"In-vitro assessment of phenolic, flavonoid, flavonolic contents and antioxidant activities of plant Pleurotus ostreatus for neurodegenerative disorders"

A research paper is submitted to the Department Of Pharmacy, East West University in conformity with the requirements for the degree of Bachelor of Pharmacy

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Declaration by the Research Candidate

I, Mahamudul Hasan Mridha, hereby declare that the dissertation entitled "**In-vitro assessment of phenolic, flavonoid, flavonolic contents and antioxidant activities of plant *Pleurotus ostreatus* for neurodegenerative disorders**" submitted by me to the Department of Pharmacy, East West University, in the partial fulfilment of the requirement for the award of the degree Bachelor of Pharmacy is a complete record of original research work carried out by me during 2017, under the supervision and guidance of Kushal Biswas, Lecturer, Department of Pharmacy, East West University. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Certificate by the Supervisor

This is to certify that the thesis entitled "**In-vitro assessment of phenolic, flavonoid, flavonolic contents and antioxidant activities of plant *Pleurotus ostreatus* for neurodegenerative disorders** " submitted to the Department of Pharmacy, East West University, in the partial fulfilment of the requirement for the degree of Bachelor of pharmacy was carried out by Mahamudul Hasan Mridha, ID: 2013-1-70-037 in 2017, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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This is to certify that the thesis submitted to the Department of Pharmacy, East West University, Dhaka-1212, entitled "**In-vitro assessment of phenolic, flavonoid, flavonolic contents and antioxidant activities of plant *Pleurotus ostreatus* for neurodegenerative disorders**" in partial fulfilment of the requirement for the Degree of Bachelor in Pharmacy, was carried out by Mahamudul Hasan Mridha ID: 2013-1-70-037.

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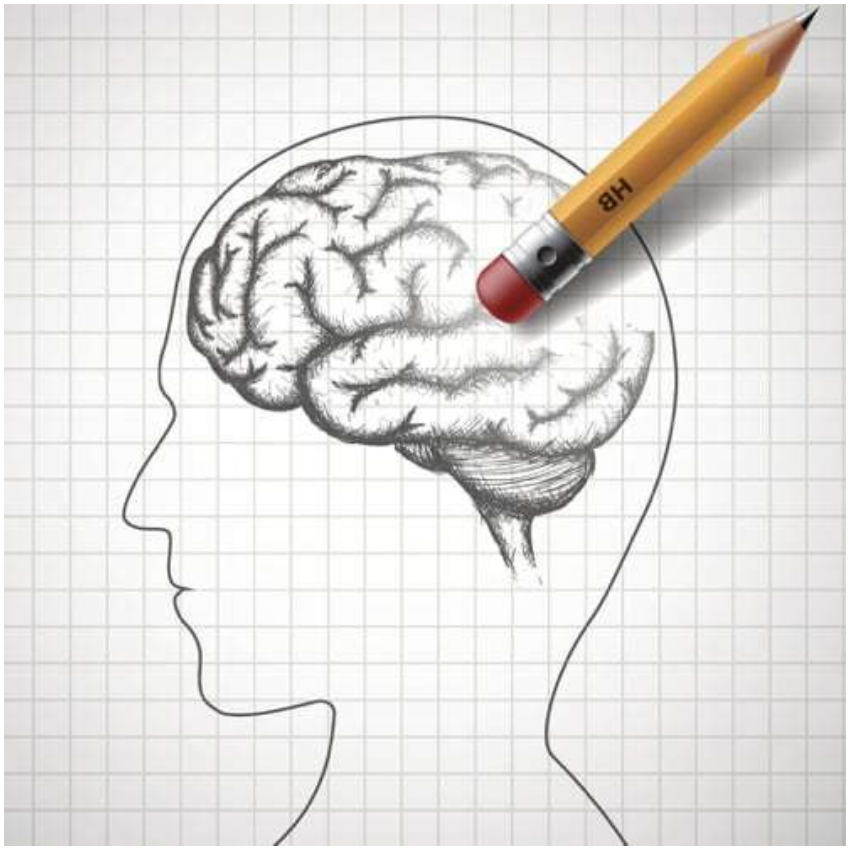
Abstract

Among the pathologic hypotheses of Alzheimer's disease (AD), cholinergic deficit and oxidative stress have been implicated as two major hallmarks. Therefore, inhibition of cholinesterase and oxidation are the two promising strategies in the development of a drug for AD. *Pleurotus ostreatus* fungi extract is used in this research to investigate its anticholinesterase and antioxidant potentials. Anticholinesterase activity was measured by modified Ellman's method. Antioxidant potentials were evaluated by the assay of reducing power, radical scavenging. The Methanolic extract showed strong antioxidant effect. Additionally, the extract exhibited pronounced reducing capacity. The fungi extract found moderate inhibitor of cholinesterase. The tested sample reflects potential antioxidative and moderate anticholinesterase inhibitory effect which may warrant its effectiveness in the treatment of AD.

Introduction



1.1 Alzheimer Disease:

Alzheimer disease affects the brain, causing memory problems and eventually severe problems with mental function. It gets worse over time, and people with Alzheimer disease have gradual memory loss, as well as loss of judgment, trouble concentrating, loss of language skills, personality changes, and a decline in the ability to learn new tasks. In advanced stages, people with Alzheimer disease may lose all memory and mental abilities. [1]

Alzheimer disease is the most common form of dementia. About 5 million Americans have Alzheimer disease, and this number is expected to grow as the population gets older. The disease progress is different for each person. If Alzheimer disease comes on quickly, it usually gets worse quickly. If it has been slow to get worse, it will often continue slowly. [2]

Alzheimer disease symptoms happen because the disease kills brain cells. In a healthy brain, billions of neurons create chemical and electrical signals that are relayed from cell to cell. They help a person think, remember, and feel. Neurotransmitters, brain chemicals, help these signals move from cell to cell. In people with Alzheimer disease, neurons in some places start to die, and the brain makes lower levels of neurotransmitters. That causes the brain to have problems with its signals. [3]

There is no cure for Alzheimer disease, but medicines can help slow the progression of the disease in some people. Herbs and supplements, and lifestyle adjustments, may also help reduce the risk or improve quality of life. [5]

Alzheimer's disease is a degenerative disease of the brain. Understanding how the anatomy of the Alzheimer's differs from a normal brain gives us insight. It can help us cope better with the changes that happen to our loved ones as a result of this debilitating disease. In Alzheimer's disease the appearance of the Alzheimer's affected brain is very different to a normal brain. [6,7]

- The cerebral cortex atrophies. That means that this area of the brain shrinks and this shrinkage is dramatically different from the cerebral cortex of a normal brain. The cerebral cortex is the outer surface of the brain. It is responsible for all intellectual functioning. There are two major changes that can be observed in the brain at autopsy:

- The amount of brain substance in the folds of the brain (the gyri) is decreased
- The spaces in the folds of the brain (the sulci) are grossly enlarged.

Microscopically there are a number of changes in the brain too. The two major findings in the Alzheimer's brain are amyloid plaques and neurofibrillary tangles. Amyloid plaques are found outside the neurons, neurofibrillary plaques are found inside the neurons. Neurons are the nerve cells within the brain. Plaques and tangles are found in the brains of people without Alzheimer's. It is the gross amounts of them that are significant in Alzheimer's disease.

The role of amyloid plaques in Alzheimer's

Amyloid plaques are mostly made up of a protein called B-amyloid protein which is itself part of a much larger protein called APP (amyloid precursor protein). These are amino acids. We do not know what APP does. But we do know that APP is made in the cell, transported to the cell membrane and later broken down.

Two major pathways are involved in breakdown of APP (amyloid precursor protein). One pathway is normal and causes no problem. The second results in the changes seen in Alzheimer's and in some of the other dementias.

1.2 Dementia

Dementia is a syndrome, not a disease. A syndrome is a group of symptoms that doesn't have a definitive diagnosis. Dementia is a group of symptoms that affects mental cognitive tasks such as memory and reasoning. Dementia is an umbrella term that Alzheimer's disease can fall under. It can occur due to a variety of conditions, the most common of which is Alzheimer's disease. [8,9]

People can have more than one type of dementia. This is known as mixed dementia. Often, people with mixed dementia have multiple conditions that may contribute to dementia. A diagnosis of mixed dementia can only be confirmed in an autopsy.As dementia progresses, it can have a huge impact on the ability to function independently. It's a major cause of disability for older adults, and places an emotional and financial burden on families and caregivers.

It’s easy to overlook the early symptoms of dementia, which can be mild. It often begins with simple episodes of forgetfulness. People with dementia have trouble keeping track of time and tend to lose their way in familiar settings.

As dementia progresses, forgetfulness and confusion grow. It becomes harder to recall names and faces. Personal care becomes a problem. Obvious signs of dementia include repetitious questioning, inadequate hygiene, and poor decision-making.

In the most advanced stage, people with dementia become unable to care for themselves. They will struggle even more with keeping track of time, and remembering people and places they are familiar with. Behavior continues to changes

Causes of dementia

You’re more likely to develop dementia as you age. It occurs when certain brain cells are damaged. Many conditions can cause dementia, including degenerative diseases such as Alzheimer’s, Parkinson’s, and Huntington’s. Each cause of dementia causes damage to a different set of brain cells.

Alzheimer’s disease is responsible for about 50 to 70 percent of all cases of dementia.

Other causes of dementia include:

- infections, such as HIV
- vascular diseases
- stroke
- depression
- chronic drug us

1.3 Difference between Dementia & Alzheimer’s Disease:

The outlook for people with dementia depends entirely on the direct cause of the dementia. Treatments are available to make symptoms of dementia due to Parkinson’s manageable, but there isn’t currently a way to stop or even slow down the related dementia. Vascular dementia can be

slowed down in some cases, but it still shortens a person's lifespan. Some types of dementia are reversible, but most types are irreversible and will instead cause more impairment over time. [10,11]

Alzheimer's is a terminal illness, and no cure is currently available. The length of time each of the three stages lasts varies. The average person diagnosed with Alzheimer's has an estimated lifespan of approximately four to eight years after diagnosis, but some people can live with Alzheimer's for up to 20 years.

Talk to your doctor if you're concerned that you have the symptoms of dementia or Alzheimer's disease. Starting treatment promptly can help you manage your symptoms.

Brain Changes Associated with Alzheimer's Disease:

Scientists have identified several hallmark brain abnormalities in people affected by Alzheimer's:Amyloid plaques, which are microscopic clumps of a protein called beta-amyloid peptide. These abnormal clusters of protein fragments build up between nerve cells which disrupts electrical signals. Diseased tissue has many fewer nerve cells and synapses compared to healthy brain tissue. [12,13]

- Dead and dying nerve cells contain neurofibrillary tangles, which are made up of twisted strands of another protein called tau (rhymes with "wow").
- These plaques and tangles tend to spread through the cortex in a predictable pattern as AD progresses.
- There is a profound loss of connections among brain cells called synapses. These connections transmit information from cell to cell and are responsible for memory, learning and communication.
- Inflammation results from the brain's efforts to fend off the lethal effects of these and other neurological changes.
- Brain cells eventually die, resulting in significant tissue shrinkage or atrophy.
- Scientists are not absolutely sure what mechanism specifically causes cell death and tissue loss in a diseased brain, but plaques and tangles are the prime suspects.

1.4 Brain where AD Develop

Scientists continue to unravel the complex brain changes involved in the onset and progression of Alzheimer's disease. It seems likely that damage to the brain starts a decade or more before memory and other cognitive problems appear. During this preclinical stage of Alzheimer's disease, people seem to be symptom-free, but toxic changes are taking place in the brain. Abnormal deposits of proteins form amyloid plaques and tau tangles throughout the brain, and once-healthy neurons stop Function connections with other neurons, and die. The damage initially appears to take place in the hippocampus, the part of the brain essential in forming memories. As more neurons die, additional parts of the brain are affected, and they begin to shrink. By the final stage of Alzheimer's, damage is widespread, and brain tissue has shrunk significantly.

1.5 Types of AD

Nearly everyone with Alzheimer's disease will eventually have the same symptoms -- memory loss, confusion, trouble with once-familiar tasks, and making decisions. Though the effects of the disease are similar, there are two main types. [14]

- **Early-onset Alzheimer's.** This type happens to people who are younger than age 65. Often, they're in their 40s or 50s when they're diagnosed with the disease. It's rare -- up to 5% of all people with Alzheimer's have early-onset. People with Down syndrome have a higher risk for it.

- Scientists have found a few ways in which early-onset Alzheimer's is different from other types of the disease. People who have it tend to have more of the brain changes that are linked with Alzheimer's. The early-onset form also appears to be linked with a defect in a specific part of a person's DNA: chromosome 14. A form of muscle twitching and spasm, called myoclonus, is also more common in early-onset Alzheimer's.

- **Late-onset Alzheimer's.** This is the most common form of the disease, which happens to people age 65 and older. It may or may not run in families. So far, researchers haven't found a particular gene that causes it. No one knows for sure why some people get it and others don't.
- **Familial Alzheimer's disease :This** is a form of Alzheimer's disease that doctors know for certain is linked to genes. In families that are affected, members of at least two generations

have had the disease. FAD makes up less than 1% of all cases of Alzheimer's. Most people who have early onset Alzheimer's have FAD.

How Alzheimer's Disease Affects the Brain at Different Ages:

Alzheimer's disease can lead to several widely divergent symptoms and, so far, its various expressions have mainly been observed through the behaviour and actions of patients. Researchers at Lund University in Sweden have now produced images showing the changes in the brain associated with these symptoms — a development which increases knowledge and could facilitate future diagnostics and treatment.

Symptoms vary in cases of Alzheimer's disease and often relate to the phase of life in which the disease first occurs. People who become ill before the age of 65 often suffer early on from diminished spatial perception and impaired orientation. Elderly patients more often suffer the symptoms traditionally associated with the disease: above all, memory impairment. Diagnostics could also be facilitated, mainly among younger patients in whom it is particularly difficult to arrive at a correct diagnosis.

1.6 Brain Neurotransmitter & Alzheimar's Disease

Scientists continue to unravel the complex brain changes involved in the onset and progression of Alzheimer's disease. It seems likely that damage to the brain starts a decade or more before memory and other cognitive problems appear. During this preclinical stage of Alzheimer's disease, people seem to be symptom-free, but toxic changes are taking place in the brain. Abnormal deposits of proteins form amyloid plaques and tau tangles throughout the brain, and once-healthy neurons stop functioning, lose connections with other neurons, and die.

The damage initially appears to take place in the hippocampus, the part of the brain essential in forming memories. As more neurons die, additional parts of the brain are affected, and they begin to shrink. By the final stage of Alzheimer's, damage is widespread, and brain tissue has shrunk significantly. [15,16]

1.7 Epidemiology:

Aging has been associated with cognitive impairment affecting working memory, processing speed, executive function and episodic memory, but different mechanisms and underlying brain changes have been related to each cognitive domain. Cognitive functions associated with frontal cortex structures and networks, particularly processing speed and working memory, have been associated with 'normal' brain aging (BA) and vascular-related white matter changes. Episodic memory impairment in turn has been attributed to Alzheimer disease (AD), the prevalence of which exponentially increases with age. AD is characterized by tau pathology spreading from the

medial temporal lobe and neocortical widespread amyloid beta deposition. Amyloid- and tau-independent mechanisms like mitochondrial dysfunction and oxidative stress have been linked to BA, although this does not exclude that the same aging-related mechanisms can lead to increased AD-related pathology.

Findings from previous studies have shown that BA in individuals without concurrent pathology is associated with pronounced gray matter loss, particularly in frontal and parietal lobes, whereas amnestic mild cognitive impairment and AD subjects have shown atrophy patterns in the temporal lobe, hippocampus and parahippocampal gyrus. In addition, co-morbid conditions such as type 2 diabetes mellitus, hypertension and arteriolosclerosis are also associated with brain atrophy and might have an additive effect on atrophy related to BA. While many studies independently showed spatially specific atrophy patterns occurring with normal aging or due to disease, structural brain changes in advanced BA (ABA), defined as significant deviation from typical BA trajectories, have not been systematically compared with AD-like brain changes in population-based studies. In addition, whether different co-morbid and genetic conditions are associated with BA and AD is still uncovered in the general community.We hypothesized that ABA will show a pattern of brain atrophy that is distinct and only partially overlapping to the one that has been described for AD. To assess ABA and AD-like patterns a traditional approach using simple radiological measures like hippocampal volume, which is commonly used to investigate brain changes related to aging and AD, might not be able to capture the complex spectrum of changes, and more sophisticated methods are required. Herein, we leverage advanced pattern analysis techniques to derive a new quantitative index for brain changes as a function of age (Spatial Pattern of Atrophy for Recognition of BA (SPARE-BA)), and to compare those with spatial brain atrophy patterns specifically found in clinically diagnosed AD cases, using the Spatial Patterns of Abnormality for Recognition of Early Alzheimer's Disease (SPARE-AD) index in a large sample from the population-based Study of Health in Pomerania (SHIP) that spanned a wide age range (20–90 years, n=2705). To our knowledge, the current study is the first to employ high-dimensional pattern recognition techniques to assess ABA patterns and to determine similarities and differences with clinical AD patterns in a large population-based cohort spanning almost the entire adulthood age range.

