Evaluation and Characterization of Duckweed Whole plant Extract along with In-silico study Against Alzheimer disease

A thesis submitted toward partial fulfilment of the requirements for the degree of

Master of Engineering in Biomedical Engineering

Course affiliated to Faculty of Engineering & Technology

Jadavpur University

Submitted by

NISHITH CHANDA

EXAM ROLL NO: M4BMD22004
CLASS ROLL NO: 002030201004
REGISTRATION NUMBER: 154629 of 2020-2021

Under the guidance of

Prof Dr. Piyali Basak
Jadavpur University
Kolkata-700032
India

School of Bioscience and Engineering

M.E. in Biomedical Engineering course affiliated to

Faculty of Engineering and Technology

Jadavpur University

Kolkata-700032

India

DEDICATION

This service is whole heartedly dedicated to God and well-being of its creations

Acknowledgements

I am glad to write this note of thanks after the end of my thesis work. It was a period of intense learning for me, not only in the scientific arena, but also on a personal level. I would like to reflect on the people who have supported and helped me so much throughout this period.

I owe a deep sense of gratitude to my respected thesis advisors ***Dr. Piyali Basak, Asst. Professor, School of Bioscience & Engineering, Jadavpur University*** *and for her esteemed guidance, invaluable suggestions, constant encouragement and affection at every stage of the entire tenure of the project without which I could not have finished the work. It has been my proud privilege to work under her guidance.*

I would also like to express deep felt gratefulness to ***Dr. Monisha Chakraborty, Professor, School of Bioscience and Engineering, Jadavpur University,*** *for their kind support.*

I would also like to special thank my respected seniors ***Pratik Das, PhD Scholar, Biomaterial and Cell culture Laboratory and Tathagata Adhikari, PhD Scholar, Biomaterial and Cell culture Laboratory*** *which is under School of Bio-Science and Engineering department at Jadavpur University, for their unconditional support and motivation which made this an enjoyable session.*

I want to convey my heartiest gratitude to all other lab members of Biomaterial and Cell Culture Lab, School of Bioscience and Engineering, Jadavpur University for their kind help, guidance and support at every step of my work.

I would also like to thank all the nonteaching staffs, all my batch mates, my seniors, and all my junior friends for their kind co-operation.

Last, but not the least, I wish to express my profound gratitude and my deep feelings for my family who have been the constant source of my energy, inspiration and determination for going ahead with my academic pursuit.

Date: **Nishith Chanda**

Declaration

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgment has been made in the text. All information in this document has been obtained and presented in accordance with academic rules and ethical conduct.

Place:

Date:

……..……

NISHITH CHANDA

Registration number:

154629 of 2020-2021

M.E. (Biomedical Engineering) course affiliated to

Faculty of Engineering and Technology

Jadavpur University

Kolkata-700032

CERTIFICATE OF RECOMMENDATION

We hereby recommend that the thesis entitled "**Evaluation and Characterization of Duckweed Whole plant Extract along with In-silico study Against Alzheimer disease**" carried out under my supervision by Nishith Chanda may be accepted in partial fulfilment of the requirement for awarding the Degree of Master in Biomedical Engineering of Jadavpur University. The project, in our opinion, is worthy for its acceptance.

_____

Dr. Piyali Basak

(Thesis Advisor)
Associate Professor
School of Bioscience and Engineering
Jadavpur University
Kolkata-700032

_____

DIRECTOR

School of Bioscience and Engineering

Jadavpur University

Kolkata-700032

_____

Dean

Faculty Council of Interdisciplinary Studies

Law and Management Jadavpur University

Kolkata – 700032

M.E. (Biomedical Engineering) course affiliated to
Faculty of Engineering and Technology
Jadavpur University
Kolkata-700032

Certificate of Approval

The forgoing thesis is hereby approved as a creditable study of an engineering subject carried out and presented in a manner satisfactory to warrant its acceptance as a prerequisite to the degree for which it has been submitted. It is understood that by this approval the undersigned do not necessarily endorse or approve any statement made, opinion expressed or conclusion drawn therein but approve the thesis only for the purpose for which it is submitted.

_____ _____

Dr. Piyali Basak
Thesis Advisor
Associate Professor
School of Bioscience and Engineering
Jadavpur University
Kolkata-700032

Signature of Examiner

**Only in case the thesis is approved.

PREFACE

Duckweed has been traditionally harnessed in different fields including food and pharmaceuticals, as well as used in industrial applications. Although the fact that duckweed provide micronutrient is well established, scientific data is needed to evaluate its extent of potency. This work highlights the positive qualities that Duckweed possesses and aims for the betterment of them.

Walking through this thesis, chapter one deals with the understanding of basic principles used in this work. All the assays and their mechanisms are discussed here. The statements given are well established and behaves as a tool to begin the work.

In chapter two, the knowledge behind this presentation in noted in a nutshell. It contains the results of different researches which are being linked or used in some way to accomplish the work.

Chapter three and four claims the motivation behind the work, its objectives and a plan along with the requirements and procedure of the experiments performed. All the findings, their probable interpretations and conclusion after the end of the work is presented in chapter five and six.

List of Figures:

List of Tables:

CONTENTS

CHAPTER ONE

INTRODUCTION

[1] INTRODUCTION:

Duckweeds are the fastest growing and smallest flowering plant that has a potential to reproduce huge amount biomass in limited period of time and broad range of applications in the field of pharmaceuticals, poultry food, biogas and biofuel.[1] Duckweed are aquatic plants which float on or remain just beneath the surface of fresh water bodies. Duckweed, a monocotyledonous water plants, commonly known as water lens, belongs to Lemnaceae family characterized as the fastest growing flowering plants. By using molecular taxonomy duckweeds are classified into five genera (*Landoltia*, *Spirodela*, *Wolffiella*, *Lemna* and *Wolffia*) which are depended on the fingerprinting techniques by Amplified fragment length polymorphism (AFLP).

The plants do not contain leaves and stem have very simple physiological structure. They consist of a frond on the water surface, with or without normal rootlet below. Reproduction is mostly done by asexual budding of new fronds, but sometimes three tiny flowers are produced and sexual reproduction Occurs. Wolffia, the flower belongs to similar of duckweed genus is the smallest flower in the earth, merely 0.33 mm long. Duckweed is very potential high-protein food source for ducks, geese, and swans etc. As well as it is eaten by some parts of Southeast Asian human beings. Duckweed is sometimes treated as a significant nutrition contained food source because duckweed contains more protein than soybeans. Duckweed has lots of commercial as well as scientific uses; including basic research in medicine field, toxicity testing organism in biotechnology, wastewater remediation, biotech protein factories, high protein animal feed and carbon cycling.

