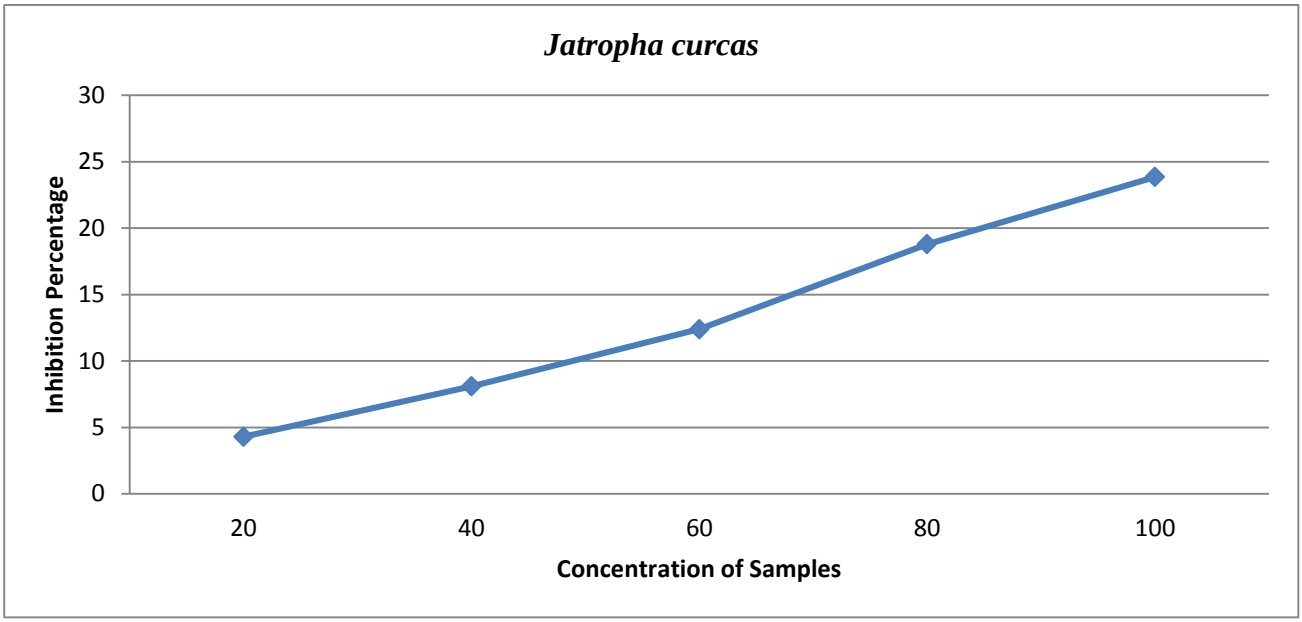
M.sylvestris showed moderate inhibitory activity as shown in the graph.