1.8 Contribution of high-dimensional pattern classification techniques

An important contribution of our study is the use of advanced methods of high dimensional pattern classification for BA assessment, which allowed us to investigate in detail the spatial patterns of atrophy, and to derive individualized indices that were further correlated with epidemiologic and clinical factors. Our approach utilizes information from all brain regions jointly, thereby capturing the structural abnormality subtleties in BA, which is high dimensional in nature and goes beyond the small number of dimensions represented in one or few volumetric measures. Recently, researcher analyzed prediction patterns for hippocampal volumes in SHIP. Interestingly the associated risk factors with hippocampal volume were similar to those associated with the aging patterns in the current study but different from the prediction patterns of SPARE-AD. This finding indicates that the hippocampus alone is unlikely to adequately reflect the complexity of neurodegeneration in AD, as previously demonstrated in the study by a researcher.

1.9 Overlap between ABA and AD spatial patterns of atrophy

Some research shows that the regions of the ABA-related spatial patterns of atrophy overlapped only partially with the clinical AD-related patterns. While ABA-like patterns were widespread in the brain (in blue), clinical AD-like patterns were spatially more localized (in red), mostly significant in several (especially medial) temporal lobe regions. The overlap between clinical AD-like and ABA-like regions (in green) existed mainly in parts of the hippocampus and in areas of the temporal lobe. These differences in the spatial distribution of atrophy patterns associated to ABA and to clinical AD suggest that ABA stems from distinct mechanisms, which potentially constitute a co-morbidity for clinical AD largely by virtue of affecting spatially overlapping brain regions.

1.10 Mortality from Alzheimer's Disease:

Data from the National Vital Statistics System

- The age-adjusted death rate from Alzheimer's disease increased by 39 percent from 2000 through 2010 in the United States.
- Alzheimer's disease is the sixth leading cause of death in the United States and is the fifth leading cause among people aged 65 years and over. People aged 85 years and over have a 5.4 times greater risk of dying from Alzheimer's disease than people aged 75–84 years.
- The risk of dying from Alzheimer's disease is 26 percent higher among the non-Hispanic white population than among the non-Hispanic black population, whereas the Hispanic population has a 30 percent lower risk than the non-Hispanic white population.
- In 2010, among all states and the District of Columbia, 31 states showed death rates from Alzheimer's disease that were above the national rate.

In 2010, Alzheimer's disease was the underlying cause for a total of 83,494 deaths and was classified as a contributing cause for an additional 26,488 deaths. Mortality from Alzheimer's disease has steadily increased during the last 30 years. Alzheimer's disease is the sixth leading cause of death in the United States and the fifth leading cause for people aged 65 years and over. An estimated 5.4 million persons in the United States have Alzheimer's disease. The cost of health care for people with Alzheimer's disease and other dementia was estimated to be 200 billion dollars in 2012, including 140 billion dollars in costs to Medicare and Medicaid and is expected to reach 1.1 trillion dollars in 2050.

Alzheimer's disease mortality varies by age, sex, race, Hispanic origin, and geographic area. This report presents mortality data on Alzheimer's disease based on data from the National Vital Statistics System from 2000 through 2010, the most recent year for which detailed data are available.

Diagnosis:

Doctors use several methods and tools to help determine whether a person who is having memory problems has "possible Alzheimer's dementia" (dementia may be due to another cause) or "probable Alzheimer's dementia" (no other cause for dementia can be found).

To diagnose Alzheimer's, doctors may:

- Ask the person and a family member or friend questions about overall health, past medical problems, ability to carry out daily activities, and changes in behavior and personality
- Conduct tests of memory, problem solving, attention, counting, and language
- Carry out standard medical tests, such as blood and urine tests, to identify other possible causes of the problem
- Perform brain scans, such as computed tomography (CT), magnetic resonance imaging (MRI), or positron emission tomography (PET), to rule out other possible causes for symptoms.

These tests may be repeated to give doctors information about how the person's memory and other cognitive functions are changing over time.

Alzheimer's disease can be *definitely* diagnosed only after death, by linking clinical measures with an examination of brain tissue in an autopsy. People with memory and thinking concerns should talk to their doctor to find out whether their symptoms are due to Alzheimer's or another cause, such as stroke, tumor, Parkinson's disease, sleep disturbances, side effects of medication, an infection, or a non-Alzheimer's dementia. Some of these conditions may be treatable and possibly reversible. If the diagnosis is Alzheimer's, beginning treatment early in the disease process may help preserve daily functioning for some time, even though the underlying disease process cannot be stopped or reversed. An early diagnosis also helps families plan for the future. They can take care of financial and legal matters, address potential safety issues, learn about living arrangements, and develop support networks.

In addition, an early diagnosis gives people greater opportunities to participate in clinical trials that are testing possible new treatments for Alzheimer's disease or other research studies.

Supportive diagnostic features include:

1.11. Alzheimer's Disease Tests

Determining whether someone has Alzheimer's disease (AD) is not an exact science. There are several tests that can help ensure an accurate diagnosis. These include:

- brain imaging
- genetic testing

- neuropsychological testing

The tests can also help rule out other possible conditions and diseases.

1.12 Types of brain imaging

MRI (magnetic resonance imaging)

An MRI takes multiple images of the brain using powerful magnets and radio waves. It can help detect:

- cysts
- tumors
- bleeding
- swelling
- structural abnormalities
- infections
- inflammatory conditions
- problems with the blood vessels

It's a pain-free, noninvasive procedure. It usually takes from 30 minutes to two hours. You lie down on a table that slides into the MRI machine. You may have a contrast dye injected into your arm to enhance the images. You will have to remove all metallic objects, such as:

- jewelry
- eyeglasses
- hair clips

In terms of preparation, you may be asked to fast, or not to eat or drink anything, for four to six hours before the MRI.

Be sure to let the doctor know ahead of time if you are uncomfortable in small spaces. They can prescribe medication to help you relax, or recommend an “open” MRI. An open MRI is less visually confining than the standard MRI machine. People with cardiac pacemakers typically cannot have an MRI and should not enter an MRI area. Make sure you tell your doctor if you have a cardiac pacemaker. You will be advised on your particular circumstances. You may not be able to have an MRI if you have any of the following metallic objects in your body:

- brain aneurysm clips
- certain types of artificial heart valves
- heart defibrillator or pacemaker
- inner ear, or cochlear, implants
- recently placed artificial joints
- certain types of vascular stents

1.13 CT (computed tomography) scan

A CT scan uses x-ray technology to create multiple images of the brain. It helps detect conditions like:

- bleeding in the brain
- inflammation
- skull fractures
- blood clots
- strokes
- brain tumors
- enlarged brain cavities
- other signs of brain disease

A CT scan is a pain-free and noninvasive test that last a few minutes. Like the MRI, you will lie down on a table that slides into the CT machine. You have to lie still during the procedure and may have to hold your breath for short periods of time. You may be asked to wear a hospital gown

and remove all metal objects. You may have a contrast dye injected into your arm to enhance the images. In terms of preparation, you may be asked to fast for four to six hours beforehand.

PET (positron emission tomography) scan: According to the Alzheimer's Association, research studies have shown that amyloid plaque buildup can bedetected with PET scan technology, even beforesymptoms are evident. It's still unknown if these plaques arerisk factors for AD, the result of the disease, or some combination thereof. Using PET scans as an early

detection diagnostic tool is still being developed and isn't ready for use by general practice clinicians. If you have diabetes, be sure to share that information with your doctor. Blood sugar or insulin levelsResearchers now know of 10 genes believed to be associated with AD. The most notable is the gene apolipoprotein E (APOE). While genetic blood tests are available, they don't provide a definitive diagnosis. Additionally, having "AD genes" only increases your risk of developing AD. It doesn't mean you have the disease. There are people with the AD genes who never develop AD.

1.14. Genetic testing (blood tests)

A positron emission tomography (PET) scan is an imaging test that can provide information on how the brain and its tissues function on a cellular level. It's used to detect changes in bodily processes that can reveal abnormalities of brain function. These include changes in:

- glucose metabolism
- oxygen metabolism
- blood flow

Like the MRI and CT scan, you will need to lie down on a table that slides into the PET scan machine. About an hour prior to the PET scan, you will be injected with or asked to inhale a small amount of radioactive material, called a "tracer." You may be asked to perform various mental tasks, such as reading or naming letters. This diagnostic tool allows the doctor to see levels of brain activity. Being required to fast for four to six hours prior to the test is not unusual. This test usually takes between 30 minutes and two hours.

Early-onset AD genetic blood test

Studies of families with a history of early-onset AD have identified defects in three different genes. They are *APP* (on chromosome 21), *PSEN1* (on chromosome 14), and *PSEN2* (on chromosome 1). People with mutations on one or more of these genes tend to develop early-onset AD. All of these can be detected with a specialized genetic blood test. There are people who suffer from early-onset AD who don't have mutations in any of these genes.

1.15 Prenatal diagnosis

Additionally, prenatal diagnosis using amniocentesis during pregnancies can detect an increased risk for the *PSEN1* mutation. However, this test is unlikely to be performed unless a family member has been diagnosed with the genetic mutation. The presence of a mutation does not guarantee an individual will develop Alzheimer's disease.

Neuropsychological testing

The most commonly used neuropsychological test is the mini-mental state exam (MMSE). During the MMSE, you are asked questions and given instructions designed to evaluate your basic mental status. You may be asked the day's date and the date of your birthday. You may also be asked to repeat a list of words or phrases and count backward from 100 by sevens. No advanced preparation is needed for this test.

1.16. Different Hypothesis for the Pathogenesis of Alzheimer's disease:

Cholinergic Hypothesis:

A variety of neuropsychiatric symptoms occur in Alzheimer's disease (AD) including agitation, psychosis, depression, apathy, disinhibition, anxiety, purposeless behavior, and disorders of sleep and appetite. Neuropsychiatric symptoms have been related to cholinergic deficiency and improve after treatment with cholinomimetic agents. Cholinergic drugs are unique among psychotropic agents in exerting disease-specific and broad-spectrum effects. These observations provide the basis for the cholinergic hypothesis of the neuropsychiatric symptoms of AD, suggesting that the cholinergic deficit of AD contributes to the neuropsychiatric symptoms of AD and that cholinomimetic therapy ameliorates the behavioral disturbances accompanying AD. [15]

Amyloid Hypothesis:

Perhaps the single greatest obstruction to maintaining a healthy brain with advancing age is the insidious accumulation of the pathological lesions that define Alzheimer's disease (AD), the most common form of dementia in the elderly. With the increasing longevity of our population, AD is already approaching epidemic proportions with no cure or preventative therapy yet available. AD is a progressive neurodegenerative disorder characterized by global cognitive decline involving memory, orientation, judgment, and reasoning. The disease is named after Alois Alzheimer, a Bavarian psychiatrist with expertise in neuropathology, whose 1906 meeting presentation of his patient, Auguste D., fueled a major paradigm shift in how we think about mental disorders. Auguste D. was a 51-year-old woman admitted to an asylum for "delerium and frenzied jealousy of her husband." Given her relatively young age of 51, she was diagnosed with what we would now refer to as "presenile dementia." In his presentation of this patient, Alzheimer made the then bold assertion that her dementia was intimately related to gross neuropathological lesions that he observed in her autopsied brain: "miliary bodies" and nerve cells whose interiors were choked by "dense bundles of fibrils." This postulate was put forward in the early days of what could be considered the "clinicopathological era" of neurological and psychiatric disease, when scientists were attempting to correlate clinical symptoms with pathological features. While the unfamiliar notion that a "mental" disorder like presenile dementia could be due to "physical" aberrations was not readily accepted at the time, the disorder would, nonetheless, be named in 1910 after Alois Alzheimer by his mentor, Emil Kraepelin.

By the end of the 1960's, autopsy of brains taken from elderly individuals who suffered from dementia would reveal that "senility" was not simply a function of advanced age but, in most cases, was consistent with the same disease presented by Alzheimer in 1906. Clearly visible upon autopsy examination of most cases of senility at the light microscopic level were extracellular deposits of β-amyloid (Alzheimer's "miliary foci") and intracellular deposits of neurofibrillary tangles (Alzheimer's "dense bundles of fibrils"). Abundant amounts of these lesions in the brain are necessary for a confirmed diagnosis of AD. Studies of the etiology of AD were not particularly fruitful over most of the 20th century, and the majority of AD cases display no discernible mode of inheritance. However, in 1981, Heston et al. first reported that relatives of 125 subjects who had autopsy-confirmed AD exhibited a significant excess of dementing illness consistent with genetic

transmission (Heston et al., 1981). Interestingly, in that same seminal study, it was observed that when compared to controls, the relatives of affected individuals derived from families with a significantly greater incidence of Down's syndrome (DS, or trisomy 21). While this connection is still not fully understood, it was particularly interesting given the high incidence of Alzheimer-type neuropathology that is inevitably observed in the brains of middle-aged patients with DS. Taken together, these observations first suggested a possible genetic link between AD and an abnormal gene or structural defect on chromosome 21. The relationship between chromosome 21 and AD pathology would become clearer a decade later, but not without the advent in the mid-1980s of critical biochemical data emanating from the analysis of AD-related β-amyloid deposits. [18-21]

Oxidative Stress Hypothesis:

Research in Alzheimer disease has recently demonstrated compelling evidence on the importance of oxidative processes in its pathogenesis. Cellular changes show that oxidative stress is an event that precedes the appearance of the hallmark pathologies of the disease, neurofibrillary tangles, and senile plaques. While it is still unclear what the initial source of the oxidative stress is in Alzheimer disease, it is likely that the process is highly dependent on redox-active transition metals such as iron and copper. Further investigation into the role that oxidative stress mechanisms seem to play in the pathogenesis of Alzheimer disease may lead to novel clinical interventions. [22-24]

Tau Hypothesis:

During the past decade, hypotheses concerning the pathogenesis of most neurodegenerative diseases have been dominated by the notion that the aggregation of specific proteins and subsequent formation of cytoplasmic and extracellular lesions represent a harbinger of neuronal dysfunction and death. As such, in Alzheimer's disease, phosphorylated tau protein, the major component of neurofibrillary tangles, is considered a central mediator of disease pathogenesis. We challenge this classic notion by proposing that tau phosphorylation represents a compensatory response mounted by neurons against oxidative stress and serves a protective function. This novel concept, which can also be applied to protein aggregates in other neurodegenerative diseases, opens a new window of knowledge with broad implications for both the understanding of

mechanisms underlying disease pathophysiology and the design of new therapeutic strategies. [25-26]

Inflammatory Hypothesis:

Alzheimer's disease is aetiologically heterogeneous, but the pathogenesis is often considered to be initiated by the deposition of amyloid fibrils, followed by neuritic tau pathology and neuronal death. A variety of inflammatory proteins has been identified in the brains of patients with Alzheimer's disease *postmortem*. In this article, Piet Eikelenboom and colleagues review evidence to suggest that the inflammatory processes are intimately involved in several crucial events in the pathological cascade. This suggests possibilities for the treatment of Alzheimer's disease with anti-inflammatory drugs. [27-29]

1.17. What Causes Alzheimer's Disease?

Scientists believe that many factors influence when Alzheimer's disease begins and how it progresses. Increasing age is the most important known risk factor for Alzheimer's. The number of people with the disease doubles every 5 years beyond age 65. About one-third of all people age 85 and older may have Alzheimer's disease. The causes of late-onset Alzheimer's, the most common form of the disease, probably include a combination of genetic, lifestyle, and environmental factors. The importance of any one of these factors in increasing or decreasing the risk of development Alzheimer's may differ from person to person.

Scientists are learning how age-related changes in the brain may harm nerve cells and contribute to Alzheimer's damage. These age-related changes include atrophy (shrinking) of certain parts of the brain, inflammation, production of unstable molecules called free radicals, and breakdown of energy production within cells. As scientists learn more about this devastating disease, they realize that genes also play an important role.

Genetics 101

Each human cell contains the instructions a cell needs to do its job. These instructions are made up of DNA, which is packed tightly into structures called chromosomes. Each chromosome has thousands of segments called genes. Genes are passed down from a person's birth parents. They carry information that defines traits such as eye color and height. Genes also play a role in keeping the body's cells healthy. Problems with genes—even small changes to a gene—can cause diseases like Alzheimer's disease. [30,31]

1.18. The Genetics of Disease

Some diseases are caused by a genetic mutation, or permanent change in one or more specific genes. If a person inherits from a parent a genetic mutation that causes a certain disease, then he or she will usually get the disease. Sickle cell anemia, cystic fibrosis, and early-onset familial Alzheimer's disease are examples of inherited genetic disorders.