[1.1] SCIENTIFIC CLASSIFICATION OF DUCKWEED:

Kingdom: Plantae kingdom

Clade: Tracheophytes, Angiosperms, Monocots

Family: Araceae Family

Subfamily: Lemnoideae

Order: Alismatales

Genus: *Lemna*

Species: *L. minor*

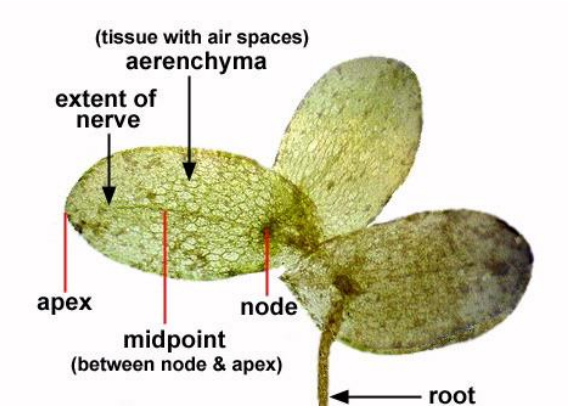

Figure1.1: Duckweed (*Lemna minor*) with its botanical view

[1.2] Major compounds of Duckweeds:

Duckweed plant is highly phytochemicals contained plant which have protein, carbohydrate, fats in adequate amount. There are further group of classification given below -

PROTEINS	
Amino acid derivatives	Citrulline, cystathionine, γ-aminobutyric acid (GABA), hydroxyproline, and taurine
Amino acids	Alanine, arginine, phenylalanine, asparagine, aspartate, isoleucine, glutamate, glycine, histidine, leucine, lysine, methionine, tryptophan, tyrosine, and valine
CARBOHYDRATES	
Polysaccharides	pectin, Inulin, and hemicellulose
Sugars	Apiose, arabinose, fructose, raffinose, galactose, glucose, rhamnose, fucose, sucrose, and xylose
Starch	Starch
FATS	
Long-chain fatty acids	Behenic acid, 2-hydroxypalmitic acid, linolenic acid, myristic acid, lignoceric acid, eicosanoic acid, α-linolenic acid, γ-linolenic acid,

	linoleic acid, nonadecylic acid, oleic acid, pentadecylic acid, stearic acid, palmitelaidic acid, palmitic acid, and stearidonic acid
Medium-chain fatty acids	Lauric acid

Table 1.1: Major compounds of Duckweeds

[1.3] ALZHEIMER'S DISEASE

Alzheimer's disease is one of the most prevalent causes of general dementia, responsible for 65% to 85% of all reported cases. According to recent investigations and, more than half of the population have brain abnormalities (pathology) as well as brain alterations and cerebrovascular illness. Impaired speech, confusion, disorientation, poor judgement, changes in behaviour, and eventually difficulties in speaking, swallowing problem, and unable to walking are some of the later signs of this disease. The build-up of β-amyloid (plaques) protein fragment outer side of neurons in the brain and distorted strands of the tau (tangles) protein inner side of neurons are characteristic of Alzheimer. Neurons died and brain tissues are damaged as a result of these alterations.[35] Alzheimer is a slowly evolving brain illness that starts few years before symptoms noticed.[36]

[1.3.1] Alzheimer Disease Flow and Phases

The development of Alzheimer's disease initiate brain changes that are not easily observed to the affected person till cause serious problems with memory and subsequently physical inability as disease continuous.[37]

There are mainly three broad phases:

a) Preclinical phase of Alzheimer's disease

b) Mild level cognitive impairment (MCI)

c) Most dangerous dementia phases

The Alzheimer's dementia can be further divided into three stages of mild, moderate and severe, which determine the level of symptoms affected in person's capability to carry out daily life activities.[38]

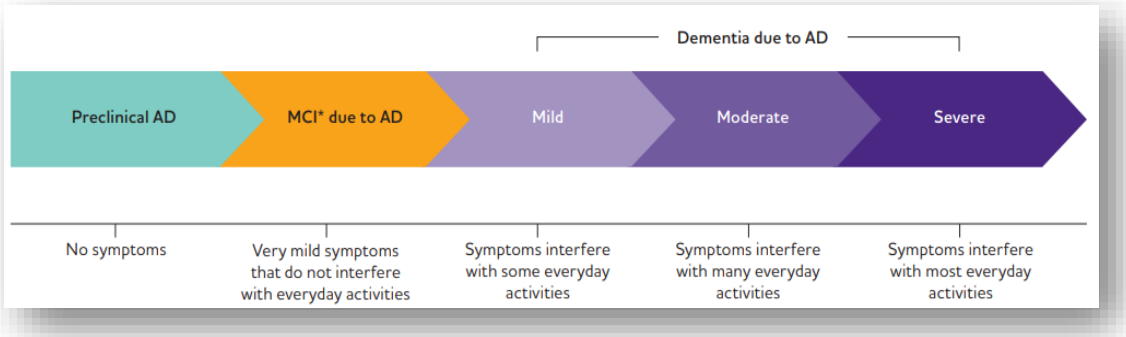

Figure 1.2: Alzheimer's disease flow diagram

Preclinical phase of Alzheimer's disease: Persons exhibit responsible brain function abnormalities in this preliminary phase, which represent the initial signs of Alzheimer's disease, though there is no such symptom like memory loss occurs.[50] Abnormal level of beta-amyloid is showed on cerebrospinal fluid analysis (called CSF) and by positron emission tomography (PET) scanning reasonable changes in brain could be detected. When the preliminary phase of Alzheimer's occurs, the brain tries to resist them by enabling individuals to continue function normally.[51] While research is being conducted to develop the tools and skills required to identify or assess some of the early brain abnormalities associated with Alzheimer's disease, further study is required to evaluate accuracy before they are made accessible for random use. In rare situations, individuals died with plaques of beta-amyloid protein yet had no memory loss or cognitive issues during their lives.[52]

Mild cognitive impairment (MCI) due to Alzheimer's disease: Alzheimer's disease is characterised by signs of Alzheimer's brain alterations (for example, aberrant levels of beta-amyloid) as well as suppressed memory and thinking problems. These issues may be visible to family and friends but not to others, and they do not affect an individual's ability to carry out daily tasks.[41] When the brain can't

handle the damage and death of nerve cells induced by Alzheimer's disease, minor abnormalities in cognitive abilities occur.[42]