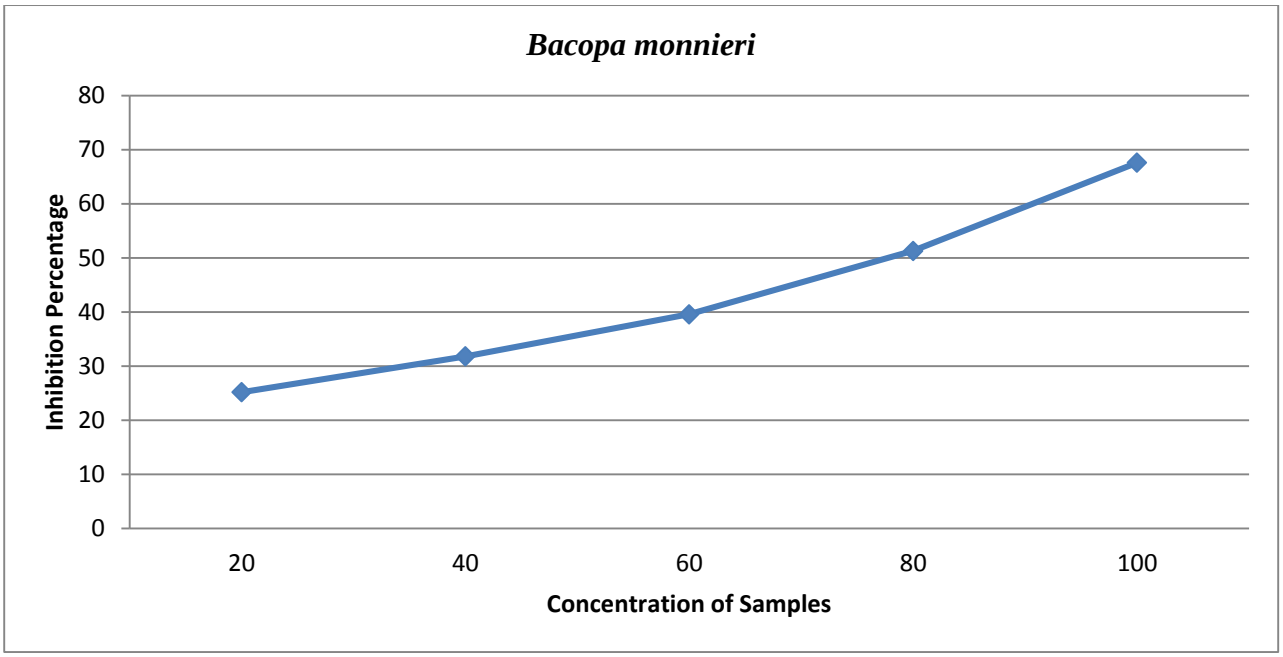
A.calamus showed good inhibitory activity as shown in the graph.



C.tinctorius showed low inhibitory activity as shown in the graph.



J.curcas showed low inhibitory activity as shown in the graph.



B.monnieri showed good inhibitory activity as shown in the graph.

6. DISCUSSIONS:

The 96-well microplate assay which is based on Ellman's method is the most commonly used method for AChE inhibitory activity detection in the studies conducted for it. It is most probably due to the reason that this method ensures the possibility of running many replicates for each determination, to improve statistical treatment of all results, and is both cheap & economical, as only small amounts of test substances and reagents are used.

Methanol was known to be the most commonly used solvent in extracting the plants. This may exhibit that most of the substances which show anticholinesterase activity are polar in nature. The plant part that was most commonly investigated was the whole plant or aerial parts (in case of herbs), indicating that bark or roots do not possess sufficient anticholinesterase inhibitory activity. Also, huge amount of evidence has showed that oxidative stress is ultimately involved in age-related neurodegenerative ailments and there are a lots of studies which have demonstrated the positive benefits of antioxidants to decrease or to block neuronal death appearing in the pathophysiology of these disorders (Loizzo *et al*, 2009).

These ten plants are tested to show there activity against acetylcholinesterase enzyme. These findings suggest that these plants which were reported to have anticholinesterase activity may have favourable pharmacological profile in the treatment of Alzheimer's disease. Since Acetylcholine plays a vital role in cognitive function including learning and memory and is evident that the anticholinesterase activity has memory enhancing properties. Clinical significance of these plants can be evolved by studying their active phytoconstituents, toxicity studies and further mechanistic studies are to be assessed and explored.

The results show ±10-12% deviations from the previous Acetylcholinesterase inhibitory tests performed on them. For example, Orhan *et al*, 2006 reported 45.5 Inhibitory Percentage in *Salvia aucheri,* similarly, Vinutha *et al*, 2007 reported 17.8 activity in *Moringa oleifera.* These changes may be due to the change in location of the plant, its age and the environmental condition it faces, change in the decoction or the solvent used for the formation of extract and change in the lab conditions. Also, it is shown that the plants belonging to families Lamiaceae, Zingiberaceae, Moringaceae, Malvaceae, Araceae, Asteraceae, Euphorbiaceae, and scrophulariaceae may show acetylcholinesterase inhibition properties.