In other diseases, a genetic variant may occur. A single gene can have many variants. Sometimes, this difference in a gene can cause a disease directly. More often, a variant plays a role in increasing or decreasing a person's risk of developing a disease or condition. When a genetic variant increases disease risk but does not directly cause a disease, it is called a genetic risk factor.

Alzheimer's Disease Genetics

There are two types of Alzheimer's—early-onset and late-onset. Both types have a genetic component. [32-36]

Late-Onset Alzheimer's Disease

Most people with Alzheimer's have the late-onset form of the disease, in which symptoms become apparent in the mid-60s.

Researchers have not found a specific gene that directly causes the late-onset form of the disease. However, one genetic risk factor—having one form of the apolipoprotein E (APOE) gene on chromosome 19—does increase a person's risk. APOE comes in several different forms, or alleles:

- APOE ε2 is relatively rare and may provide some protection against the disease. If Alzheimer's disease occurs in a person with this allele, it usually develops later in life than it would in someone with the APOE ε4 gene.
- APOE ε3, the most common allele, is believed to play a neutral role in the disease—neither decreasing nor increasing risk.
- APOE ε4 increases risk for Alzheimer's disease and is also associated with an earlier age of disease onset. A person has zero, one, or two APOE ε4 alleles. Having more APOE ε4 alleles increases the risk of developing Alzheimer's.

APOE ε4 is called a risk-factor gene because it increases a person's risk of developing the disease. However, inheriting an APOE ε4 allele does not mean that a person will definitely develop Alzheimer's. Some people with an APOE ε4 allele never get the disease, and others who develop Alzheimer's do not have any APOE ε4 alleles.

Early-Onset Alzheimer's Disease

Early-onset Alzheimer's disease occurs between a person's 30s to mid-60s and represents less than 10 percent of all people with Alzheimer's. Some cases are caused by an inherited change in one of three genes, resulting in a type known as early-onset familial Alzheimer's disease, or FAD. For other cases of early-onset Alzheimer's, research suggests there may be a genetic component related to factors other than these three genes.

A child whose biological mother or father carries a genetic mutation for early-onset FAD has a 50/50 chance of inheriting that mutation. If the mutation is in fact inherited, the child has a very strong probability of developing early-onset FAD.

Early-onset FAD is caused by any one of a number of different single gene mutations on chromosomes 21, 14, and 1. Each of these mutations causes abnormal proteins to be formed. Mutations on chromosome 21 cause the formation of abnormal amyloid precursor protein (APP). A mutation on chromosome 14 causes abnormal presenilin 1 to be made, and a mutation on chromosome 1 leads to abnormal presenilin 2.

Each of these mutations plays a role in the breakdown of APP, a protein whose precise function is not yet fully understood. This breakdown is part of a process that generates harmful forms of amyloid plaques, a hallmark of Alzheimer's disease.

1.19 Health, Environmental, and Lifestyle Factors

Research suggests that a host of factors beyond genetics may play a role in the development and course of Alzheimer's disease. There is a great deal of interest, for example, in the relationship between cognitive decline and vascular conditions such as heart disease, stroke, and high blood pressure, as well as metabolic conditions such as diabetes and obesity. Ongoing research will help us understand whether and how reducing risk factors for these conditions may also reduce the risk of Alzheimer's.

A nutritious diet, physical activity, social engagement, and mentally stimulating pursuits have all been associated with helping people stay healthy as they age. These factors might also help reduce the risk of cognitive decline and Alzheimer's disease. Clinical trials are testing some of these possibilities.

Treatments for Alzheimer's disease:

Currently, there is no cure for Alzheimer's. But drug and non-drug treatments may help with both cognitive and behavioral symptoms. Researchers are looking for new treatments to alter the course of the disease and improve the quality of life for people with dementia.

1.20 Types of drugs:

a. Cholinesterase inhibitors (Aricept, Exelon, Razadyne)

b. Memantine(Namenda) — to treat the cognitive symptoms (memory loss, confusion, and problems with thinking and reasoning) of Alzheimer's disease.

There is also a medication that combines one of the cholinesterase inhibitors (donepezil) with memantine called Namzaric.
Three cholinesterase inhibitors are commonly prescribed:

- Donepezil (Aricept) is approved to treat all stages of Alzheimer's.
- Rivastigmine (Exelon) is approved to treat mild to moderate Alzheimer's.
- Galantamine (Razadyne) is approved to treat mild to moderate Alzheimer's.

Donepezil:

Donepezil (Aricept), is a centrally acting reversible acetyl cholinesterase inhibitor. Its main therapeutic use is in the treatment of Alzheimer's disease where it is used to increase cortical acetylcholine. Donepezil is postulated to exert its therapeutic effect by enhancing cholinergic function. This is accomplished by increasing the concentration of acetylcholine through reversible inhibition of its hydrolysis by acetylcholinesterase. If this proposed mechanism of action is correct, donepezil's effect may lessen as the disease process advances and fewer cholinergic neurons remain functionally intact. Donepezil has been tested in other cognitive disorders including Lewy body dementia and Vascular dementia, but it is not currently approved for these indications. Donepezil has also been studied in patients with Mild Cognitive Impairment, schizophrenia, attention deficit disorder, post-coronary bypass cognitive impairment, cognitive impairment associated with multiple sclerosis, and Down syndrome. [36,37]

Galantamine:

A benzazepine derived from norbelladine. It is found in galanthus and other amaryllidaceae. Galantamine is a cholinesterase inhibitor that has been used to reverse the muscular effects of gallamine triethiodide and tubocurarine, and has been studied as a treatment for Alzheimer's disease and other central nervous system disorders. [38,39]

Rivastigmine:

Rivastigmine is a parasympathomimetic or cholinergic agent for the treatment of mild to moderate dementia of the Alzheimer's type. Rivastigmine is a cholinesterase inhibitor that inhibits both butyrylcholinesterase and acetylcholinesterase. [40,41]

Memantine:

Memantine is an amantadine derivative with low to moderate-affinity for NMDA receptors. It is a noncompetitive NMDA receptor antagonist that binds preferentially to NMDA receptor-operated cation channels. It blocks the effects of excessive levels of glutamate that may lead to neuronal dysfunction. It is under investigation for the treatment of Alzheimer's disease, but there has been no clinical support for the prevention or slowing of disease progression. [42,43]

Antihypertensive drugs:

The most frequently studied antihypertensive agents were calcium channel blockers (7 studies), diuretics (6 studies), and angiotensin-converting enzyme (ACE) inhibitors (6 studies). Overall, these medications appeared to be beneficial in dementia, but only ACE inhibitors and diuretics significantly reduced the risk for and progression of dementia in the majority of studies. Antihypertensive medications—particularly ACE inhibitors and diuretics—may be helpful in reducing the risk for and progression of dementia. Large randomized clinical trials are warranted to further explore the relationship between antihypertensive drugs and dementia.

Secretase inhibitor:

The 4-kDa amyloid β-peptide (Aβ) is strongly implicated the pathogenesis of Alzheimer's disease (AD), and this peptide is cut out of the amyloid β-protein precursor (APP) by the sequential action of β- and γ-secretases. γ-Secretase is a membrane-embedded protease complex that cleaves the transmembrane region of APP to produce Aβ, and this protease is a top target for developing AD therapeutics. A number of inhibitors of the γ-secretase complex have been identified, including peptidomimetics that block the active site, helical peptides that interact with the initial substrate docking site, and other less peptide-like, more drug-like compounds. To date, one γ-secretase inhibitor has advanced into late-phase clinical trials for the treatment of AD, but serious concerns remain. The γ-secretase complex cleaves a number of other substrates, and γ-secretase inhibitors cause *in vivo* toxicities by blocking proteolysis of one essential substrate, the Notch receptor. Thus, compounds that modulate γ-secretase, rather than inhibit it, to selectively alter Aβ production without hindering signal transduction from the Notch receptor would be more ideal. Such modulators have been discovered and advanced, with one compound in late-phase clinical trials, renewing interest in γ-secretase as a therapeutic target.

Anti-inflammatory drugs:

evaluate the role of nonsteroidal anti-inflammatory drugs (NSAIDs) on clinical features and progression of the disease. Patients taking NSAIDs or aspirin on a daily basis (N = 32) to non-NSAID patients (N = 177) on clinical, cognitive, and psychiatric measures. The NSAID group had a significantly shorter duration of illness at study entry. Even after controlling for this difference, the NSAID group performed better on the Mini-Mental State Examination, Boston Naming Test, and the delayed condition of the Benton Visual Retention Test. Furthermore, analysis of longitudinal changes over 1 year revealed less decline among NSAID patients than among non-NSAID patients on measures of verbal fluency, spatial recognition, and orientation. These findings support other recent studies suggesting that NSAIDs may serve a protective role in Alzheimer's disease.

Etanercept:

TNF-alpha inhibitor that acts by blocking the binding of this cytokine to its receptors. This outcome is concordant with recent basic science studies suggesting that TNF-alpha functions *in vivo* as a gliotransmitter that regulates synaptic function in the brain. We hypothesized that

perispinal etanercept had the potential to improve verbal function in Alzheimer's disease, so we included several standarized measures of verbal ability to evaluate language skills in a clinical trial of perispinal etanercept for Alzheimer's disease.

1.21.Brain-Derived Neurotrophic Factor:

Brain-derived neurotrophic factor (BDNF) has a neurotrophic support on neuron of central nervous system (CNS) and is a key molecule in the maintenance of synaptic plasticity and memory storage in hippocampus. However, changes of BDNF level and expression have been reported in the CNS as well as blood of Alzheimer's disease (AD) patients in the last decade, which indicates a potential role of BDNF in the pathogenesis of AD. Therefore, this review aims to summarize the latest progress in the field of BDNF and its biological roles in AD pathogenesis. We will discuss the interaction between BDNF and amyloid beta (Aβ) peptide, the effect of BDNF on synaptic repair in AD,

and the association between BDNF polymorphism and AD risk. The most important is, enlightening the detailed biological ability and complicated mechanisms of action of BDNF in the context of AD would provide a future BDNF-related remedy for AD, such as increment in the production or release of endogenous BDNF by some drugs or BDNFmimics.

1.22 Immunizations:

AD pathogenesis has been associated with the accumulation, aggregation, and deposition of amyloid beta (Abeta) peptides in the brain. Hallmarks of AD are the amyloid plaques consisting of fibrillar Abeta and neurofibrillary tangles which are intracellular fibrils of hyperphosphorylated tau protein that develop later in this disease. The amyloid cascade hypothesis postulates that Abeta deposition is an initial event in the multifactorial pathogenesis and Abeta deposition may precede AD symptoms in some patients by at least 20 years. Amyloid beta therapy with active and passive immunizations against Abeta has a high possibility to be effective in removing Abeta from brain and might thus prevent the downstream pathology.

Flavanoids and other novel plant constituent

Huperzine:

Huperzine A may have cognition-enhancing activity, can maintains acetylcholine levels for healthy cognitive function and promotes enhanced memory and cell to cell communication,it is potentially ward off the devastating effect of the memory-robbing disease Alzheimer's.

Polyphenols:

Brain aging and the most diffused neurodegenerative diseases of the elderly are characterized by oxidative damage, redox metals homeostasis impairment and inflammation. Food polyphenols can counteract these alterations in vitro and are therefore suggested to have potential anti-aging and brain-protective activities, as also indicated by the results of some epidemiological studies. Despite the huge and increasing amount of the in vitro studies trying to unravel the mechanisms of action of dietary polyphenols, the research in this field is still incomplete, and questions about bioavailability, biotransformation, synergism with other dietary factors, mechanisms of the antioxidant activity, risks inherent to their possible pro-oxidant activities are still unanswered. Most of all, the capacity of the majority of these compounds to cross the blood–brain barrier and reach brain is still unknown. This commentary discusses recent data on these aspects, particularly focusing on effects of curcumin, resveratrol and catechins on Alzheimer's disease.

Curcumin:

Curcumin has a long history of use as a traditional remedy and food in Asia. Many studies have reported that curcumin has various beneficial properties, such as antioxidant, antiinflammatory, and antitumor. Because of the reported effects of curcumin on tumors, many clinical trials have been performed to elucidate curcumin's effects on cancers. Recent reports have suggested therapeutic potential of curcumin in the pathophysiology of Alzheimer's disease (AD).

In *in vitro* studies, curcumin has been reported to inhibit amyloid-β-protein (Aβ) aggregation, and Aβ-induced inflammation, as well as the activities of β-secretase and acetylcholinesterase. In *in vivo* studies, oral administration of curcumin has resulted in the inhibition of Aβ deposition, Aβ oligomerization, and tau phosphorylation in the brains of AD animal models, and improvements in behavioral impairment in animal models. These findings suggest that curcumin might be one of the most promising compounds for the development of AD therapies. At present, four clinical trials concerning the effects of curcumin on AD has been conducted. Two of them that were performed in China and USA have been reported no significant differences in changes in cognitive function between placebo and curcumin groups, and no results have been reported from two other clinical studies. Additional trials are necessary to determine the clinical usefulness of curcumin in the prevention and treatment of AD.

Resveratrol

Resveratrol, a red wine polyphenol, is known to protect against cardiovascular diseases and cancers, as well as to promote antiaging effects in numerous organisms. It also modulates pathomechanisms of debilitating neurological disorders, such as strokes, ischemia, and Huntington's disease. The role of resveratrol in Alzheimer's disease is still unclear, although some recent studies on red wine bioactive compounds suggest that resveratrol modulates multiple mechanisms of Alzheimer's disease pathology. Emerging literature indicates that mechanisms of aging and Alzheimer's disease are intricately linked and that these mechanisms can be modulated by both calorie restriction regimens and calorie restriction mimetics, the prime mediator of which is the SIRT1 protein, a human homologue of yeast silent information regulator (Sir)-2, and a

member of NAD^+-dependent histone deacetylases. Calorie restriction regimens and calorie restriction-mimetics trigger sirtuins in a wide variety of organisms, ranging from bacteria to mouse. In a mouse model of Huntington's disease, resveratrol-induced SIRT1 was found to protect neurons against ployQ toxicity and in Wallerian degeneration slow mice, resveratrol was found to protect the degeneration of neurons from axotomy, suggesting that resveratrol may possess therapeutic value to neuronal degeneration. This paper mainly focuses on the role of resveratrol in modulating AD pathomechanisms.

Tacrine:

Tacrine produced an improvement in key outcome measures roughly equivalent to the deterioration which might have occurred over 6-12 months. The clinical relevance of the findings is a matter for individual judgment.

Herbal supplement

Ginkgo biloba:

The flavonoid components of ginkgo appear to be useful in animal models in preventing some types of oxidative and peroxidative neuronal injury. Another hypothesis of a cause of AD centers around an inflammatory process.[20,21] Ginkgo being a PAF antagonist has anti-inflammatory effects. Another reason for the plausibility of use of ginkgo in individuals with AD also relates to its activity as a PAF antagonist. The effect of PAF antagonism directly on brain function is fairly unexplored.

Panax ginseng:

After ginseng treatment, the cognitive subscale of ADAS and the MMSE score began to show improvements and continued up to 12 weeks (P=0.029 and P=0.009 vs. baseline, respectively). After discontinuing ginseng, the improved ADAS and MMSE scores declined to the levels of the control group. These results suggest that *Panax ginseng* is clinically effective in the cognitive performance of AD patients .

Withania somnifera :

W. somnifera mediated through up-regulation of liver LRP indicates that targeting the periphery offers a unique mechanism for Aβ clearance and reverses the behavioral deficits and pathology seen in Alzheimer's disease models.

Nutrients:

Alpha-lipoic acid:

Oxidative stress and energy depletion are characteristic biochemical hallmarks of Alzheimer's disease (AD), thus antioxidants with positive effects on glucose metabolism such as thioctic (α-lipoic) acid should exert positive effects in these patients.

Alpha-lipoic acid(Chemical structure)

Omega-3 Fatty Acid :

Epidemiological studies suggest that increased intake of the omega-3 (n-3) polyunsaturated fatty acid (PUFA) docosahexaenoic acid (DHA) is associated with reduced risk of Alzheimer's disease (AD). DHA levels are lower in serum and brains of AD patients, which could result from low dietary intake and/or PUFA oxidation.

Acetyl-L-carnitine:

Normalization of high-energy phosphate levels was observed in the acetyl-L-carnitine-treated but not in the placebo-treated patients. This is the first direct in vivo demonstration of a beneficial effect of a drug on both clinical and CNS neurochemical parameters in AD.

Vitamins and minerals

Vitamin B_{12}:

Vitamin B_{12} may help protect the brain against Alzheimer's disease, according to new evidence that suggests the vitamin and an amino acid called homocysteine may both be involved in the development of Alzheimer's.

Vitamin A:

The deposition of amyloid β-protein (Aβ) in the brain is an invariant feature of Alzheimer's disease (AD). Vitamin A, which has been traditionally considered an anti-oxidant compound, plays a role in maintaining higher function in the central nervous system. Plasma or cerebrospinal fluid concentrations of vitamin A and β-carotene have been reported to be lower in AD patients.