Individuals in this stage demonstrate significant brain alterations that are indicative of Alzheimer's disease. Abnormal amounts of beta-amyloid protein, as detected by Positron emission tomography scans (PET) and cerebrospinal fluid (CSF) analyses, are examples of identifiable brain alterations.[45] When early Alzheimer's symptoms appear, the brain attempts to prevent them, allowing people to function normally.[47] While researchers have the techniques and skills to detect some of the early brain abnormalities associated with Alzheimer's disease, further study is required to determine accuracy before they are made accessible for random use in hospitals, physicians offices, and other clinical settings.[48] It is essential to emphasize that not all the people who have basic signs of Alzheimer's related brain alterations experience side effects of MCI or dementia as a result of the disease. In rare circumstances, people die with beta-amyloid protein plaques but don’t have memory problems or other cognitive issues.[49]

Most dangerous dementia phase due to Alzheimer: Alzheimer's disease (AD) Dementia is characterised by significant memory, cognitive, or behavioural impairments, as well as signs of Alzheimer's disease-related brain changes. Persons having Alzheimer’s disease variety of alternative symptoms are noticed over time. These symptoms reflect severity of brain nerve cell injury.[51]

A) Mild level Alzheimer’s Dementia: Most of the people suffering in mild Alzheimer's dementia may operate independently in any areas, although they will merely need assistance with other tasks to maintain independence for daily routine task and safety. They could also be capable of driving, working, and participate in cultural program.

B) Moderate level Alzheimer’s Dementia: Individuals having Alzheimer's dementia in the moderate stage, become incontinent and develop odd personality and behavioural changes which is suspicious and agitative.

C) Severe level Alzheimer's Dementia: Individuals those having severe Alzheimer's disease mostly require assistance with everyday routine tasks and round-the-clock care is needed. The physical impacts of Alzheimer on individuals become more prominent at this stage. When parts of brain become injured that impacts in movement of body and become bed-ridden. Being bedridden patients feel various kind of illnesses such as skin infections, blood clots, and sepsis, which causes organ failure and severe inflammation.[52]

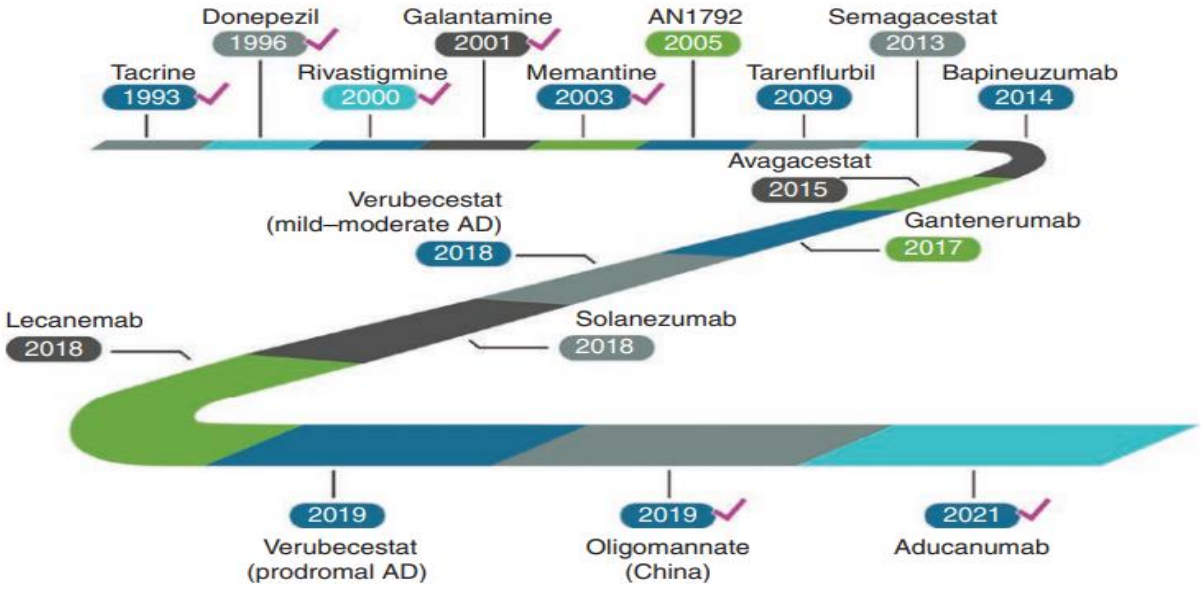

Figure 1.3 Timeline for the major clinical trials for medicines of Alzheimer's disease.

[1.3.2] Atrophy in Alzheimer's Disease:

The appearance of senile plaques (amyloid deposition) and tangles of neurofibril in autopsied brains are neuropathological markers of the atrophy process involves in Alzheimer's disease (AD). Neurofibrillary tangles are mostly generated by hyperphosphorylated tau protein found within neurons and progressed to different temporal, parietal, and finally frontal association cortices. Alzheimer's disease first manifests itself in poorly myelinated limbic neurons in memories and learning-related

system locations including the hippocampus and the association cortex. Only in the later stage of illness highly myelinated neurons are damaged. [11]

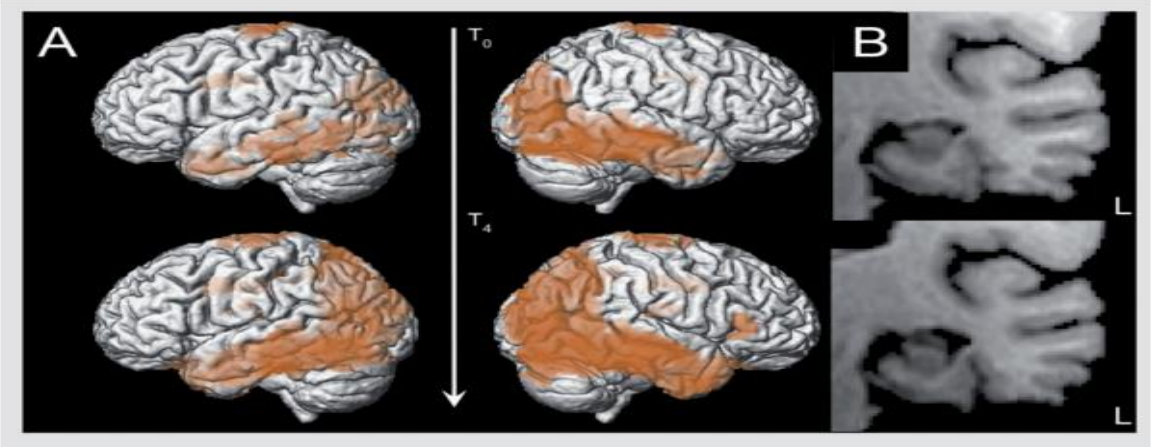

Figure1.4: Atrophy in a case of AD over 4 years. (A) Lateral view of grey matter Reduction, (B) Left hippocampus at baseline in coronal view (hippocampal grey matter).