7. CONCLUSION :

Current efforts are focussed towards the treatment of AD, ataxia, senile dementia, myasthenia gravis and Parkinson's disease are centered around the decreasing of cholinergic deficit by the use of BChE and AChE inhibitors. Various drugs are in the market, including the plant alkaloid which is galanthamine. However, findings for more efficient agents with lesser side effects has resulted in the screening of various medicinal plants for possible activity as shown in this review. It is easy to perceive the potential in these plants as an attractive and less harming targets for future studies, to recognise the active constituents and possibly to disclose new alternatives to the existing neurodegenerative diseases therapies. Also, *in vivo* activity of the active substances needs to be focussed in animal models and human subjects, so as to find their efficacy in a metabolic environment. Such future studies will be important for expansion of the existing, limited therapeutic arsenal for the almost all of neurodegenerative diseases, especially for those therapies with adverse effects that limit their effectiveness.

The 96 micro-well plate Acetylcholinesterase activity assay has shown to be simple, spectrophotometric, accurate, sensitive, and colorimetric, and superior to the Ellman's method, and thus can be used efficiently for quantitative and qualitative analysis of AChE inhibitory activities of extracts of plants of a wide range of different plant species and to give high detection rates from a range of plant parts. *R.officinalis, S.aucheri, B.monnieri* extracts were proved to have a great potential and should be used for further studies to find the constituents responsible for the Acetylcholinesterase inhibitory activity, which can be finally utilized in the prevention, treatment and medication of AD.

Among the tested plants, *Bacopa* showed the best activity and *Mentha* showed poor activity. These plants clinical significance may be stablished using their active constituents and their pharmacological profile. Also, the results produced *in vitro* may differ from *in vivo* and can be established with the help of animal studies which will help in the establishment of medication using plants. The pathophysiological process of Alzheimer's is thought to begin many years before the Alzheimer's disease is diagnosed. This long "preclinical" phase of Alzheimer's disease would give a significant opportunity for therapeutic intervention. It is hoped that plants with strong reversible antioxidant activities & Acetylcholinesterase inhibitory activity will aid in earlier intervention at a stage of Alzheimer's when some disease-improvising therapies may be most efficacious.

8. REFERENCES :

1. Abou-Donia AH, Darwish FA, Toaima SM, Shawky E, Takla SS (2014). A new approach to develop a standardized method for assessment of acetylcholinesterase inhibitory activity of different extracts using HPTLC and image analysis. Journal of Chromatography B , 955:50-57.

2. Albuquerque, U.P.; Medeiros, P.M.; Almeida, A.L.S.; Monteiro, J.M.; Neto, E.M.F.; Melo, J.G. (2007); Medicinal plants of the caatinga (semi-arid) vegetation of NE Brazil: A quantitative approach. *Journal of Ethnopharmacology.*, v.114, p.325-354.

3. Achten WMJ, Nielsen Lr, Aets R, Lengkeek AG, Kjaer ED, Trabucco A, Hansen JK, Males WH, MuysB., 2010. Towards domestication of Jatropha curcas *Journal of Jatropha curcas.* Biofuels Vol.No.1 pp 91-107

4. Ali-Shtayeh MS, Jamous RM. (2006); Ethnobotany of Palestinian herbal medicine in the Northern West Bank and Gaza Strip: Review and a comprehensive field study. Biodiversity and Environmental Sciences Studies Series, 4:1-122.

5. Ali-Shtayeh MS, Jamous RM: (2014); Traditional Arabic Palestinian Herbal Medicine, TAPHM.Til, Nablus: Biodiversity & Environmental Research Center-BERC; 2008. Functional Foods in Health and Disease; 4(9):381-400 Page 396 of 400

6. Andersen JB, Engeland A, Owe JF, Gilhus NE (2010);. Myasthenia gravis requiring pyridostigmine treatment in a national population cohort. *European Journal Neurology* ;17:1445–1450.