Vitamin E :

It has been suggested that oxidative stress may contribute to the pathogenesis of Alzheimer's disease. Vitamin E is a potent antioxidant, and the results of some epidemiological studies have suggested that high intake of vitamin E through food is inversely associated with the incidence of Alzheimer's disease. Randomized controlled studies have shown that treatment with vitamin E could delay functional decline in patients with mild to moderate Alzheimer's disease. However, vitamin E had no cognitive benefits in patients with mild cognitive impairment or in generally healthy older women. Well-designed clinical trials or preventive interventions with vitamin E are necessary to establish its efficacy as therapeutic or preventive agents for Alzheimer's disease.

Lithium:

Lithium is a well-established therapeutic option for the acute and long-term management of bipolar disorder and major depression. More recently, based on findings from translational research, lithium has also been regarded as a neuroprotective agent and a candidate drug for disease-modification in certain neurodegenerative disorders, namely, Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and, more recently, Parkinson's disease (PD). The putative neuroprotective effects of lithium rely on the fact that it modulates several homeostatic mechanisms involved in neurotrophic response, autophagy, oxidative stress, inflammation, and mitochondrial function. Such a wide range of intracellular responses may be secondary to two key effects, that is, the inhibition of glycogen synthase kinase-3 beta (GSK-3β) and inositol monophosphatase (IMP) by lithium.

Hormones

Melatonin:

It's a powerful antioxidant, acting to counter harmful chemical reactions that can damage cells.It appears to have specific neuroprotective qualities, meaning that it helps protect nerve and brain cells from damage. There's also evidence that having an insufficiency of melatonin plays a role in depression.

Cholinesterase & their mechanism:

Acetylcholinesterase :

AChE or **acetylhydrolase**, is the primary cholinesterase in the body. It is an enzyme that catalyzes the breakdown of acetylcholine and of some other choline esters that function as neurotransmitters. AChE is found at mainly neuromuscular junctions and in chemical synapses of the cholinergic type, where its activity serves to terminate synaptic transmission. It belongs to carboxylesterase family of enzymes. It is the primary target of inhibition by organophosphoruscompounds such as nerve agents and pesticides. [42,45]

Enzyme structure & mechanism:

AChE is a hydrolase that hydrolyzes choline esters. It has a very high catalyticactivity - each molecule of AChE degrades about 25000 molecules of acetylcholine(ACh) per second, approaching the limit allowed by diffusion of the substrate. The active site of AChE comprises 2 subsites - the anionic site and the esteratic subsite. The structure and mechanism of action of AChE have been elucidated from the crystal structure of the enzyme.

The anionic subsite accommodates the positive quaternary amine of acetylcholine as well as other cationic substrates and inhibitors. The cationic substrates are not bound by a negatively charged amino acid in the anionic site, but by interaction of 14 aromatic residues that line the gorge leading

to the active site. All 14 amino acids in the aromatic gorge are highly conserved across different species.[13]Among the aromatic amino acids, tryptophan 84 is critical and its substitution with alanine results in a 3000-fold decrease in reactivity.[14] The gorge penetrates halfway through the enzyme and is approximately 20 angstroms long. The active site is located 4 angstroms from the bottom of the molecule. [46-48]

The esteratic subsite, where acetylcholine is hydrolyzed to acetate and choline, contains the catalytic triad of three amino acids: serine 200, histidine 440 and glutamate 327. These three amino acids are similar to the triad in other serine proteasesexcept that the glutamate is the third member rather than aspartate. Moreover, the triad is of opposite chirality to that of other proteases.[16] The hydrolysis reaction of the carboxyl ester leads to the formation of an acyl-enzyme and free choline. Then, the acyl-enzyme undergoes nucleophilic attack by a water molecule, assisted by the histidine 440 group, liberating acetic acid and regenerating the free enzyme.

Biological function:

During neurotransmission, ACh is released from the presynaptic neuron into the synaptic cleft and binds to ACh receptors on the post-synaptic membrane, relaying the signal from the nerve. AChE, also located on the post-synaptic membrane, terminates the signal transmission by hydrolyzing ACh. The liberated choline is taken up again by the pre-synaptic neuron and ACh is synthesized by combining with acetyl-CoA through the action of choline acetyltransferase.
A cholinomimetic drug disrupts this process by acting as a cholinergic neurotransmitter that is impervious to acetylcholinesterase's lysing action.

Distribution of AChE:

AChE is found in many types of conducting tissue: nerve and muscle, central and peripheral tissues, motor and sensory fibers, and cholinergic and noncholinergic fibers. The activity of AChE is higher in motor neurons than in sensory neurons.Acetylcholinesterase is also found on the red blood cell membranes, where different forms constitute the Yt blood groupantigens.[34] Acetylcholinesterase exists in multiple molecular forms, which possess similar catalytic properties, but differ in their oligomeric assembly and mode of attachment to the cell surface.

AChE gene:

In mammals, acetylcholinesterase is encoded by a single AChE gene while some invertebrates have multiple acetylcholinesterase genes. Diversity in the transcribed products from the sole mammalian gene arises from alternative mRNA splicing and post-translational associations of catalytic and structural subunits. There are three known forms: T (tail), R (read through), and H(hydrophobic)

$AChE_T$:

The major form of acetylcholinesterase found in brain, muscle, and other tissues, known as is the hydrophilic species, which forms disulfide-linked oligomers with collagenous, or lipid-containing structural subunits. In the neuromuscular junctions AChE expresses in asymmetric form which associates with ColQ or subunit. In the central nervous system it is associated with PRiMA which stands for Proline Rich Membrane anchor to form symmetric form. In either case, the ColQ or PRiMA anchor serves to maintain the enzyme in the intercellular junction, ColQ for the neuromuscular junction and PRiMA for synapses.

$AChE_H$:

The other, alternatively spliced form expressed primarily in the erythroid tissues, differs at the C-terminus, and contains a cleavable hydrophobic peptide with a PI-anchor site. It associates with membranes through the phosphoinositide (PI) moieties added post-translationally.[

$AChE_R$

The third type has, so far, only been found in *Torpedo* sp. and mice although it is hypothesized in other species. It is thought to be involved in the stress response and, possibly, inflammation.

Butyrylcholinesterase:

It is very similar to the neuronal acetylcholinesterase, which is also known as RBC or erythrocyte cholinesterase. The term "serum cholinesterase" is generally used in reference to a clinical test that reflects levels of both of these enzymes in the blood. Assay of butyrylcholinesterase activity in plasma can be used as a liver function test as both hypercholinesterasemia and hypocholinesterasemia indicate pathological processes. The half-life of BCHE is approximately 10 to 14 days. Butyrylcholine is a synthetic compound that does not occur in the body naturally. It is used as a tool to distinguish between acetylcholinesterase and butyrylcholinesterase. [50]

Fungus Introduction and Literature Review

Plant Name: Pleurotus ostreatus.

Alternative Titles:

Oyster mushroom**.** Both the Latin and common names refer to the shape of the fruiting body. The Latin pleurotus (sideways) refers to the sideways growth of the stem with respect to the cap, while the Latin ostreatus (and the English common name, oyster) refers to the shape of the cap which resembles the bivalve of the same name. Many also believe that the name is fitting due to a flavor resemblance to oysters. The name oyster mushroom is also applied to other Pleurotus species, so P. ostreatus is sometimes referred to as the tree oyster mushroom or the grey oyster mushroom to differentiate it from other species in the genus.

Common Names of Pleurotus ostreatus(Oyster Mushrooms):

Pearl Oyster – *Pleurotus ostreatus*

Pearl Oyster mushrooms are also an abundant native to North America, found locally on hardwood snags, stumps and logs. Oyster mushrooms have a milder flavor and more tender consistency than Shiitake but they do hold their own. With a sweet woodsy taste, Oyster mushrooms are also quite versatile and substitute well into many mushroom recipes. People consider them quite exemplary in any egg dish, great in omelets, quiches and fritattas.

Golden Oyster – *Pleurotus citrinopileatus*

The Golden Oyster is native to northern areas in the Asian continent it is a popular cultivar in China. An aggressive saprophytic mushroom, there are reports of it naturalizing in North America. It boasts vibrant yellow clusters of mushrooms with a thin delicate flesh. The Golden oyster is distinctly fragrant and offers a complex but subtle aromatic flavor. They are great braised or in soups or stir fry. A farm favorite is on a white pizza with an olive oil garlic base, mozzarella and diced sweet red peppers. As with the other oysters, the Golden oyster also excels in egg dishes, cream sauces, or sautéed until crispy and served as a garnish.

Blue Oyster – *Pleurotus ostreatus var columbines*

The Blue Oyster mushroom is a sub species of the Pearl Oyster that exhibits a notable blue-gray hue. The color contrast between the darker caps and pale gills give them a truly stunning appearance. Native to Western Europe, the Blue Oyster thrives in cooler temperatures, helping Mycoterra Farm extend our growing season. Easily grown on rye grain and straw, our production of the Blue Oyster is identical to the process we use for other Oyster species. Look for them at one of our winter markets, or in the early spring or late fall at our main season markets.We've found the Blue Oyster is identical in taste and texture to the Pearl Oyster and have yet to be able to distinguish the two in a blind taste test. They are just as versatile in a wide range of recipes

Pink Oyster- *Pleurotus salmoneo stramineus*

The Pink Oyster mushroom boasts a vibrant pink color and ruffled appearance. Other common names include Flamingo Oyster, Salmon Oyster and Strawberry Oyster. Native to the tropics, the Pink Oyster fruits abundantly in warmer temperatures; we've found it extremely productive mid-summer when other varieties are stunted by extreme heat. As with our other Oyster species, we produce the pink oyster on rye grain and pasteurized straw. Although similar to other Oysters in flavor, The Pink Oyster mushroom tends to be more pungent and woody with a tougher texture. Unfortunately the pink color fades upon cooking. In soups, the Pink oyster is a great addition to potato leek soup or substitute for the seafood component in a cream based chowder recipe.

The Fungus Family Pleurotaceae:

The Pleurotaceae are a family of small to medium-sized mushrooms which have white spores. The family contains four genera and 94 species. Members of Pleurotaceae can be mistaken for members of Marasmiaceae. Perhaps the best known member is the oyster mushroom, Pleurotus ostreatus.Many species in the genera Pleurotus and Hohenbuehelia are nematophagous, that is, they derive nutrition by consuming nematodes. This is made possible by hyphae that may have adhesive knobs that attach to passing nematodes and secrete nematotoxic compounds.

Classification of Plant Family:

Genus: Antromycopsis. It is a genus of fungi in the Pleurotaceae family. The genus,an anamorphic form of Pleurotus, has a widespread distribution and contains three species.

Fungus names

A.fuscosquamulosa	*A. guzmanii*	*A.macrocarpa*

Genus: Hohenbuehelia. It is a pleurotoid genus of agaric fungi characterized by gelatinous-sheathed bowling-pin-shaped cystidia, on conidia, basidiospore germ tubes, and mycelium that adhere to and capture nematodes. The fruitbodies bear thick-walled cystidia (metuloids) in the hymenium along the gill sides and that differentiate the genus from Pleurotus in the Pleurotaceae family. The genus has a widespread distribution and contains about 50 species.

Fungus names

H. abietina	***H. aciculospora***	***H. amazonica***
H. angustata	***H. approximans***	***H. atrocoerulea***
H. aurantiocystis	***H. auriscalpium***	***H. austrocedri***
H. barbatula	***H. brunnea***	***H. unguicularis***
H. tropicalis	***H. tremula***	***H. testudo***
H. silvana	***H. sciadia***	***H. reniformis***
H. podocarpinea	***H. recedens***	***H. pinicola***
H. pinacearum	***H. minutissima***	***H. myxotricha***
H. panelloides	***H. luteola***	***H. longipes***
H. leightonii	***H. izonetae***	***H. inversa***
H. espeletiae	***H. culmicola***	***H. cyphelliformis***
H. delasotae	***H. elegans***	***H. fluxilis***

Genus: Pleurotus.It is a genus of gilled mushrooms which includes one of the most widely eaten mushrooms, P. ostreatus. Species of Pleurotusmay be called oyster, abalone, or tree mushrooms, and are some of the most commonly cultivated edible mushrooms in the world. Pleurotus fungi have been used in mycoremediation of pollutants such as petroleum and polycyclic aromatic hydrocarbons. Pleurotus means "side ear", from Greek.

Fungus Names

P. ostreatus **(oyster or pearl oyster mushroom)**	***P. pulmonarius*** **(phoenix or Indian oyster mushroom)**	***P. columbines***
P. populinus	***P. eryngii*** **(king oyster mushroom)**	***P. ferulae***
P. fossulatus	***P. nebrodensis***	***P. cornucopiae*** **(branched oyster mushroom)**
P. citrinopileatus **(golden oyster mushroom)**	***P. euosmus*** **(tarragon oyster mushroom)**	***P. djamor*** **(The pink oyster mushroom)**
P. cystidiosus **(abalone mushroom)**	***P. australis*** **(brown oyster mushroom)**	***P. tuber-regium*** **(king tuber mushroom)**

The plant Genus:

Pleurotus is a genus of gilled mushrooms which includes one of the most widely eaten mushrooms, P. ostreatus. Species of Pleurotus may be called oyster**,** abalone**,** or tree mushrooms, and are some of the most commonly cultivated edible mushrooms in the world. Pleurotus fungi have been used in mycoremediation of pollutants such as petroleum and polycyclic aromatic hydrocarbons. Pleurotus means "side ear", from Greek. The caps may be laterally attached with no stem. If there is a stem, it is normally eccentric and the gills are decurrent along it. The term pleurotoid is used for mushrooms having this general shape. The spores are smooth and elongated described as "cylindrical". Where hyphae meet, they are joined by clamp connections. Pleurotus is not considered to be a bracket fungus, and most of the species

are monomitic with a soft consistency. However, remarkably, Pleurotus dryinus can sometimes be dimitic, meaning that it has additional skeletal hyphae, which give it a tougher consistency like bracket fungi. Pleurotus fungi are found in both tropical and temperate climates throughout the world. Most species of Pleurotus are white-rot fungi on hardwood trees, although some also decay conifer wood. P. eryngii is unusual in its association with herbaceous plants, and P. tuber-regium produces underground sclerotia. In addition to being saprotrophic, all species of Pleurotus are also nematophagous, catching nematodes by paralyzing them with a toxin.

Classification for Down to Genus pleurotus:

Serial no.	Species-	Common name	Global Habitat
01.	*P. ostreatus* *P. florida*	Oyster or pearl oyster mushroom	North America and northern Eurasia
02.	*P. pulmonarius* *P. columbinus* *P. sapidus*	Phoenix or Indian oyster mushroom	North America, Eurasia, and Australasia
03.	*P. populinus*		North America
04.	*P. cornucopiae*	Branched oyster mushroom	Europe
	P. citrinopileatus	Golden oyster mushroom	Eastern Asia
	P. euosmus	Tarragon oyster mushroom	
05.	*P. djamor* *P. flabellatus* *P. salmoneo-stramineus* *P. salmonicolor*	The pink oyster mushroom	Pantropical
06.	*P. eryngii*		Europe and the Middle East
	P. ferulae	King oyster mushroom	

	P. fossulatus		Afghanistan
	P. nebrodensis		
07.	*P. cystidiosus*	Abalone mushroom	Global
	P. abalonus		Taiwan
	P. fuscosquamulosus		Africa, Europe
	P. smithii		Mexico
08.	*P. dryinus*		North America, Europe, and New Zealand
09.	*P. tuber-regium*	King tuber mushroom	Africa, Asia, Australasia
10.	*P. opuntiae*		North America, New Zealand
11.	*P. abieticola*		Asia
12.	*P. albidus*		Caribbean, Central America, South America
13.	*P. australis*	Brown oyster mushroom	Australia and New Zealand
14.	*P. purpureo-olivaceus*		Australia and New Zealand
	P. rattenburyi		

Pleurotus ostreatus Taxonomy:

Scientific classification:

Domain: Eukaryota

Kingdom: Fungi

Division: Basidiomycota

Subdivision: Agaricomycotina

Class: Agaricomycetes

Subclass: Agaricomycetidae

Order: Agaricales

Family: Pleurotaceae

Genus: *Pleurotus*

Species: *Pleurotus ostreaus*

Fungus Description:

Pleurotus ostreatus is easily recognized by the way it grows on wood in shelf-like clusters; its relatively large size; its whitish gills that run down a stubby, nearly-absent stem; and its whitish to lilac spore print. It appears between October and early April across North America, and features a brown cap. A number of very similar species are closely related, including Pleurotus pulmonarius which is often paler, and appears between late April and September, and Pleurotus populinus which is found on the wood of quaking aspen.The mushroom has a broad, fan or oyster-shaped cap spanning 5–25 cm; natural specimens range from white to gray or tan to dark-brown; the margin is inrolled when young, and is smooth and often somewhat lobed or wavy. The flesh is white, firm, and varies in thickness due to stipe arrangement. The gills of the mushroom are white

to cream, and descend on the stalk if present. If so, the stipe is off-center with a lateral attachment to wood. The spore print of the mushroom is white to lilac-gray, and best viewed on dark background. The mushroom's stipe is often absent. When present, it is short and thick.