[1.3.3] Statistical presentation of Alzheimer Disease

Alzheimer disease is spreading drastically on year-to-year basis. Analysis showing that no. of Alzheimer effected people form low- and middle-income countries is more than high income countries thought out world. Data is given below -

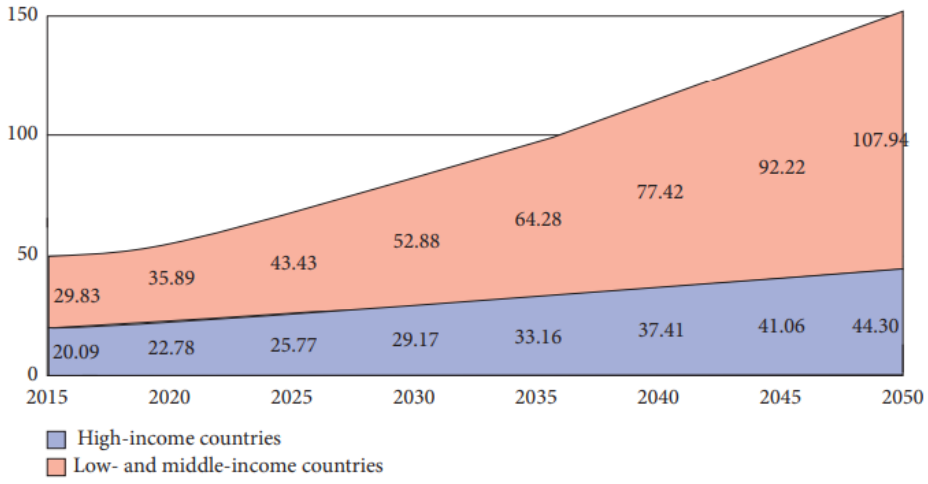

Figure1.5: Number of people predicted with Dementia in millions worldwide.[11]

[1.4] MOLECULAR DOCKING:

Molecular docking is an optimization process that predicts an intermolecular complex produced between two or more molecules by describing the "best-fit" configuration of a ligand that binds to a certain protein of interest.[48] Because of its potential implications in medicine, the protein ligand interaction is the most relevant aspect. Ligands are small molecules that interact with the binding sites of proteins. There are numerous mutual ordinary procedures in which binding can occur. These are known as binding modes.[49] Molecular docking is commonly used in current drug design to analyse drug-receptor interactions. For many decades, humans have been constantly exposed to microbes. Invasive microbial infections are a big issue all throughout the world, particularly among immunocompromised people. There is a current increase in the new antimicrobial drug development for getting new antimicrobial medicines that are more selective, powerful, and less toxic than existing medications used in clinical trials.[50]

Antibacterial and antifungal properties have been documented for heterocycles containing an azole ring structure. Because of their extensive biological activities and usage in synthetic chemistry, imidazole and its derivatives have gained tremendous relevance. Imidazole compounds have several pharmacological actions, including anti-inflammatory, analgesic, anticonvulsant, antitubercular, antibacterial, anticancer, and anti- Parkinson's. The primary objective of our in-silico experiments (virtual molecular docking and molecular dynamics simulation) is to find bioactive chemicals from Lamna minor that can efficiently block the Alzheimer-causing protein beta amyloid, thereby giving a way out of this devastating illness. This study may be the first to show how duckweed may be utilised to prevent the effects of a fatal illness that is threatening human survival. More research is needed to confirm its activity on beta amyloid, which is responsible for the growth of the devastating illness Alzheimer's, but this effort aims to give a first step. Further extensive research is required to prove its efficacy as a therapeutic agent to treat Alzheimer.[52]

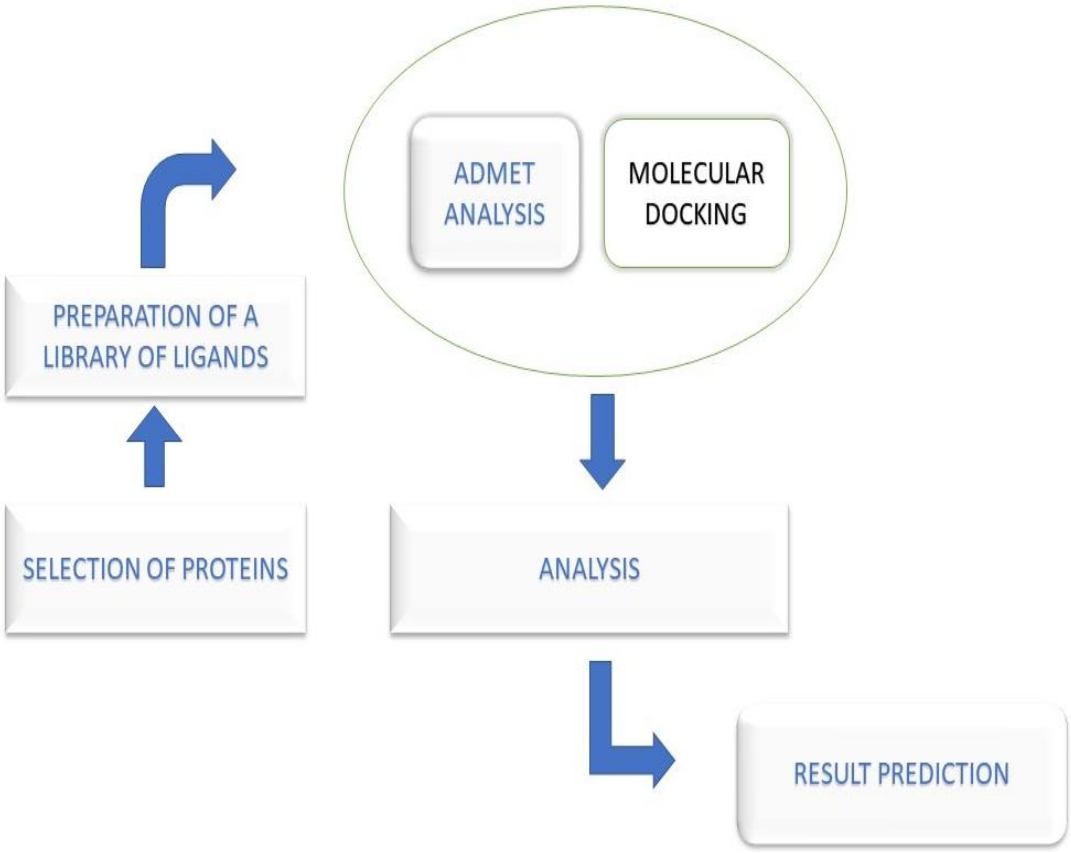

Figure 1.6: Flow diagram of Molecular Docking

CHAPTER TWO

OBJECTIVES AND PRPOSED PLAN OF WORK

[2.1] MOTIVATION & OBJECTIVES

Duckweed had chosen as a plant to be studied as aquaculture are many times more productive than terrestrial agriculture and have the potential to significantly increase production [4]. Now a days wetland plants like Duckweeds which is used for construction of wetlands and the removal of toxic trace elements from the wastewater [5].