7. Atta-Ur-Rahman, Akhtar MN, Choudhary MI, Tsuda Y , Sener B, Khalid A, et al. (2002). New steroidalalkaloids from Fritillaria imperialis and their cholinesterase inhibiting activities. *Chem Pharm Bull* (Tokyo), 50:1013–6.

8. Bennett DA, et al. (2005); Mild cognitive impairment related to Alzheimer disease pathology and cerebral infarctions. *Journal of Neurology*. ;64(5):834–41.

9. Blumberg S, Silman I. (1978); Inactivation of electric eel acetylcholinesterase by acylation with N-hydroxysuccinimide esters of amino acid derivatives. *Journal of Biochemistry*. ;17:1125–1130.

10. Catto M, Pisani L, Leonetti F, Nicolotti O, Pesce P, Stefanachi A, Cellamare S, Carotti A. (2013); Design synthesis and biological evaluation of coumarin alkylamines as potent and selective dual binding site inhibitors of acetylcholinesterase. *Bioorganisms Medicinal Chemistry* ;21:146–152

11. Chacho LW, Cerf JA. (1960); Histochemical localization of cholinesterase in the amphibian spinal cord and alterations following ventral root section. *Journal of Anatomy ;*94:74–81.

12. Chattipakorn S, Pongpanparadorn A, Pratchayasakul W, Pongchaidacha A, Ingkaninan K, Chattipakorn N. (2007). Tabernaemontana divaricata extract inhibits neuronal acetylcholinesterase activity in rats. *Journal of Ethnopharmacology*,110:61–68.

13. Delamare APL, Moschen-Pistorello IT, Artico L, Atti-Serafini L, Echeverrigaray S (2007). Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. Food Chem. 100: 603-608.

14. Erkinjuntti T, Kurz A, Gauthier S, Bullock R, Lilienfeld S, Damaraju CV (2002). Efficacy of galantamine in probable vascular dementia and Alzheimer's disease combined with cerebrovascular disease: a randomised trial. The Lancet, 359(9314):1283-1290.

15. Ertas, A.C. Goren, N. Hasimi, V. Tolan and U. Kolak (2012). Phytochemical and biological investigations on *Mentha longifolia* subsp. *noeana*, Special Issue: *Journal of Planta Med.* **78**, 1171-1171.

16. Ferreira A, C. Proença C, Serralheiro MLM, Araújo (2006); MEM. The in vitro screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. *Journal of Ethnopharmacology,* 108(1):31-37.

17. Folnegović-Šmalc V, Mimica N, Makarić G, Varda R, Silić A (2006): Croatian therapeutic algorithm for treatment of Alzheimer's disease. Neurologia Croatica; 55(suppl 4):38.

18. Folnegović-Šmalc V, Uzun S, Kozumplik O, Folnegović-Grošić P, Henigsberg N, Makarić G, Gottwald, M.D., Rozanski, R.I., (1999); Rivastigmine a brainregion selective acetylcholinesterase inhibitor for treating Alzheimer's disease: review and current status. *Expert Opinion Investigatory Drugs*, 8: 1673–1682.

19. F. Orhan, O. Barıs, D. Yanmıs, T. Bal, Z. Guvenalp and M. Gulluce (2012). Isolation of some luteolin derivatives from *Mentha longifolia* (L.) Huds. subsp. *longifolia* and determination of their genotoxic potencies, *Journal of Food Chem.* **135**, 764-769.

20. Greenblatt HM, Kryger G, Lewis T, Silman I, Sussman JL. (1998); Structure of acetylcholinesterase complexed with (âˆ’)-galanthamine at 2.3 resolution. Febs Letters, 463(3):321-326.