Ecology: They are Saprobic; growing in shelf-like clusters on dead logs and living trees primarily hardwoods, but sometimes on conifers; causing a white rot; occuring late fall October through early spring early April; widely distributed in North America. The illustrated and described collections are from California and Illinois.

Chemical Reactions: KOH negative on cap surface.

Spore Print: White to faintly yellowish, or lilac

Origin and geographical distribution:

The oyster Mushroom was first described scientifically in 1775 by Dutch naturalist Nikolas Joseph Freiheer von Jacquin in 1727-1871 and named Agaricus Ostreatus. In 1871 German mycologist Paul Kummer transferred the Oyster Mushroom to the genus Pleurotus, giving it its currently accepted scientific name. It occurs throughout the Britain and Ireland as well as in most parts of mainland Europe. It is also widely distributed throughout much of Asia, including Japan, and is present in parts of North America.

Habitat:

The oyster mushroom is widespread in many temperate and subtropical forests throughout the world, although it is absent from the Pacific Northwest of North America, being replaced by P. pulmonarius and P. populinus. It is a saprotroph that acts as a primary decomposer of wood, especially deciduous trees, and beech trees in particular. It is a white-rot wood-decay fungus.The oyster mushroom is one of the few known carnivorous mushrooms. Its mycelia can kill and digest nematodes, which is believed to be a way in which the mushroom obtains nitrogen.The standard oyster mushroom can grow in many places, but some other related species, such as the

branched oyster mushroom, grow only on trees. They may be found all year round in the UK.While this mushroom is often seen growing on dying hardwood trees, it only appears to be acting saprophytically, rather than parasitically. As the tree dies of other causes, P. ostreatus grows on the rapidly increasing mass of dead and dying wood. They actually benefit the forest by decomposing the dead wood, returning vital elements and minerals to the ecosystem in a form usable to other plants and organisms. Despite this, the belief that P. ostreatus could damage New Zealand's forestry industry has led New Zealand to ban its importation.

Propagation:

Mushrooms have been recorded as a source of vegetable and medicines for human beings throughout the world. The oyster mushroom Pleurotus ostreatus is edible and an important ingredient of pizza and many other popular bakery dishes. Oyster mushroom is cultivated on different agricultural wastes due to its compatibility and produce high yield in diversified climate. Studies revealed that the joint portion of cap and stipe produced vigorous mycelium growth in minimum time, The average maximum growth was obtained on Malt Extract Agar (MEA) than on Potato Dextrose agar (PDA) medium at 25 ºC under humid (65 – 80 RH) conditions. For the substrate, out of three types of grains viz., wheat, sorghum and oat; sorghum

was found to be best for mycelium propagation and the time period for optimum growth was 7 days.

Pleurotus ostreatus symbiosis:

P. ostreatus can be found on both living and dead hosts. Though mostly saprophytic, it can become a parasite upon a stressed host. Preferred hosts include cottonwood, oak, alder, maple, aspen, ash, beech, birch, elm, willow, poplar, and sycamore. Colonies may also be found on other decomposing material such as hay bales or discarded coffee grounds. Colonies of fruiting bodies are rarely singular, and are more commonly found in shelving masses and gregarious clusters. A single infected host can be capable of producing hundreds of pounds of mushroom biomass. Mushrooms form in temperatures between 40 and 75 degrees Fahrenheit (Arora 1979, Stamets 2005). P ostreatus often forms a symbiosis with the Gram-negative soil bacteria Bradyrhizobium, in which it trades carbohydrates for atmospheric nitrogen fixed by the bacteria.It is generally reported that fungi like Pleurotus spp. can fix nitrogen (N2). The way they do it is still not clear.

The present study hypothesized that only associations of fungi and diazotrophs can fix N2. This was tested in vitro. Pleurotus ostreatus was inoculated with a bradyrhizobial strain nodulating soybean and P. ostreatus with no inoculation was maintained as a control. At maximum mycelial colonization by the bradyrhizobial strain and biofilm formation, the cultures were subjected to acetylene reduction assay (ARA). Another set of the cultures was evaluated for growth and nitrogen accumulation. Nitrogenase activity was present in the biofilm, but not when the fungus or the bradyrhizobial strain was alone. A significant reduction in mycelial dry weight and a significant increase in nitrogen concentration were observed in the inoculated cultures compared to the controls. The mycelial weight reduction could be attributed to C transfer from the fungus to the bradyrhizobial strain, because of high C cost of biological N2 fixation. This needs further investigations using 14C isotopic tracers. It is clear that mushrooms alone cannot fix atmospheric N2. But when they are in association with diazotrophs, nitrogenase activity is detected because of the diazotrophic N2 fixation. It is not the fungus that fixes N2 as reported earlier. Effective N2 fixing systems, such as the present one, may be used to

increase protein content of mushrooms. This study has implications for future identification of as yet unidentified N2 systems occurring in the environment.

Botanical Features:

The oyster mushrooms have three distinct parts- a fleshy shell or spatula shaped cap (pileus) , a short or long lateral or central stalk called stipe and long ridges and furrows underneath the pileus called gills or lamellae. The gills stretch from the edge of the cap down to the stalk and bear the spores. The spores are smooth, cylindrical and germinate very easily on any kind of mycological media within 48-96 hrs. The mycelium of Pleurotus is pure white in colour

Cap: 3–15 cm across; broadly convex, becoming flat or shallowly depressed; kidney-shaped to fan-shaped in outline, or nearly round if growing on the tops of logs; somewhat greasy when young and fresh; bald; pale to dark brown; fading to buff; sometimes fading slowly and becoming two-toned; the margin somewhat inrolled when young.

Gills: Running down the stem or pseudostem; close; short-gills frequent; whitish or with a gray tinge, becoming yellowish in age and sometimes developing brownish edges; often filled with black beetles, in my collecting areas.

Stem: Usually rudimentary and lateral or nearly absent when mushrooms are growing from the sides of logs or trees, but sometimes more or less central when growing on the tops of logs or branches; 1–7 x 1–3 cm; whitish; hairy to velvety; tough.

Flesh: Thick; white; unchanging when sliced.

Odor and Taste: Odor distinctive but hard to describe; taste mild.

Traditional Uses:

Oyster mushroom is an edible mushroom which was originated from Germany during the World War I. Pleurotus ostreatus is commonly known as Oyster Shelf, Tree Oyster, Tamogitake and Straw Mushroom, is a mushroom very similar to Pleurotus pulmonarius with some differences. The cap of Oyster mushroom are broad and fan or oyster shape. Pleurotus ostreatus prefer temperate and sub-tropical climate. This fungi grows on dead hardwood trees. It was firstly sophisticated in Germany during the World War I. It is grown economically in the world as a food. It is cultivated as similar to the king Oyster mushroom. These mushrooms are also used for industrial purposes. These fungi are cultivated worldwide generally in the India, South East Asia, Africa and Europe. Oyster mushrooms are mostly found in the Chinese foods. Due to its high amount of nutrients, it is widely popular. Oyster mushroom is sautéed with garlic and olive oil. They are loaded with various amounts of nutrients and vitamins which makes them a healthy diet. It is free from fat, cholesterol, gluten, low in calories and sodium. The iron found in Oyster mushrooms is high in comparison to the meat.

Medicinal uses of the fungi Pleurotus ostreatus:

Nutritional benefits of Oyster Mushrooms:

Oyster mushrooms are:

- Low in calories
- Fat free
- Cholersterol-free
- Gluten-free
- Very low in sodium

At the same time, they're also high in:

- Protein & fibre
- Iron, Zinc, Potassium, Phosphorus & Selenium
- Calcium
- Folic acid
- Vitamins B1, B3, B5 & B12
- Vitamin C & Vitamin D

Medicinal benefits of Oyster Mushrooms:

But the benefits don't end there. Recent studies have shown that Oyster mushrooms also have the following medicinal properties:

- Cholestoerol lowering agent
- Anti-oxidant
- Anti-cancer

Health Benefits of Oyster mushroom:

Maintain the levels of blood sugar:

The insufficient amount of insulin in the body results in diabetes. The calcium is required for the insulin secretion. Vitamin D helps to reduce inflammation and raise the insulin sensitivity. The studies shows that Vitamin D helps to prevent both type 1 and type 2 diabetes.

Immunity system:

Vitamin D helps to replicate the healthy cells and protects the autoimmune conditions such as flu and common cold. It also prevents the excessive or prolonged inflammatory responses. Inflammation is the cause for autoimmune disorders and chronic diseases such as rheumatoid arthritis, multiple sclerosis, irritable bowel syndrome, high blood pressure and digestive problems.

Cardiovascular conditions:

Besides the balance of triglycerides and cholesterol levels, Vitamin B3 helps to reduce atherosclerosis that is hardening of arteries which may lead to the heart disease. It also assists to reduce the histamine production and inflammation along with the improvement in circulation.Vitamin B3 reduces the chances of reoccurring of heart disease or cardiac arrest in those who have already experienced. Additionally, Vitamin B3 helps to treat pellagra which is the health condition that occurs from the deficiency of niacin.

Skin problems:

Vitamin B3 which is in the form of niacinamide helps to clear acne when it is applied topically in the skin. Niacin assists in the reduction of flare ups, skin inflammation, redness and irritation. It also helps to treat granuloma annulare and bullous pemphigoid which is a skin disease that blisters the skin, cause pain and infection.

Brain health:

The studies show that copper has an impact on the brain pathways such as galactose and dopamine which helps to maintain the energy, mood, focus and outlook. The deficiency of copper results in

fatigue, low metabolic activity, poor mood and problem in concentration. Copper also helps to utilize the antioxidants such as superoxide dismutase, Vitamin C, tyrosinase and ascorbate oxidase. This helps to prevent the damage made by free radicals in the brain and also slows down the process of ageing, neuro-degenerative diseases and cancer.

Cures anemia:

An inadequate amount of oxygen in the blood, low red bloods cell production and the blood loss are the causes of Anemia. Vitamin B2 helps to prevent the anemia. Vitamin B2 is vital for production of red blood cells and steroid hormone synthesis. It assists in transporting the oxygen in the body and also mobilizes iron. The people with low Vitamin B2 have the high chances of sickle cell anemia and anemia. The research shows that Vitamin B2 helps to reduce the homocysteine levels in the blood.

Provides energy:

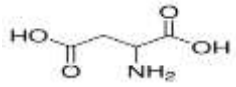
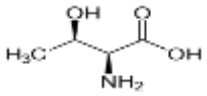
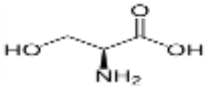
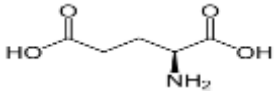
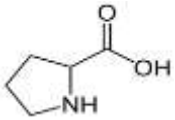
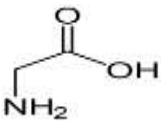
Vitamin B converts the carbohydrate into glucose which is used as a fuel to produce energy. Vitamin B synthesizes the body and metabolizes proteins and fats. Vitamin B5 assist to rebuild muscles, tissues and organs. Vitamin B5 helps to maintain the optimum level of metabolism.

Principal Constituents of Pleurotus ostreatrus:

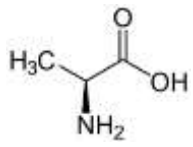
Pleurotus ostreatus was divided into two groups, one was mature group with more than 1cm diameter pileus and the other was young group with less than 1cm diameter. The contents of water, ash, minerals, free fatty acids, organic acid, free amino acids and saccharides in pileus, stipe, and base of stipe were determined. Water and ash contents were from 86 percent to 92 percent and from 0.9 percent to 1.3 percent respectively for all of the parts examined. K, P, Na, Mg, Cu, Zn and Mn were measured. The content of K (361mg/100g) was the highest, then P (121mg/100g), Na (28.0mg/100g) and Mg (17.4mg/100g) followed. As for free fatty acids, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, $C_{18'}$ and $C_{18''}$ were detected. Among them $C_{18''}$ was the main one which accounted for more than 60 percent of total fatty acid. With ten prevalent organic acids, pyroglutamic acid was the largest, followed by malic acid and succinic acid. All of them were found to be higher in a stipe part. The total free amino acid content was 337.4mg/100g in average. Twenty nine free amino acids were detected. Glutamic acid was the highest, followed by ornithine and alanine. These three amino acids accounted for about 35 percent of total free amino acid. Glutamic acid content was high in a

mature pileus and in a base of stipe. Similarly ornitine and alanine contents were high in a stipe. Mannitol was contained mainly in a base of stipe. In every part tested, mannitol was contained more than glucitol Mannose and glucose were contained mainly in a stipe part.

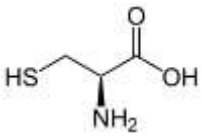
Phytochemical constituents in Pleurotus ostreatus fungus extract-

Amino Acid constituents:	**Structure:**
Aspartic acid	
Threonine	
Serine	
Glutamic acid	
Proline	
Glycine	

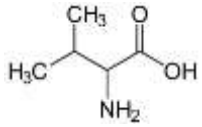
Alanine



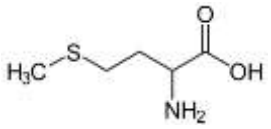
Cysteine



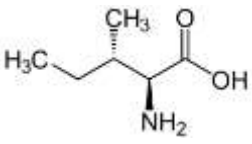
Valine



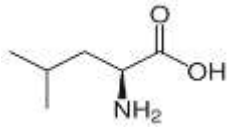
Methionine



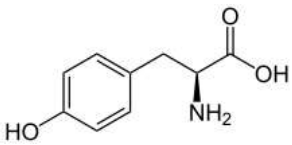
Isoleucine



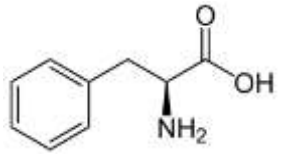
Leucine



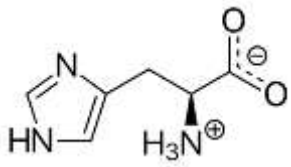
Tyrosine



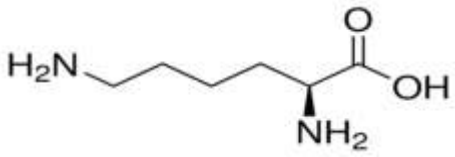
Phenylalanine



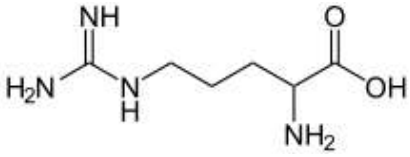
Histidine



Lysine

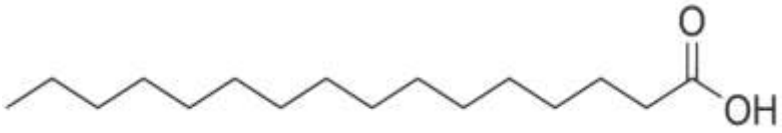


Arginine

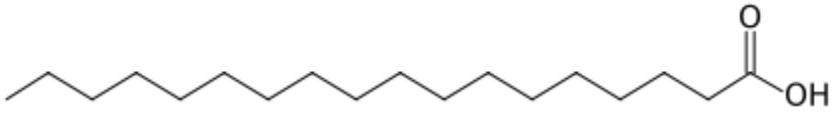


Fatty Acids constituents: **Structure:**

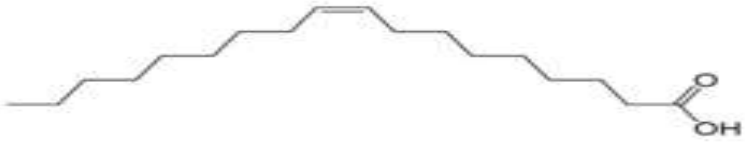
Palmitic acid



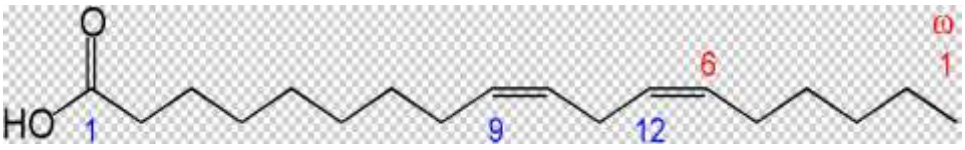
Stearic acid



Oleic acid

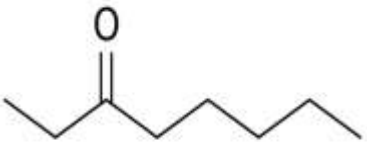


Linoleic acid

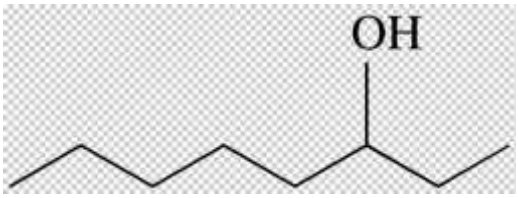


Volatile compounds: **Structures:**

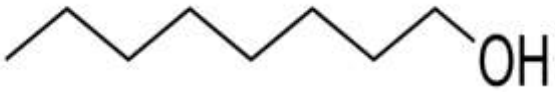
3-octanone



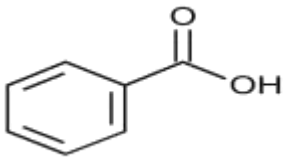
3-octanol



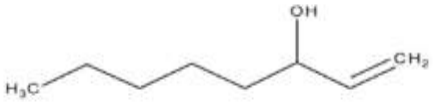
1-octanol



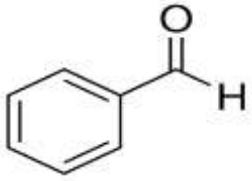
Benzoic acid



1-octan-3-ol



Benzaldehyde



Literature review:

Psychiatric effect:

The capability of Pleurotus ostreatus mushroom to accumulate lithium (Li) and the accessibility of this Li compared with lithium carbonate (Li_2CO_3), often used as psychiatric medicine, were investigated. Mushrooms were produced on a substrate-based on coffee husk, with different added concentrations of lithium chloride (LiCl). Biological efficiency , the crude protein content, the concentration of Li and other elements present in mushrooms were determined. The sequential extraction and in vitro test were used to verify the accessibility and the degree of solubility of this element. Li concentration in mushrooms was directly influenced by increasing LiCl concentration in the substrate ($P < 0.05$). The biological efficiency was not affected by different concentrations of LiCl. Li present in enriched mushrooms showed greater accessibility than in Li_2CO_3. Therefore, P. ostreatus mushrooms, enriched with lithium can be an alternative source of Li, as well as being a food with high nutritional value.