Alzheimer's disease is the sixth biggest cause of mortality, responsible for 70% among all dementia cases. The worldwide pervasiveness of Alzheimer's disease is anticipated from 266 lakhs cases in a year by 2006 to 1068 lakhs cases in a year by 2050[6].

There is no proper treatment developed for Alzheimer’s disease till now. Until now, anti-dementia drugs and disease-modifying therapies have shown little efficacy and have mostly been designated for Alzheimer's disease, regardless of the fact that multiple novel treatments are being studied in various phases of clinical trials.

The main objective of this thesis was to evaluate the anti-Alzheimer potential of Duckweed plant extract as well as the in-silico study of Duckweed phytochemicals against Alzheimer responsible beta amyloid protein.

[2.2] Proposed plan to work:

- The initial step was to collect and prepare Duckweed whole plant crude extract.

- The work was initiated by extracting bioactive components from Duckweed whole plant by solvent extraction and preparing samples of crude extract to carry out assays used in this work

- Quantitative phytochemical analysis was carried out to establish the best solvent for its extraction process.

- Preparation of different concentration of Duckweed sample using lyophilized Duckweed crude extract powder.

- Comparative analysis of antimicrobial effect between different solvent extracted samples and the synthesized nano-composite.

- Percentage of free radical activity of Duckweed whole plant extract was calculated using DPPH method.

- Cytotoxicity assessment by MTT assay on PBMC (which was separated from whole blood)

CHAPTER THREE

LITERATURE REVIEW

[3.1] LITERATURE REVIEW:

Plants are multicellular creatures in the most basic sense. Flowering plants, ferns, conifer gymnosperms, and green algae are all members of the clade viridiplantae. There are around 320 thousand plant species, of which 260-290 thousand are seeded plants. Green plants gradually contribute a share of the world's molecular oxygen demand. Plants have various cultural applications such as decorations, psychotropic drugs. Most plants gain energy via photosynthesis, which uses sunlight, water, and carbon dioxide to produce food[45].

Plants produce a large number of bioactive substances for a variety of purposes, including defense against bacteria, viruses, insects, fungus, and illness. Many phytochemicals have been discovered to have potential or demonstrated biological action. The bioactive phytochemical of Azolla is a pteridophyte. Azolla is a tiny fern with small leaves that can grow up to 2.5 cm in length. They are covered with small hairs and are green, bluish green, or dark red in colour. The plant's microscopic hairs are responsible for the velvet look and making the upper surface of the leaf water resistant.[46]

From recent studies we get that Duckweeds are potent sources of bio-active compounds e.g. antioxidant, anti-diarrheal, anti-inflammation, anti-diabetic and anticancer compounds. Plant based natural products having anti-inflammatory properties are divided into 12 distinct chemical groups. Those groups are as follows- 1) alkaloids, 2) terpenoids, 3) phenylpropanoids, 4) aldehydes, 5) glycosides, 6) saponified, 7) lignans, 8)lipids, 9) nucleic acids, 10) polysaccharides, 11) proteins and 12) unidentified compounds. The secondary metabolites of plants have been used as medicines from ancient era. Alkaloids, phenolic compounds, steroids, terpenoids etc. are products of secondary metabolism and these chemical compounds have pharmacological importance which can be used to design potential drugs[47].

Based on the findings of a recent study, most widely used marketed drugs for Alzheimer are Donepezil, Galantamine and Rivastigmine. There chemical structures are given below-

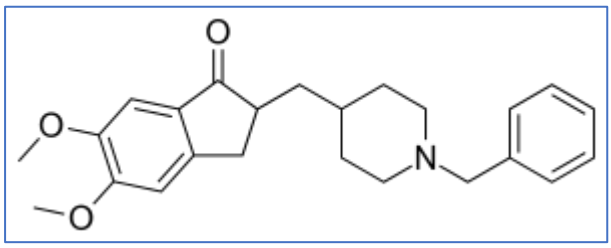

Fig 3.1: Structure of Donepezil

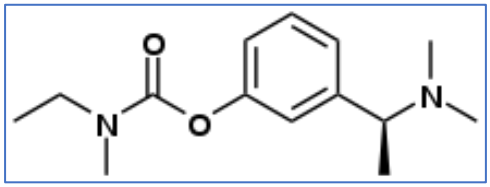

Fig 3.2 Structure of Rivastigmine

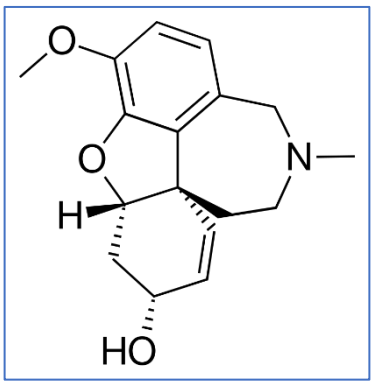

Fig 3.3: Structure of Galantamine

In a previous study it shows that an in-silico evaluation of anti-Alzheimer compounds found in various alcoholic extract of Neolamarckia cadamba bark using GC-MS indicated the potential lead compounds against the Alzheimer targets AChE in a prior work. As a result of our study, we will be able to uncover novel chemical elements of potentially more effective anti-Alzheimer lead molecules than now available.[51]

Another study shows that Alzheimer's disease is the leading cause of dementia due to two primary factors: acetylcholinesterase enzyme build-up at cholinergic synapse and -amyloid protein deposition at neurofibrillary. The current study investigates the affinities of phytochemicals discovered by gas chromatography-mass spectrometry in the methanolic leaf extract of Erythroxylon monogynic against chosen targets, namely -amyloid and acetylcholinesterase.[52]

CHAPTER FOUR

MATERIALS AND METHODS

[4] Material and Methodology:

[4.1] Sample collection Whole plant part of duckweed was collected from Jadavpur University Lake, Jadavpur, Kolkata. Duckweed whole Plant was separated out from unwanted particles with through wash of water and knur ware picked up by hand. Then fresh clean Duckweed was divided into two parts- one used for extraction immediately which is highly moisture contained (group A) and the other part was kept at 37℃ for two days (group B) to make sure duckweed extract should be moisture free compound. After 2 days group B was crushed using mortar and pestle into coarse powder.