21. Ganguly R, Guha D. (2008); Alteration of brain monoamines and EEG wave pattern in rat model of Alzheimer's disease and protection by *Moringa oleifera*. *Indian J Med Res* 128:744-751.

22. Hachiro Sugimoto, Hiroo Ogura, Yasuo Arai, Youichi Iimura and Yoshiharu Yamanishi (2002); Research and Development of Donepezil Hydrochloride, a New Type of Acetylcholinesterase Inhibitor *Journal of Alzheimer's Research 56-217.*

23. H. Ozturk, U. Kolak and C. Meric (2011). Antioxidant, anticholinesterase and antibacterial activities of *Jurinea consanguinea* DC, *Rec. Nat. Prod.* **5**, 43-51.

24. Heinrich M, Lee Teoh H. (2004); Galanthamine from snowdrop—the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. Journal of Ethnopharmacology, 92(2):147-162.

25. Hostettmann K, Borloz AU, Marston A. (2006). Natural product inhibitors of Acetylcholinesterase. Curr. Org. Chem, 10: 825-847.

26. Houghton PJ, Howes MJ (2005). Natural Products and Derivatives affecting neurotransmission relevant to Alzheimer's and Parkinson's disease. Neurosignals, 14: 6-22.

27. Howes M-JR, Houghton PJ. (2003); Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. Pharmacology Biochemistry and Behavior , 75(3):513-527.

28. Hiramatsu M, Takahashi T, Komatsu M, Kido T, Kasahara. (2009); M. Antioxidant and neuroprotective nactivities of Mogami-benibana (Safflower, Carthamus tinctorius Linne). *Neurochemical Resource Journal*; 34: 795– 805.

29. Huang YJ, Huang Y, Baldassarre H, Wang B, Lazaris A, Leduc M, Bilodeau AS, Bellemare A, Cote M, Herskovits P, Touati M, Turcotte C, Valeanu L, Lemee N, Wilgus H, Begin I, Bhatia B, Rao K, Neveu N, Brochu E, Pierson J, Hockley DK, Cerasoli DM, Lenz DE, Karatzas CN, Langermann S. (2007); Recombinant human butyrylcholinesterase from milk of transgenic animals to protect against organophosphate poisoning.Proc. Natl. Acad. Sci. U.S.A;104(34):13603–13608.

30. Hung TM, Ngoc TM, Youn UJ, Min BS, Na M, Thuong PT, Bae K (2008). Anti-amnestic activity of pseudocoptisine from Corydalis tuber. *Journal of Biological Pharmacological Bull*. 31: 159-162.

31. In Kyung Rhee, Natalie Appels,Teus Luijendijk,Hubertus Irth and Robert Verpoorte. (2008); Determining Acetylcholinesterase Inhibitory Activity in Plant Extracts Using a Fluorimetric Flow Assay *Journal of Bioresource*. 31: 159-187.

32. Jann MW, Small GW (2005): Cholinesterase inhibitors and similarly acting compounds. In: Kaplan & Sadock's comprehensive textbook of psychiatry. VIII edition. Volume [2]. Sadock BJ, Sadock VA. (eds). Lippincott Williams & Wilkins, Philadelphia, , 2808-2817.

33. Jongschaap REE, Corre WJ, Bindraban PS and Brandenburg WA. (2007). Claims and Facts on jatropha curcas L : Global Jatropha curcas evaluation, breeding and propagation Programme. Plant Research International, B.V. Wageningen, The Netherlands.

34. Jicha GA, et al. (2006); Neuropathologic outcome of mild cognitive impairment following progression to clinical dementia. *Journal of Arch Neurology* ;63(5):674–81.

35. Kalauni SK, Choudary MI, Khalid A, Manandhar MD, Shaheen F, Atta-ur-Rahman, et al. (2002). New cholinesterase inhibiting steroidal alkaloids from the leaves of Sarcococca coriacea og Nepalese origin. Chem Pharm Bull (Tokyo), 50: 1423-6.