Usages as food source:

Oyster Mushrooms are also considered as functional foods because they elicit their positive effect on human being in several ways. Functional food comprises products of microbial, plants and animals origin containing physiologically active compounds beneficial for human health and reducing the risk of chronic diseases. It includes dietary supplements, nutriceuticals, medicinal foods, vita foods, pharma foods, phytochemicals, mycochemicals and so on.

Antibacterial effect:

Oyster Mushroom has been explored to combat simple and multiple drug resistant isolates of Eschrichia coli, Staphylococcus epidermidis, S. aureus and species of Candida, Streptococcus, Enterococcus. Methanolic extracts of Pleurotus species demonstrated an inhibition in growth of Bacillus megaterium, S. aureus, E. coli, Klebsiella pneumoniae, C. albicans, C. glabrata, species of Trichophyton and Epidermophyton to different degrees that was lower with respect to two antifungal agents: Streptomycin and Nystatin . Antimicrobial and antifungal activity of OM depended upon the nature of the solvent, ether extract were more active against Gram negative

bacteria as compared to acetone extract . Ether and acetone extracts of Oyster Mushroom was effective against B. subtilis, E. coli and S. cerevisiae. Later, Nithya and Ragunathan using extracts of P. sajor-caju reported higher antibacterial activity against Pseudomonas aeruginosa and E. coli when compared with the Gram positive organism S. aureus.

Antiviral effect:

Pleurotus mushroom contain substances that exert direct or indirect antiviral effects as a result of immune-stimulatory activity. Ubiquitin, an anti-viral protein was isolated and identified from fruiting body of oyster mushroom. Water-insoluble S-glucans isolated from sclerotia of P. tuber-regium and their corresponding water-soluble sulphated derivatives were active against herpes simplex virus type-1 and type-2. The anti-viral activity was due to binding of sulphated S-glucans to viral particles thereby preventing them from infecting the host cells. Not only intracellular proteins of P. ostreatus but its extracellular extract also contains polysaccharides that have immuno-modulating effects

Anti-Human Immunodeficiency Virus (HIV):

Ribonucleases (RNases: mol. wt. 10.7 kDa) have been isolated and characterized from the P. ostreatus that has the potentiality to neutralize HIV through degradation of viral genetic material.

Antineoplastic effect:

In 1969, Wantanabe detected antineoplastic activity of polysaccharide extracted from the fruiting body of P. ostreatus. These polysaccharides are components of the cell wall of Oyster Mushroom. Polysaccharide extracted from P. ostreatus culture broth when injected intra-peritoneally (i.p.) in the female Swiss albino mice caused 76% reduction in the number of neoplastic cells. Extracts from mushrooms including species Pleurotus may modulate the response of host immune system; in particular, various mushroom polysaccharides are likely to effect promotion and progression stages towards cancer as reviewed by Chatterjee et al.

Antitumor effect:

Hot water extract, showed a remarkable host mediated antitumor activity against Sarcoma, S-180, extracted from the fruiting body of polyporaceae family due to presence of S-D-glucan. Later the antitumor properties of mushroom-derived S-glucan were reviewed by Wasser. Similar activity was also observed by Choi et al. with hot water and ethanol extracts from fruiting body of oyster mushroom that exerted positive effect on three human solid carcinomas, a lung carcinoma (A549) and two cervical carcinomas (SiHa and HeLa). P. ostreatus extracts against cancer cell (HL-60), the cytotoxic effect was reported due to presence of higher content of flavonoids in fruiting body. Cibacron blue affinity purified protein, protein fraction extracted from P. ostreatus, has been shown to have potent antitumor activity against different tumors using mice model.

Antimutagenic effect:

Filipic et al. tested extracts of 89 different mushrooms species for their antigenotoxic and bio-antimutagenic activities on S. typhimurium and E. coli amongst them P. cornucopiae was found to be most effective. Methanolic extracts of P. ostreatus var. florida showed significant inhibition of mutagenicity elicited through mutagens requiring activation. Dried P. ostreatus, in diet, reduced pathological changes in dimethylhydrazine-induced colon cancer, in rats.

Antioxidant effect:

Fruiting bodies of Pleurotus possessed higher concentration of antioxidants than other commercial mushrooms. This activity was mainly due to presence of polysaccharide pleuran that has been isolated from P. ostreatus showing a positive effect on rat colon with pre-cancerous lesions. P. ostreatus increased the activities of important antioxidant enzymes (superoxide dismutase, catalase and peroxidase) thereby reducing oxidative damage in humans. Recently, Venkatakrishnan et al. have shown that extract from P. ostreatus inhibited the growth of HL-60 cells by cell cycle arrest i.e. by the induction of apoptosis by their experiments due to the presence of flavonoid (quercetin equivalent) and phenolics components (catechin equivalent) in fruiting bodies.

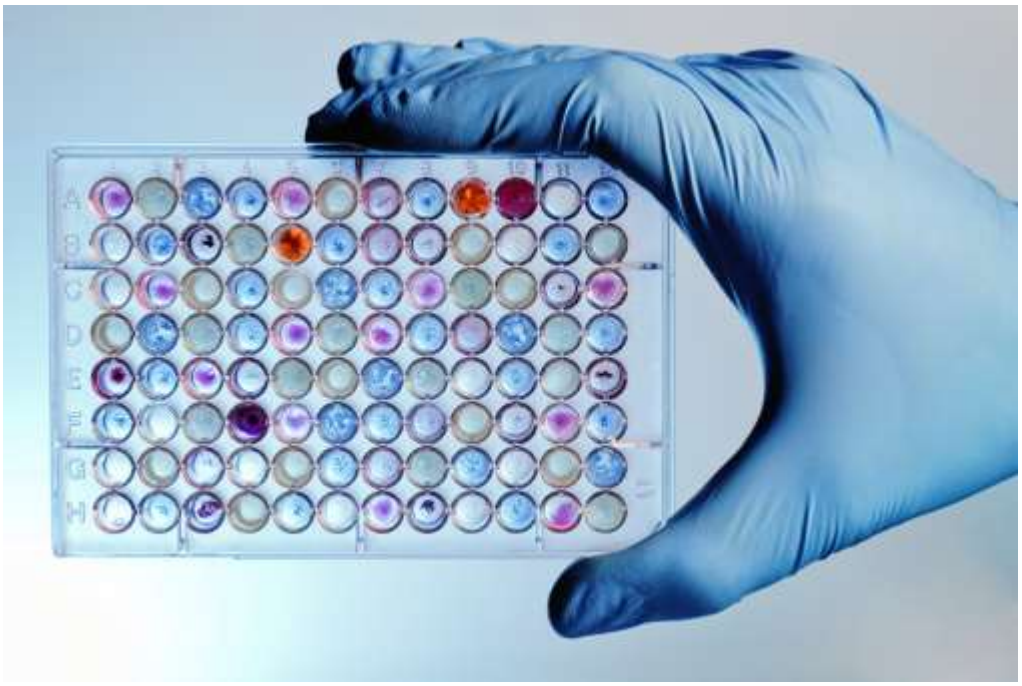
Hypoglycemic effect :

Guanide, a compound related to the bi-guanide class of oral anti-diabetic drugs was isolated from the Pleurotus species that exerted anti-hypoglycemic effect. Endo-polymer from submerged mycelia cultures of P. ostreatus possesses hypoglycemic effects. High fibre and proteins content and low fat content of edible mushrooms make it ideal food for diabetic patient.

Hypocholesterolemic effect:

Preliminary reports indicated that diet containing 4-10 % dried fruiting body of Pleurotus species show more reduction in the arterial pressure and blood cholesterol level when compared to normal diet in rabbits and rats. Lovastatin, a drug, used in the lowering blood cholesterol level, produced by P. ostreatus was approved by FDA in 1987. When dried mushroom mixed in the diet of experimental animal acted as accelerator of HDL (high density lipoprotein), reduced production of VLDL (very low density lipoprotein), LDL (low density lipoprotein), cholesterol, reduced cholesterol absorption and reduced HMG-CoA reductase activity in the liver.

Materials and Methods



Materials and Methods

3.1. Chemical study:

Generally the following methods are used throughout the experimental work-

- Collection and proper identification of the plant sample,
- Preparation of the plant material,
- Extraction,
- Solvent-solvent partitioning of the crude extract,
- Determination of total phenolics,
- Determination of total flavonoids,
- Determination of Cholinesterase inhibition,

3.1.1. In-vitro studies:

a. In- vitro antioxidant studies

b. In- vitro acetyl cholinesterase inhibitory studies

3.1.2. Material:

The fresh parts of the plant was selected for the chemical and biological investigations.

3.1.3. Collection of Plant Materials:

The whole plant was collected from Rajshahi and other districts of Bangladesh, in March, 2016 and identified by an expert taxonomist. A voucher specimen was submitted to the herbarium of the Department of Pharmacy, East West University.

3.1.4: Preparation of Plant Material:

The collected barks were first washed with water to remove adhering dirt and then shade dried for several days with occasional sun drying. These were then dried in an oven for 24 hours at considerably low temperature for better grinding. The dried barks were ground into coarse powder by a grinding machine in the Department of Pharmacy, East West University.

3.1.5. Cold extraction of the plant materials:

Powdered plant materials (barks) having a weight of about 1.6 kg were taken in an amber colored reagent bottle and soaked in 6.0 liter of methanol. The bottle with its contents were sealed and kept for a period of about 7 days with occasional shaking and stirring. The whole mixture was then filtered through cotton and then through Whatman No.1 filters paper and was concentrated with a rotary evaporator under reduced pressure at 50°C temperature to afford crude methanolic extract (CME).

3.2: Determination of Total Phenolics:

Total phenolic content of the different extractives of the plant were determined employing the method as described by Singleton in 1965 involving Folin-Ciocalteu reagent as oxidizing agent and catechin as standard.

3.2.1. Principle:

The content of total phenolic compounds of different fractions in the plant was determined by Folin–Ciocalteu Reagent (FCR). The FCR actually measures a sample's reducing capacity. The exact chemical nature of the FC reagent is not known, but it is believed to contain heteropolyphosphotungstates–molybdates. Sequences of reversible one or two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}O_{40})_4$. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo (VI):

$$Mo\ (VI) + e^{-} \rightarrow Mo\ (V)$$

3.2.2. Materials:

- Folin – ciocalteu reagent (Sigma chemical company, USA),
- Sodium carbonate (Sigma chemical company, USA),
- Methanol (Sigma chemical company, USA),
- Gallic acid (Wako pure chemicals Ltd., Japan),
- Micropipette (10-100 µl),
- Pipette (1-10 ml),
- UV-spectrophotometer (Shimadzu, USA).

3.2.3. Experimental procedure:

The amount of total phenolics in extract was determined according to the Folin-cio calteu procedure. Samples (500µl) were introduced into test tubes. 2.5mL of Folincio-calteu reagent and 2.5 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absobance at 760 nm was measured. The total phenolic content was expressed as Gallic acid equivalents (GAE) in milligrams per gram extract as calculated from standard Gallic acid graph by the following formula.

$$C = (c \times V)/m$$

Where,

C = total content of phenolic compounds, mg/g plant extract, in GAE;

c = the concentration of gallic acid established from the calibration curve, mg/ml;

V = the volume of extract, ml;

m = the weight of different pure plant extracts, gm.

3.3. Determination of Total Flavonoids (TF):

Total flavonoid content of the different extractives of the plant was determined by aluminum chloride colorimetric method. Catechin was used as standard and the flavonoid content of the extractives was expressed as mg of catechin equivalent/gm of dried extract.

3.3.1. Principle:

The content of total flavonoids in different extractives of plant extract was determined by the well-known aluminum chloride colorimetric method.[181] In this method aluminum chloride forms complex with hydroxyl groups of flavonoids present in the samples. This complex has the maximum absorbance at 510 nm.

3.3.2. Materials:

- Aluminum Chloride (Sigma chemical company, USA)
- 5% $NaNO_2$
- 1 mM NaOH
- Methanol (Sigma chemical company, USA)
- Catechin (Wako pure chemicals Ltd., Japan)
- Micropipette (10-100 µl)
- Pipette (1-10 ml)
- UV-spectrophotometer (Shimadzu, Japan)

3.3.3. Experimental procedure:

Total flavonoid (TF) was determined using the procedure by Dewanto, Wu, Adom, and Liu, (2002). One milliliter of extract containing 0.1 g/mL of dry matter was placed in a 10 mL volumetric flask and then 5 mL of distilled water added followed by 0.3mL of 5% $NaNO_2$. After 5 min, 0.6 mL of 10% $AlCl_3$ was added. After another 5 min 2 mL of 1M NaOH was added and volume made up with distilled water. The solution was mixed and absorbance measured at 510 nm. TF amounts were expressed as catechin equivalents per dry matter. All samples were analyzed thrice and result averaged.

The total content of flavonoid compounds in plant extracts in catechin equivalents was calculated by the following formula equation

$$C = (c \times V)/m$$

Where,

C = total content of flavonoid compounds, mg/g plant extract, in catechin equivalent (GAE);

c = the concentration of catechin established from the calibration curve, mg/ml;

V = the volume of extract, ml;

m = the weight of pure plant extracts, gm.

3.4 Total Flavanol Content Determination

Total Flavanol content of the methanol extract of the plant is determined by a method named aluminum chloride colorimetric method. This test requires gallic acid was as standard. The flavanol content of the extractives was denoted by mg of Gallic acid equivalent/gm of dried extract.

3.4.1 Principle

The amount of total flavanols in methanoic extract of the plant was determined by the popular aluminum chloride colorimetric method. In this process, aluminum chloride forms complex with hydroxyl groups of flavanols which may be present in the samples. This formed complex has the highest absorbance at 440 nm.

3.4.2 Materials

- Aluminum Chloride 2% solution (Sigma chemical company, USA)
- Sodium acetate 5% solution
- Gallic acid
- Micropipette (20-200 µl, 100-1000 µl)
- Pipette (1-10 ml)
- UV-spectrophotometer (Shimadzu, Japan)

3.4.3 Procedure

Total flavanol content was identified by using aluminum chloride. As a standard Gallic acid was used. Initially, 300 µl sample was taken from the stock solution. This was made upto 1ml by adding methanol. Then 1ml of 2% aluminium chloride solution which was made with ethanol is added with the sample. After that 1.5 ml 5% sodium acetate solution was added. This mixture was incubated at room temperature for 2.5 hours. And finally, absorbance was taken at 440 nm.

The total content of flavonoid in plant extracts in Gallic acid equivalents was calculated by the following formula

$$C = (c \times V)/m$$

Where,

C = total content of flavonoid compounds, mg/g plant extract, in catechin equivalent (GAE);

c = the concentration of catechin established from the calibration curve, mg/ml;

V = the volume of extract, ml;

m = the weight of pure plant extracts, gm.

3.5 DPPH (1, 1-diphenyl-2-picrylhydrazyl) Free Radical Scavenging Assay.

DPPH was used to evaluate the free radical scavenging activity of various fractions, isolated pure compounds and column subfractions.