Sample preparation

Instruments used- Rotary evaporator (Manufacturer: IKA), lyophilizer (Manufacturer: Biobas)

Material used- Filter paper is used (Whatman No.1)

To extract the bioactive compounds, the duckweed whole plant (Group A) was blended using mixer grinder at required speed and the freshly prepared extract hence filtered under vacuum pressure using Whatman Filter paper no 1 and concentrated using Rotary evaporator. Filtrate extracts solution lyophilized for 24 h and temperature kept at -80 ºC. Lyophilized powder, which was obtained used as test sample and stored at -23º C. Group B had also followed the same process to get extract.[105]

[4.2] Extraction of the bioactive components

Material used: Methanol, ethanol, acetone, and 2-propanol (isopropanol).

15 grams of Duckweed whole plant from group A and group B was macerated with 60% of 50 mL methanol solution for consecutive 3 days to get optimum phytochemicals. This was followed by digestion process.[111] In digestion process, freshly prepared 10 gm powder added in the solution then heated and then to increase concentration of bioactive components kept at 3 ºC temperature for 1 day.[113]

Same process is followed for the powder from group B with solvents namely acetone, propan-2-ol and ethanol accordingly.

[4.3] Phytochemical Screening of Extract

All phytochemical analyses were carried out in accordance with standards set. The presence of phenolic compounds, tannin, alkaloids and flavonoids, in crude extracted from entire Duckweed plant was determined.[96]

a. Test for Phenols

Ferric chloride test for phenol: 5% concentrated ferric chloride solution was poured drop wise in 1 mL of diluted extract solution. The appearance of a greenish blue colour confirms phenol is present.

b. Test for alkaloids

1 mL of crude extract was poured to 2 mL of hexane then shaken well for proper mixture. Following this, 3 mL of 2 % HCL was added to the extract with heat and then filtered out.[92] 1 drop of picric acid was added to the filtrate, and the presence of alkaloid is indicated by the presence of yellow precipitate.

c. Test for reducing sugars

Fehling's test: 0.2 g plant sample is added in 1 mL ethanol combined with 3 mL distilled water. In a test tube, 1 mL of Fehling's solutions A and B were heated to boiling before being put in the aqueous methanolic plant extract. The presence of reducing sugars was determined by a change in colour response.

d. Test for flavonoids

The aqueous filtrate from each plant extract was combined with 3 mL of dilute ammonia. The solution was then treated with 1 mL of concentrated sulphuric acid (H_2SO_4). Flavonoids were detected in each extract by the presence of yellow color.

e. Tannins

2mL of the extract was diluted with water to which 10% ferric chloride solution was added drop wise. A blue colour is formed for the presence of **gallic tannins** on other hand green colour formation indicates presence of **catecholic tannins**.

[4.4] Phytochemical analysis

[4.4.1] Evaluation of total Phenolic content

Instrument required: UV-visible Spectrophotometer (Agilent Technologies, Cary 60)

Materials required: Folin-Ciocalteu reagent (FCR), gallic acid, sodium carbonate (Na_2CO_3), distilled water

Methods: By using Folin - Ciocalteu (FC) reagent total phenolic content of the plant extract was determined. 0.5 mL of the whole plant extract was mixed with 0.5 mL of FC reagent (2x diluted with water) and the mixture was allowed to incubate for 5 minutes at 25 ºC and it was followed by addition of 2 mL of 20% Na_2CO_3. After 90 minutes of incubation at 25 ºC, the absorbance at 650 nm was measured.[64] Gallic acid was used as a reference to determine the total phenolic content (g/mL).[65]

[4.4.2] Evaluation of total Flavonoid content

Instrument required: UV-visible Spectrophotometer (Agilent Technologies, Cary 60)

Materials required: sodium nitrite, aluminum chloride, sodium hydroxide, quercetin

Methods: In this approach, 0.5 mL of extract (concentration 100 g/mL) or quercetin (concentration 0-500 g/mL range) was added with 0.5 mL of distilled water. After that, 5% concentrated 0.3 mL sodium nitrite solution was added and allowed to stand at normal room temperature for 5 minutes before adding 0.3 mL of 10% aqueous aluminium chloride solution.[66] After 5 minutes, 2 mL of a 1M NaOH solution was added, the absorbance at 510 nm was measured using a UV-visible Spectrophotometer. Spectrophotometric analysis is done for those extracted sample with reference of quercetin standard curve.

[4.5] Antioxidant activity quantification method using DPPH (1,1-diphenyl-2-picrylhydrazyl)

Using DPPH method free radical scavenging activity (RSA) was performed. Different concentration of the Duckweed extracts (25, 50, 75, 100 µg/mL) were used to do so. 50 µl of all concentrations of extracts was mixed with 200 µl of 0.02% DPPH solution separately. Then kept it in dark place at room temperature for 30 min, finally the

absorbance is found through UV spectrophotometry carried out at 595 nm in triplicates for better accuracy for each concentration. Ascorbic acid was used as a standard antioxidant.[68] As a control water is used. The percentage inhibition of free radical formation was calculated by following equation:

$$\textbf{RSA (\%)} = \frac{(CONTROL - SAMPLE)}{CONTROL} \text{ X } 100\ \%$$

[4.6] In-vitro activity study

Cell culture

L929 cell culture: The L strain was created from healthy subcutaneous adipose and areolar tissue from a 100-day-old male C3H/An mouse. L929 fibroblast cell line was obtained from the National Centre for Cell Science in Pune[112]. The growth medium was made with Dulbecco's Modified Eagle's Medium, 10% foetal bovine serum (Gibco), and 2% penicillin-streptomycin as antibiotics, and was kept at 37° C in an incubator with 5% CO2. The cells were grown in T-15 flasks until they achieved around 80% confluence.