36. Kamatou GPP, Viljoen AM, Makunga NP, Ramogola WPN (2008). South African *Salvia* species: a review of biological activities and phytochemistry. *Journal of Ethnopharmacology* 119:667-672.

37. Kasa, P., Rakonczay, Z, Gulya, K., (1997). The cholinergic system in Alzheimer's disease. *Progressive Neurobiology Journal*, 52: 511–535.

38. Kelly, C.A., Harvey, R.J., Cayton, H., (1997). Drug treatments for Alzheimer's disease. *Bioresource Medicine Journal*, 314: 693–694.

39. Kelen M, Tepe B (2008). Chemical composition. antioxidant and antimicrobial properties of the essential oils of three *Salvia* species from Turkish flora. *Bioresource Technology*. 99:4096-4104.

40. Koedam ELGE, Lauffer V, van der Vlies AE, van der Flier WM, Scheltens P, Pijnenburg YAL. (2010); Early-versus late-onset Alzheimer's disease: more than age alone. *Journal of Alzheimer's Disease*, 19(4):1401-1408.

41. K. Kubota, Y. Ueda, M. Yasuda, A. Masuda. (2009); Occurrence and antioxidative activity of 1'-acetoxychavicol acetate and its related compounds in the rhizomes of *Alpinia galanga* duringcooking. Food flavors and chemistry: advances of the new millennium. Proceedings of the 10th International Flavor Conference, Paros, Greece, 4-7 July 2000. 2001, 601-607.

42. Lahiri DK, Farlow MR, Greig NH, Sambamurti K. (2009); Current drug targets for Alzheimer's disease treatment. *Journal of Drug Development Research* 56(3):267-281.

43. Loizzo MR, Menichini F, Conforti F, Tundis R, Bonesi M, Saab AM, Statti GA, Cindio B, Houghton PJ, Menichini F, Frega NG (2009). Chemical analysis, antioxidant, anti-inflammatory and anticholinesterase activities of Origanum ehrenbergii Boiss and Origanum syriacum L. essential oils. *Journal of Food Chem*. 117: 174-180.

44. Machado, D.G.; Bettio, L.E.B.; Cunha, M.P.; Capra, J.C.; Dalmarco, J.B.; Pizzolatti, M.G.; Rodrigues, (2009); A.L.S. Progress in neuro-antidepressant-like effect of the extract of *Rosmarinus officinalis* in mice: involvement of the monoaminergic system. *Psychopharmacol*ogy *Biology Psychiatry*, v.33, p.642-650.

45. Mirjana B Colovic, Danijela Z Krstic, Tamara D Lazarevic-Pasti, c, and Vesna M Vasic,(2009); Acetylcholinesterase Inhibitors: Pharmacology and Toxicology.*Journal of Neurology* 14(4):289-300

46. Mormino EC, et al. (2009). Episodic memory loss is related to hippocampal-mediated beta-amyloid deposition in elderly subjects. Brain. *Journal of Phytomedicine* ;132(Pt 5):1310–23.

47. Mukherjee PK, Kumar V, Mal M, Houghton PJ. (2007); Acetylcholinesterase inhibitors from plants. *Journal of Phytomedicine*, 14(4):289-300.

48. Müller WE, Fürstl H, (2001): Pharmacological and nonpharmacological approaches to the treatment of Dementia. In: Contemporary Psychiatry: Psychiatry in Special Situations. Volume (2), 36-44. Henn F, Sartorius N, Helmchen H, Lauter H. (eds). Springer- Verlag, Berlin,.

49. M. Gulluce, F. Sahin, M. Sokmen, H. Ozer, D. Daferera, A. Sokmen, M. Polissiou, A. Adiguzel and H.Ozkan (2007). Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. *longifolia, Food Chem.* **103**, 1449-1456.