Principle:

The 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) has been widely used to evaluate the free radical scavenging capacity of antioxidants. DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. DPPH can make stable free radicals in aqueous or methanol solution. With this method it was possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in the absorbance of DPPH at 517 nm. Resulting from a color change from purple to yellow the absorbance decreased when the DPPH was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule.

Materials:

- DPPH (Sigma chemical company, USA)
- Methanol (Sigma chemical company, USA)

- Catechin
- Pipette (1-10 ml)
- UV spectrophotometer (Shimadzu, Japan)

Experimental procedure:

The free radical scavenging activity of the extracts, different subcolumn fractions and isolated compounds of L. globosus was detected based on the method described by Braca et al. (2001, J. Nat. Prod., 64, 892-895). Sample (2 ubml) will be added to 3ml of a 0.004% methanol solution of DPPH. Absorbance at 517 nm will be determined after 30 mins and the percentage inhibition activity was calculated from

$$\mathbf{I\% = [(A_0 – A_1)/A_0] \times 100,}$$

Where,

I% is the percentage of scavenging activity

A_0 is the absorbance of the control, and

A_1 is the absorbance of the extract/standard.

Then % inhibitions were plotted against concentration and from the graph IC_{50} was calculated.

3.6 Determination of Total Antioxidant Capacity:

Total antioxidant capacity of the different extractives, column subfractions and the isolated compounds of L. globosus was determined by the method of Prieto et al., (1999) with some modifications.

Principle:

The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, α-tocopherol and carotenoids. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo (VI) and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm.

$$\mathbf{Mo\ (VI) + e \rightarrow Mo\ (V)}$$

Materials

- Sulphuric acid (Merck, Germany)
- Sodium Phosphate (Sigma chemical company, USA)
- Ammonium Molybdate (Sigma chemical company, USA)
- Ascorbic acid (Analytical or Reagent grade)
- Methanol (Sigma chemical company, USA)
- Water bath
- Micropipette (100-1000 µl)
- Pipette (1-10 ml)
- UV-spectrophotometer (Shimadzu, Japan)

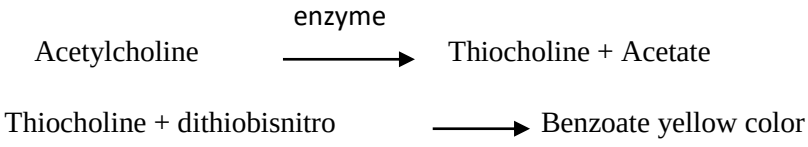
Experimental procedure:

The sample (0.5 mL) was mixed with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) . The tubes were incubated at 95^0C for 90 min. The mixture was cooled to room temperature, then the absorbance of the solution was measured at 695 nm against blank. A typical blank solution contained 3 mL of reaction mixture and the same volume of solvent used for the sample, and it is incubated under the same conditions as the rest of the sample solution. The total antioxidant activity was expressed as compared with ascorbic acid .

3.7 In-Vitro Acetyl Cholinesterase Inhibitory Studies:

Principle:

The acetylcholinesterase inhibitory activity of different extractives, column subfractions and isolated compounds of the plant was determined by Ellman's method. This method estimates AchE using acetylcholine iodide (substrate) and dithiobisnitro benzoic acid (DTNB). The enzymatic activity was measured by the yellow color compound produced by thiocholine when it reacts with dithiobisnitro benzoate ion.



The color intensity can be measured on a spectrophotometer and the enzyme activity expressed as the rate of reaction per minute.

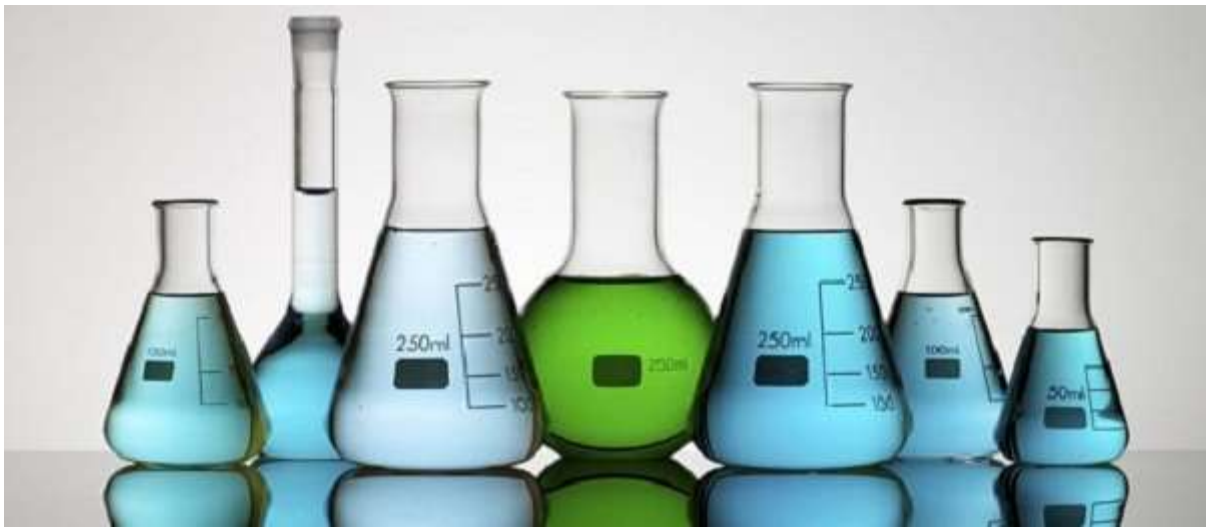
Materials:

- 5, 5´-dithio-bis-(2-nitro) benzoic acid (DTNB) (Sigma-Aldrich, Japan)
- Acetylthiocholine iodide (Sigma-Aldrich, Japan)
- Rat brain homogenate (Crude enzyme)
- Tris-Hcl buffer (Merck, Germany)
- Triton X-100 (Sigma chemical company, USA)
- BCA kit (bicinchoninic acid; Sigma Co., USA)
- Bovine serum albumin (Merck, India)
- Donepezil (Sigma-Aldrich, Japan)
- Micropipette (100-1000 µl)
- UV spectrophotometer (Shimadzu, USA)

Experimental Procedure:

The acetylcholinesterase (AChE) inhibitory assay was performed according to the colorimetric method of Ellman using acetylthiocholine iodide as a substrate. For the enzyme source, the rat brains were homogenized in a homogenizer with 5 volumes of a homogenation buffer [10 mM Tris-HCl (pH 7.2), which contained 1 M NaCl, 50 mM $MgCl_2$ and 1% Triton X-100] and centrifuged at 10,000 rpm for 30 min. The resulting supernatant was used as an enzyme source. All of the extraction steps were carried out at 4°C. Protein concentration was determined using the BCA kit (bicinchoninic acid; Sigma Co., USA) with bovine serum albumin (BSA) as a protein standard. The rates of hydrolysis by acetylcholinesterase were monitored spectrophotometrically. Each sample or standard (500 µl) was mixed with an enzyme solution (500 µl) and incubated at 37°C for 15 min. Absorbance at 405 nm was read immediately after adding an Ellman's reaction mixture [3.5 ml; 0.5 mM acetylthiocholine, 1 mM 5, 5´-dithio-bis (2-nitro benzoic acid)] in a 50 mM sodium phosphate buffer (pH 8.0) to the above reaction mixture. Reading was repeated for 10 min at 2 min intervals to verify that the reaction occurred linearly. The blank reaction was measured by substituting saline for the enzyme.

Results



4.1 Determination of Total Phenolics

Phenolic content of the crude methanolic extract, pet ether and chloroform fraction were determined using Folin-Ciocalteu reagent. Phenolic content of the samples were calculated on the basis of the standard curve for gallic acid as shown in Table 4.2 and in figure 4.1. The results were expressed as mg of gallic acid equivalent (GAE)/gm of dried extractives.

Table 4.1: Absorbance of gallic acid at different concentrations after treatment with FCR.

Concentration (µg/ml)	Absorbance			Mean ±STD
	A	b	c	
1	0.098	0.103	0.096	0.099 ± 0.003606
2	0.176	0.179	0.182	0.179 ± 0.003
4	0.403	0.411	0.401	0.405 ± 0.005292
8	0.785	0.789	0.792	0.789 ± 0.003512
16	1.452	1.456	1.432	1.447 ± 0.012858
32	2.654	2.664	2.659	2.659 ± 0.005

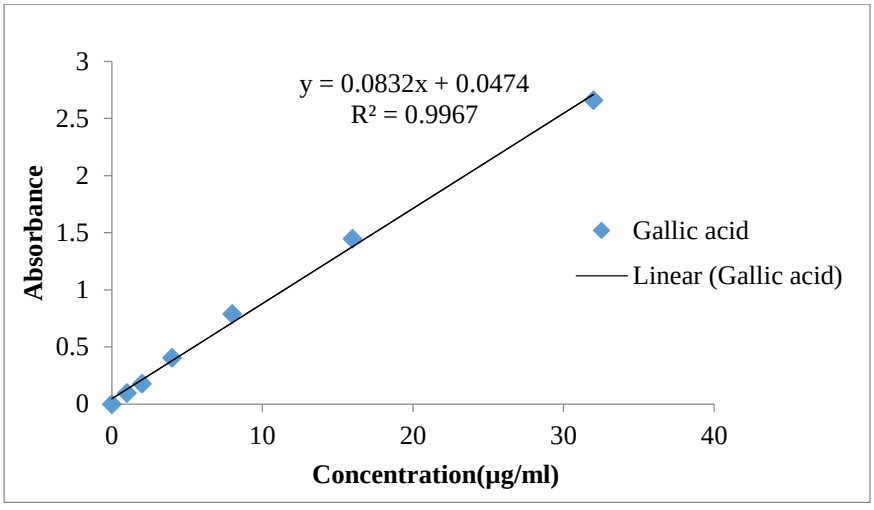


Figure 4.1: Standard curve of gallic acid for the determination of total phenolics.

Table 4.2: Determination of total phenolic content

Plant Name	Sample	Conc. (µg/ml)	Absorbance	GAE/gm of dried sample
Whole Plant	**CME**	200	0.402	54.81
Whole Plant	**CME**	500	1.78	183.30

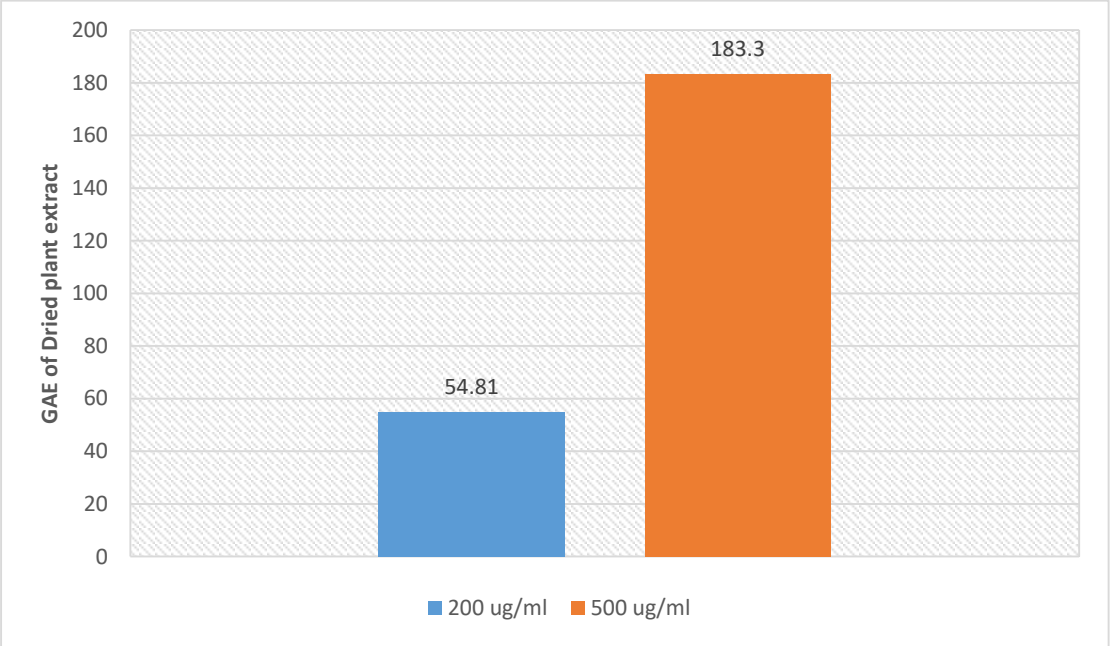


Figure 4.2: Total phenolic content (mg/gm plant extract in gallic acid equivalent)

4.2 Determination of Total Flavonoids

Total flavonoids content of crude methanol extract (CME), pet ether and chloroform fractions were determined using much known aluminum chloride colorimetric method. Flavonoid content of the samples was calculated on the basis of the standard curve for catechin as shown in Table and in Fig. The results were expressed as mg of catechin equivalent (CE)/gm of dried sample.

Table 4.3: Absorbance of catechin at different concentrations for quantitative determination of total flavonoids

Concentration (µg/ml)	Absorbance			Absorbance Mean ± STD
	a	b	c	
31.25	0.241	0.238	0.244	0.241 ± 0.003
62.5	0.380	0.378	0.382	0.38 ± 0.002
125	0.726	0.720	0.732	0.726 ± 0.006
250	1.476	1.472	1.480	1.476 ± 0.004
500	2.667	2.657	2.677	2.667 ± 0.007

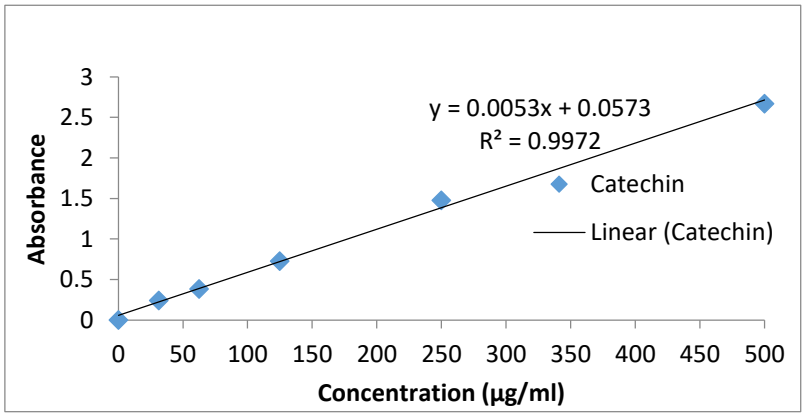


Figure 4.3: Standard curve of catechin for the determination of total flavonoids

Table 4.4: Determination of total flavonoid content

Plant Name *D. blancoi*	Sample	Conc. (µg/ml)	Absorbance	CE/gm of dried sample
Whole Plant	**CME**	200	0.032	2.28
Whole Plant	**CME**	500	0.332	12.62

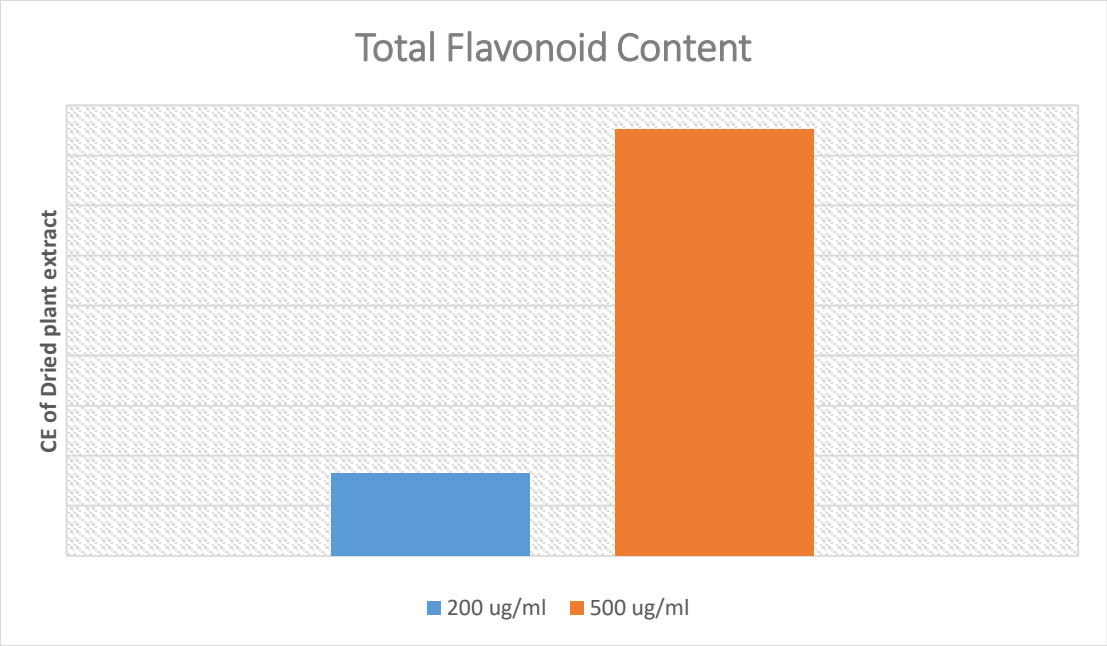


Figure 4.4: Total flavonoid content (mg/gm plant extract in catechin equivalent)

4.3 Determination of total flavanol

Flavanol content of the crude methanolic extract, pet ether and chloroform fraction were determined using reagents. The results were expressed as mg of gallic acid equivalent (GAE)/gm of dried extractives.