[4.7] Cytotoxicity test – MTT assay

Materials required: DMEM, FBS, penicillin-streptomycin, MTT, PBS

L929 was cultured in DMEM. Cells were added on 96 well plates. Cells were treated with different concentrations of duckweed extract solution in 1 % concentration of extract 50, 100, 200 and 400 µg/mL named as D.E-1, D.E-2, D.E-3, and D.E-4 respectively and incubated for 24 h at 37 ºC and 5 % CO2.[72] After that, 10 µl of MTT of concentration 5 mg/mL in PBS was added to each well and incubated for another 4 hours. Then MTT along with the media was removed carefully and dissolve the formed formazan using 100µl of DMSO. At 590 nm the absorbance was taken and % viability was calculated.[58]

$$\textbf{\% viability} = \frac{\textbf{Absorbance measured of treated cells}}{\textbf{Absorbance measured of untreated cells}} \times 100$$

[4.8] Antibacterial assay

Materials required: Nutrient broth, Agar agar, streptomycin, dimethyl sulfoxide (DMSO)

Test Microorganisms: Standard strains of S. aureus and E. coli

To test the efficacy of Duckweed whole plant extracts against chosen bacterial strains, 50 mg of each lyophilized sample were poured in 1 mL of DMSO, with DMSO serving as the reference solvent for each sample.[58]ak Antibacterial activity against E. coli and S. aureus was determined using both well-diffusion and disc-diffusion techniques. Streptomycin was employed as the standard and DMSO as the control. After incubating the plates for 24 hours, the antibacterial zone of inhibition was evaluated. The avg. value of two separate approaches was then noted down.

[4.9] In-silico Study:

[4.9.1] Phytochemicals found in Duckweed whole plant extract

From the scientific reports till published in different journals a list of phyto chemicals (‘phyto’ means plant & ‘phytochemical’ means the chemical compounds exclusively produced by the plants) present in Duckweed plant has been prepared as given in Table 4.1. After sorting down all the available phytochemical found throughout the whole body of the plant all the phytochemicals are selected.[84]

After the phytochemicals found in duckweed were selected, we took help from another website i.e. ***‘PubChem’*** website which is a treasury for the details of any chemical compound that has been discovered till now. To be more specific, PubChem is a database for chemical substances that also includes the activities of the molecules against biological studies. The entire system is managed by the National Center for Biotechnology Information (NCBI), which is part of the National Library of Medicine (NLM), which is part of the National Institutes of Health in the United States (NIH). PubChem is a free public access portal accessible via a web user interface.[85]

More than 75 database authorised vendors regularly contribute to the growing PubChem database. So, with the help of this vast database website, the details of our chemicals of interest were downloaded and put in the same table. [86]

In the PubChem website the name of each compound was put in the search box and the respective details were collected for each of the molecules.[87] The details like- ***Canonical SMILES***, molecular weight, and three dimensional (3D) structures of all the chemicals under study, have been gathered from the above-mentioned website. The whole data set is provided in the Table 4.1 below. SMILES is the abbreviation of 'Simplified Molecular Input Line Entry System'. It is one type of chemical notation of the chemical compound. This unique notation allows the user to represent a chemical structure in a way that can be used/ read by the computer. The canonical form of the SMILES is a linear text format. This particular orderly arrangement of meaningful texts can describe the connectivity and chirality of a molecule of interest.[88]

[4.9.2] Screening of phytochemicals through ADMET property checking and Selection of phytochemicals for docking

ADMEtlab2.0 is a freely available, user-friendly website for checking ADMET properties of chemical compounds based on a huge and comprehensive database. Here the canonical SMILES of all the mentioned phytochemicals were put and based on their individual properties, some were selected for docking purpose and others got rejected. [89]

The comparative table prepared below is based on the following properties-

Physicochemical property

Molecular Weight

Optimal range of MW:100~600 g/mol, based on Drug-Like Soft rule.

It is a very important physiochemical property which is the first criterion for selection of a molecule as a candidate for potential drug.[70]

Volume

Volume based on Van der Waals volume.

Density

Density = Molecular Weight / Volume

nHA and nHD

Optimal range: 0~12, based on Drug-Like Soft rule.

nHA and nHD define Number of H-bond acceptors and doners present respectively in molecule. Sum of all O and N. [71]

fChar

Formal charge is a hypothetical charge of an atom present in molecules. The atom gets that charge if we alter electron distribution in bonds even atoms also. When we go for preparation of ligand have to subtract formal charge as is fake charge.[72]

Calculating Formal Charges

Formal Charge = (valence electrons of atom) - (no of unshared electrons) - (present bonds).

Optimal: -4 ~4, based on Drug-Like Soft rule

Stereo Centers

Number of stereocenters present in molecule had to be consider.

Optimal: ≤ 2, based on Lead-Like Soft rule.

TPSA

Full form of TPSA is Topological polar surface area.

Optimal value lies between 0 to 140, based on Veber rule.[73]

logP

The n-octanol/water distribution coefficient's logarithm. Log P has a major impact on membrane permeability as well as hydrophobic binding to macromolecules such as the target receptor. and additional proteins such as plasma proteins, transporters, or metabolising enzymes.[75]

A compound's expected logP is expressed as the logarithm of the molar. Compounds with concentrations ranging from 0 to 3 log mol/L will be regarded suitable.[77]

Medicinal Chemistry

Quantitative estimate of drug-likeness (QED)

A drug-likeness metric founded on the idea of desire. QED is determined by integrating the outputs of eight drug-likeness-related desirability functions, In this case, QED was calculated using average descriptor weights. The geometric mean of the individual desirability functions yields the QED score, given by -

$$QED = exp\left(\frac{1}{n}\sum_{i=1}^{n} \ln d_i\right)$$

where d_i represents the d_{th} desirability and n = 8 represents the no. of drug-likeness associated attributes.[78]

Results interpretation: The average QED for attractive compounds is 0.67, 0.49 for unattractive compounds, and 0.34 for unattractive compounds consider complicated.

Results decision: ≤ 0.67: poor (red); > 0.67: excellent (green)

SAscore

The scale runs from 1 (easy to make) to 10. (Very tough to achieve.) The synthetic accessibility score (SAscore) is made up of two parts:

$$SAscore = fragmentScore - complexityPenalty.$$

Results interpretation: when SAscore is high: ≥ 6, difficult to synthesize; when SAscore is low: < 6, easy to synthesize

Empirical decision: > 6: poor (red); ≤ 6 : excellent (green)

Lipinski Rule

The Lipinski rule of five can allow differentiate between drug-like compounds and non-drug-like compounds. It indicates a high likelihood of accepted or rejected due to drug likeness for compounds that fulfil 2 or more than 2 of the following conditions.[79]

- Less than 5 H bond donors present
- Less than 10 H bond acceptors present
- Molar refractivity index would be between 40-130
- High lipophilicity (expressed as Log P less than 5)
- Molecular mass less than 500 Dalton

Results: If two qualities are out of range, poor permeability or absorption may occur.