50. Neary D.(1990); Non Alzheimer's disease forms of cerebral atrophy. *Journal of Neurological Neurosurgery Psychiatry*.1990;53(11):929–31.

51. Ordentlich A, Barak D, Kronman C, Ariel N, Segall Y, Velan B, Shafferman A. (1995); Contribution of Aromatic Moieties of Tyrosine 133 and of the Anionic Subsite Tryptophan 86 to Catalytic Efficiency and Allosteric Modulation of Acetylcholinesterase. *Journal of Biology Chem.* ;270:2082–2091.

52. Orhan I, Sener B, Choudhary MI, Khalid A (2004). Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some Turkish medicinal plants. *Journal of Ethnopharmacology.* 91: 57-60.

53. Ozarowski, M.; Mikolajczak, P.Ł.; Bobkiewlcz-kozlowska, T.; Kujawski, R.; Mrozikiwiecz, P.M. (2009); Neuroactive compounds from medicinal plants of the Lamiaceae family showing potentially beneficial activity in treatment of Alzheimer's disease. *Journal of Herba Polonica*, v.55, p.148-163.

54. Pratt JH, Henry EMT, Mbeza HF, Mlaka E and Satali LB. (2002). Malawi Agroforestry Extension Project Marketing & Enterprise Program. Main Report. *Malawi Agroforestry Publication No.*47 pp44-46

55. Prediger, D.S.R.; Fernandes, S.M.; Rial, D.; Wopereseis, S.; Pereira, S.P.; Bosse, S.T.; Silva, B.C.;(2008) Effects of acute administration of the hydroalcoholic extract of mate tea leaves (*Ilex paraguariensis*) in animal models of learning and memory. *Journal of Ethnopharmacology.*, v.120, p.465-473,.

56. Pengelly, A.; Snow, J.; Mills, S.Y.; Scholey, A.; Wesnes, K.; Butler, L.R , (2012). Short-term study on the effects of rosemary on cognitive function in an elderly population. *Journal of Medicinal Food*., v.15, p.10-17.

57. P.S. Dhivya, M.Sobiya, P.Selvamani, S.Latha (2014); An Approach to Alzheimer's Disease Treatment with Cholinesterase Inhibitory Activity from Various Plant Species Department of Pharmaceutical Technology, Anna University Chennai, Regional Office, BIT Campus Tiruchirappalli, India.

58. Pehkonen SO, Zhang Q.(2002); The degradation of organophosphorus pesticides in natural waters: a critical review. *Critical Review of Environmental Science Technology* ;32:17–72.

59. Personeni, C.D., Bentley, P.D., Fletcher, R.J., Kinkaid, A., Kryger, B.P., Taylor, A., Taylor, R., Taylor, I., Viner, R., Silman, I., Sussman, X.L., Greenblatt, H.M., Lewis,

T. (2001); A structure-based design approach to the development of novel, reversible AChE inhibitors. *Journal of Medicinal Chemistry*, 44: 3203–3215.

60. Pilger C, Bartolucci C, Lamba D, Tropsha A, Fels G. (2001); Accurate prediction of the bound conformation of galanthamine in the active site of torpedo californica acetylcholinesterase using molecular docking. *Journal of Molecular Graph*. Model. ;19:288–296.

61. Pisani L, Catto M, Giangreco I, Leonetti F, Nicolotti O, Stefanachi A, Cellamare S, Carotti A.(2010); Design synthesis and biological evaluation of coumarin derivatives tethered to an edrophonium-like fragment as highly potent and selective dual binding site acetylcholinesterase inhibitors. *Journal of Chemical Medicine* Chem.;5:1616–1630.

62. Rabinovici GD, et al. (2008); Abeta amyloid and glucose metabolism in three variants of primary progressive aphasia. *Journal of Ann Neurology* ;64(4):388–401.

63. Rehman EU, (2006). Indigenous knowledge on medicinal plants Kass and its allied areas, District Kotli Azad Jammu and Kashmir, Pakistan. *Journal of Ethno Leaflets*, 10:254–264.