Table 4.5: Absorbance of gallic acid at different concentrations

Concentration (µg/ml)	Absorbance			Mean ±STD
	A	b	c	
1	0.098	0.103	0.096	0.099 ± 0.003606
2	0.176	0.179	0.182	0.179 ± 0.003
4	0.403	0.411	0.401	0.405 ± 0.005292
8	0.785	0.789	0.792	0.789 ± 0.003512
16	1.452	1.456	1.432	1.447 ± 0.012858
32	2.654	2.664	2.659	2.659 ± 0.005

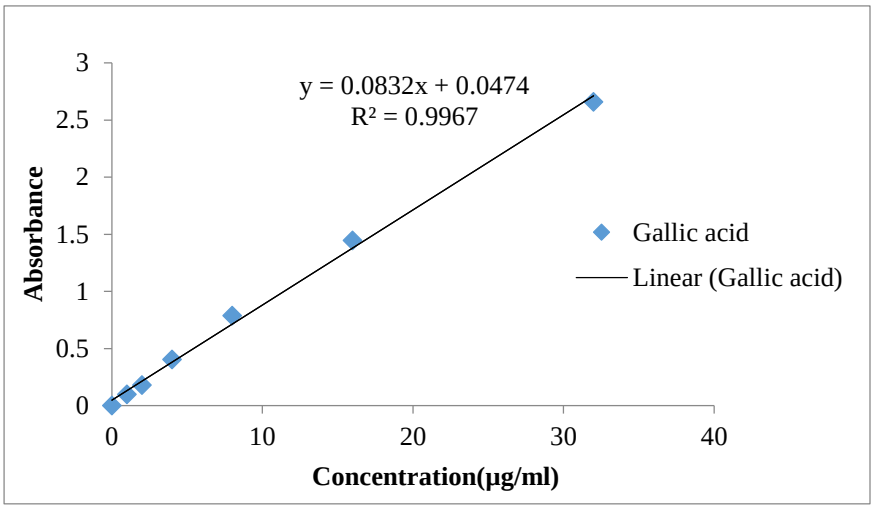


Figure 4.5: Standard curve of gallic acid for the determination of total flavanols.

Table 4.6: Determination of total flavanol content

Plant Name ***D. blancoi***	**Sample**	**Conc. (µg/ml)**	**Absorbance**	**GAE/gm of dried sample**
Whole plant	**CME**	200	0.128	16.02
Whole Plant	**CME**	500	0.531	36.91

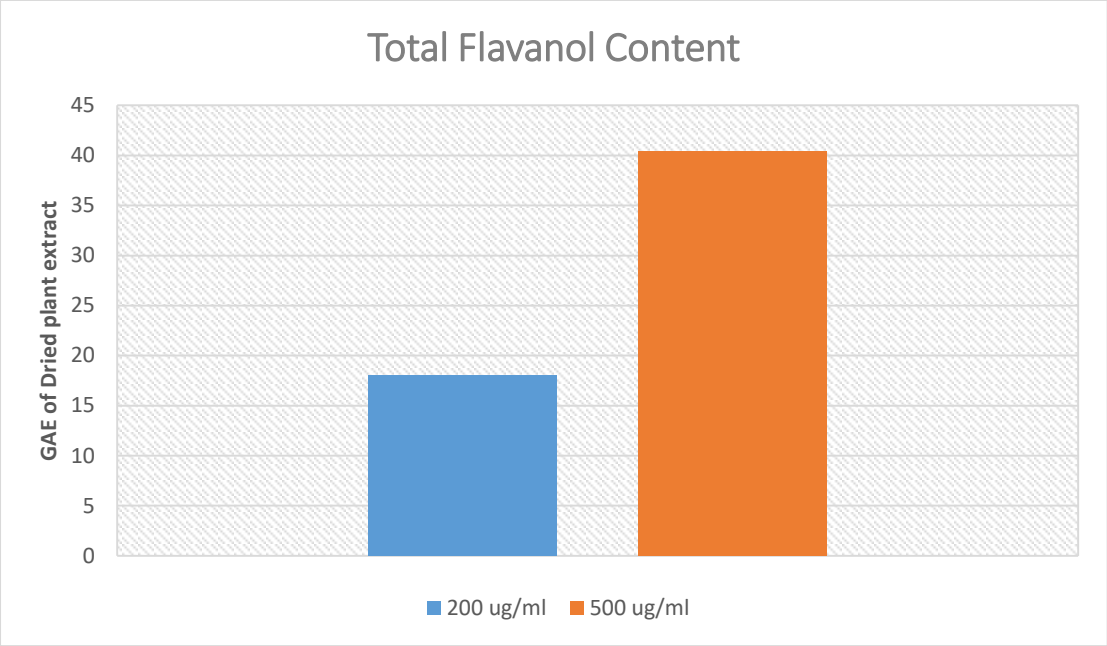


Figure 4.6: Total flavonol content (mg/gm plant extract in gallic acid equivalent)

4.4 DPPH Radical Scavenging Activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples including plant extracts. DPPH antioxidant assay is based on the ability of 1, 1 diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the change in absorbance and % of scavenging activity is calculated.

Table 4.7: % of inhibition of different parts of the plant

Name of Plant Part	Concentration (µg/µg)	Absorbance	% of Inhibition

Whole Plant CME	20	1.992	4.10
	50	1.879	7.53
	100	1.798	11.38
	200	1.591	20.12
Catechin (Standard)	20	0.19	87.39
	50	0.14	93.87
	100	0.11	94.16
	200	0.09	95.77

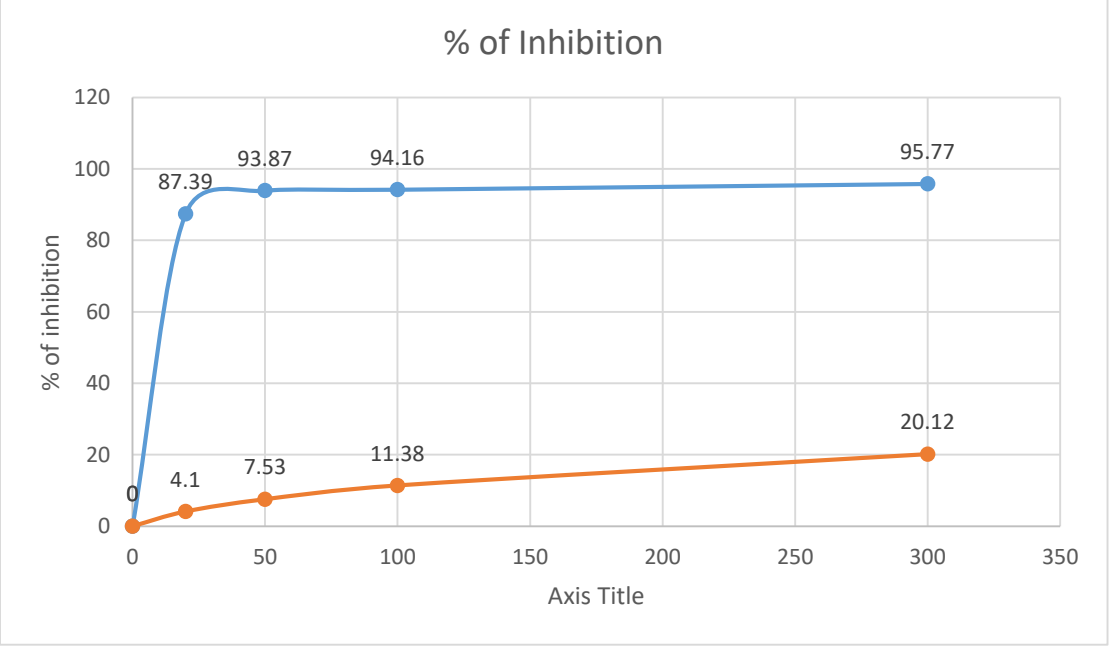


Figure 4.7: % of inhibition of different parts of plants by DPPH radical scavenging activity

4.5 Acetyl cholinesterase inhibitory activity assay

Inhibition of acetylcholinesterase, which enhances cholinergic transmission by reducing the enzymatic degradation of acetylcholine, is a widely accepted strategy for the development of AD drug. In this study, the acetylcholinesterase inhibitory activity of the crude methanol extract was assessed by modified Ellman'smethod and compared with the reference standard donepezil. This method estimates acetylcholinesterase (AChE) using acetylcholine iodide (substrate) and dithiobisnitro benzoic acid (DTNB). The enzymatic activity was measured by the yellow color compound produced by thiocholine when it reacts with dithiobisnitro benzoate ion.

Table 4.9: % of inhibition for acetyl cholinesterase inhibitory activity assay

Name of sample	Conc. (µg/ml)	% of inhibition Mean
Donepezil (Std)	20	78.34
	50	86.12
	100	91.44
	200	92.14
Whole Plant (CME)	20	5.26
	50	9.90
	100	16.38
	200	26.52

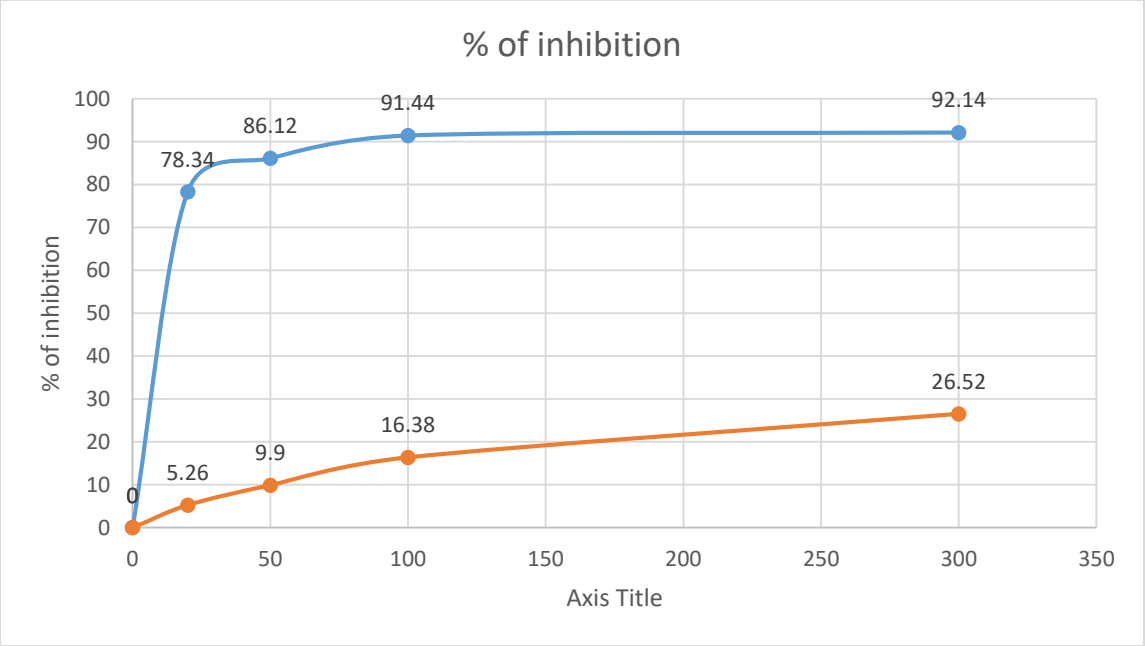


Figure 4.8: % of inhibition for acetyl cholinesterase inhibitory activity assay

4.6 Determination of Total Antioxidants

Antioxidants are one of the major components of this plants.

Table 4.11: Determination of total phenolic content

Plant Name	Sample	Conc. (µg/ml)	Absorbance	GAE/gm of dried sample
Whole Plant	**CME**	200	0.168	10.11
Whole Plant	**CME**	500	0.593	48.19

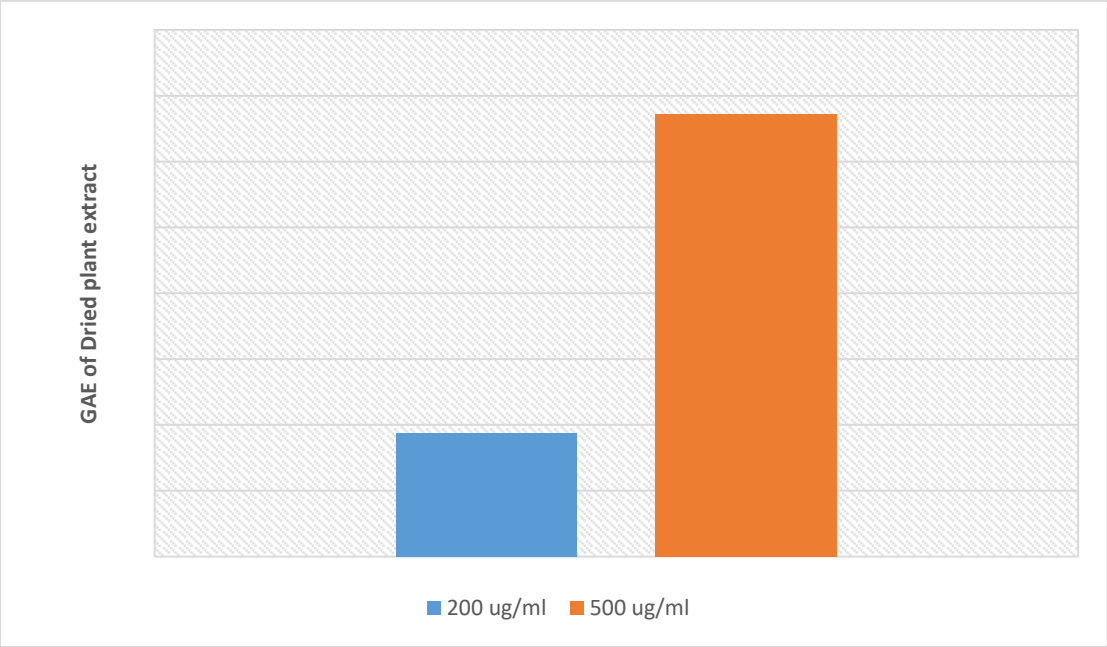
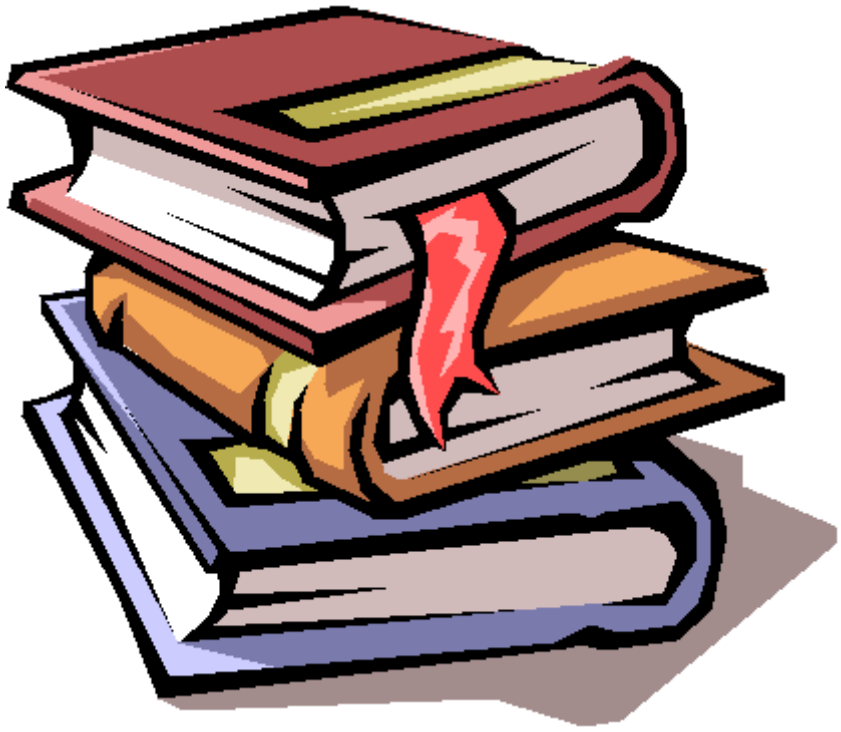


Figure 4.2: Total Antioxidant content of the plant Extract

Conclusion

On the basis of all result it can be concluded that the plant is rich in total phenolic components, flavonoid content as well as falvonol contents. But its total antioxidant content and free radical capability is not that much appreciable at all. Though it possesses moderate cholinesterase inhibitory activity.

References



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