64. Rollinger JM, Hornick A, Langer T, Stuppner H, Prast H. (2004); Acetylcholinesterase inhibitory activity of scopolin and scopoletin discovered by virtual screening of natural products. *Journal of Medicinal Chemistry*, 47(25):6248-6254.

65. Sancheti S, Sancheti S, Um BH, Seo SY. (2009): 1,2,3,4,6-penta-Ogalloyl- _-D-glucose: A cholinesterase inhibitor from Terminalia chebula. *South African Journal of Botany*..

66. Scott, L.J., Goa, K.L., Galantamine (2000): a review of its use in Alzheimer's disease. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, Drugs, 60: 1095–1122.

67. Singhal AK, Naithani V, Bangar OP. (2010). Medicinal plants with a potential to treat Alzheimer and associated symptoms. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, 2(2):84-91.

68. Singh R, Sharma PK, Malviya. (2011). Pharmacological properties and Ayurvedic value of Indian plant (*Acorus calamus*): A short review. *Journal of Biology Res*; 5: 145-154.

69. Tang H, Zhao LZ, Zhao HT, Huang SL, Zhong SM, Qin JK, Chen ZF, Huang ZS, Liang H. (2011). Hybrids of oxoisoaporphine-tacrine congeners: Novel acetylcholinesterase and acetylcholinesterase-induced β-amyloid aggregation inhibitors. *European Journal of Medicine Chemistry* ;46:4970–4979.

70. Tang M, Wang Z, Zhou Y, Xu W, Li S, Wang L, Wei D, Qiao Z. (2013) A Novel Drug Candidate for Alzheimer's Disease Treatment: gx-50 Derived from Zanthoxylum Bungeanum. *Journal of Alzheimer's Disease* 34(1):203-213.

71. Terry RD, et al(1991). Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurology*. ;30(4):572–80.

72. Tenore GC, Ciampaglia R, Arnold NA, Piozzi F, Napolitano F, Rigano D, Senatore F. (2010). Antimicrobial and antioxidant properties of the essential oil of *Salvia lanigera* from Cyprus. *Food Chemical Toxins*. 49(1):238-243.

73. Vinutha B, Prashanth D, Salma K, Sreeja SL, Pratiti D, Padmaja R, Radhika S, Amit A, Venkateshwarlu K, Deepak M. (2007). Screening of selected Indian medicinal

plants for acetylcholinesterase inhibitory activity. *Journal of Ethnopharmacology* 109(2):359-363;.

74. Venkateswaran S, Pari L.(2010). Effect of *Coccinia indica* leaves on antioxidant status in streptozotocininduced diabetic rats. *Journal of Ethnopharmacology* 84:163-168.

75. Wszelaki N, Kuciun A, Karolina Kiss A. (2010). Screening of traditional European herbal medicines for acetylcholinesterase and butyrylcholinesterase inhibitory activity. *Acta Pharmacology*, 60:119–128. 2010.

76. Wang Y, Chen P, Tang CY, Wang Y, Li YZ, Zhang H. (2014). Antinociceptive and anti-inflammatory activities of extract and two isolated flavonoids of Carthamus tinctorius L. *Journal of Ethnopharmacology* 151: 944–950.

77. Yaari R, Tariot PN, Schneider LS, Tasman A, Kay J, Lieberman JA, First MB, Maj M. (2008) Cognitive enhancers and treatments for Alzheimer's disease. *In: Psychiatry, Third Edition. Volume (2),* 2294- 2317. (eds). John Wiley & Sons, Chichester.

78. Zhang Y, Gu D, Mao S, Chen W. (1998) Protective effects of Ginkgo biloba and C.tinctorius extract on focal cerebral ischemia and thrombogenesis of carotid artery in rats, Yao Xue Xue Bao 33: 901-905.