Pfizer Rule

Content: log P should be greater than 3; TPSA should be less than 75

Results interpretation: Compounds having a high log P (>3) and a low TPSA (75) are toxic.

Results decision: two conditions satisfied: poor (red denoted); otherwise: excellent (green denoted)

Sr. No.	Phytochemical Name	Molecular Weight	PPB	CYP1A2-inh	CYP1A2-sub	Lipinski Rules	Pfizer Rules

Absorption

BBB Penetration

To reach their molecular target, drugs that function in the CNS must penetrate the blood–brain barrier (BBB). In contrast, medicines with a peripheral target may require minimal or no BBB penetration to prevent CNS adverse effects.[69]

Result interpretation: BBB penetration is measured in centimetres per second. Molecules with logBB more than -1 were classed as BBB+ (Category 1), whereas those with logBB less than -1 were classified as BBB- (Category 0).

Empirical decision: 0-0.3 indicates excellent (green); 0.3-0.7 indicates medium (yellow); 0.7-1.0(++) indicates poor (red)

EXCRETION

Clearance

Clearance is an essential pharmacokinetic characteristic that, along with volume of distribution, governs a drug's half-life and, hence, the frequency of dose.[67]

Result: if >15 mL/min/kg: high clearance; else 5- 15 mL/min/kg: moderate clearance; else <5 mL/min/kg: low clearance.

Results decision: ≥ 5 means excellent (green);< 5 means poor (red).

Table 4.1: Comparative table for Duckweed (L. minor) phytochemicals based on ADMET properties

1	Vitexin	432.11	88.82%	0.05	0.042	Accepted	Accepted
2	Niacin	123.03	16.07%	0.027	0.371	Accepted	Accepted
3	Nicotinamide	122.05	22.02%	0.31	0.756	Accepted	Accepted
4	Phenylalanine	165.08	31.09%	0.062	0.049	Accepted	Accepted
5	Leucine/Isoleucine	131.09	13.38%	0.026	0.072	Accepted	Accepted
6	Tryptophan	204.09	33.61%	0.126	0.079	Accepted	Accepted
7	Valine	117.08	7.19%	0.029	0.057	Accepted	Accepted
8	Tyrosine	181.07	25.19%	0.028	0.044	Accepted	Accepted
9	Proline	115.06	11.22%	0.013	0.079	Accepted	Accepted
10	Glutamic acid	147.05	10.64%	0.004	0.022	Accepted	Accepted
11	Aspartic acid	133.04	9.95%	0.004	0.023	Accepted	Accepted
12	Di-L-Alanine	160.08	6.58%	0.004	0.043	Accepted	Accepted
13	4-Methoxy cinnamic acid	178.06	89.42%	0.121	0.532	Accepted	Accepted
14	Alanine	89.05	7.26%	0.012	0.055	Accepted	Accepted
15	Threonine	119.06	10.76%	0.014	0.068	Accepted	Accepted
16	Serine	105.04	28.05%	0.01	0.063	Accepted	Accepted
17	Apigenin-6,8-di-Cglucopyranoside	594.16	73.59%	0.004	0.012	Rejected	Accepted
18	Robinetin	302.04	94.64%	0.89	0.106	Accepted	Accepted
19	Apigenin-6-Carabopyranoside-8- C-glucopyranose	564.15	75.67%	0.006	0.016	Rejected	Accepted
20	Luteolin-30 ,7-di-Oglucoside	610.15	70.73%	0.016	0.017	Rejected	Accepted
21	Saponarin	594.16	70.57%	0.007	0.014	Rejected	Accepted
22	Isoorientin	448.1	89.40%	0.041	0.041	Rejected	Accepted
23	Isovitexin	432.11	90.31%	0.043	0.042	Accepted	Accepted
24	Norwogonin	270.05	98.74%	0.977	0.155	Accepted	Accepted
25	Quercetin-3-Oglucoside	464.1	86.41%	0.073	0.036	Rejected	Accepted
26	Apiin	564.15	81.36%	0.021	0.054	Rejected	Accepted
27	Umbelliferone	162.03	85.68%	0.973	0.847	Accepted	Accepted
28	Quercetin	302.04	95.50%	0.943	0.115	Accepted	Accepted
29	Luteolin	286.05	95.44%	0.981	0.154	Accepted	Accepted
30	Naringenin-7-Oglucoside	434.12	86.90%	0.028	0.039	Accepted	Accepted
31	Myricetin	318.04	92.77%	0.846	0.108	Accepted	Accepted
32	Orientin	448.1	87.86%	0.05	0.04	Rejected	Accepted
33	Peonidin	301.07	96.58%	0.904	0.852	Accepted	Accepted
34	Chrysoeriol	300.06	95.93%	0.98	0.867	Accepted	Accepted
35	Tricin	330.07	91.48%	0.954	0.953	Accepted	Accepted
36	Apigenin	270.05	97.25%	0.988	0.145	Accepted	Accepted
37	Acacetin	284.07	97.23%	0.984	0.86	Accepted	Accepted
38	Kaempferol	286.05	97.86%	0.972	0.11	Accepted	Accepted

39	Galangin	270.05	98.59%	0.976	0.126	Accepted	Accepted
40	Flavone	222.07	96.90%	0.988	0.529	Accepted	Rejected
41	6-Methoxyflavone	252.08	95.64%	0.984	0.916	Accepted	Rejected
42	5-Hydroxy-6-Methoxyflavone	268.07	95.78%	0.976	0.915	Accepted	Rejected

[4.9.2] Receptor and ligand preparation

The protein data bank (PDB) website (http://www.rcsb.org/pdb) has been used to get the crystal structure of Glycogen synthase kinase 3 in conjunction with inhibitor. (PDB ID 1Q5K) Previously found major phytochemicals of Duckweed (*L. minor*) were received in SDF format from https://pubchem.ncbi.nlm.nih.gov/. Using Discovery Studio Visualizer 2017 R2, the ligand's SDF format was converted to mol2 format (Figure 4.2). Prior to docking, the receptor and ligands were built using UCSF Chimera's Dock Prep programme. The ligands and receptors were employed specifically by adding hydrogen atoms to the procedure. Atoms have been charged in order for them to be associated with partial charges and other force field attributes.[74]

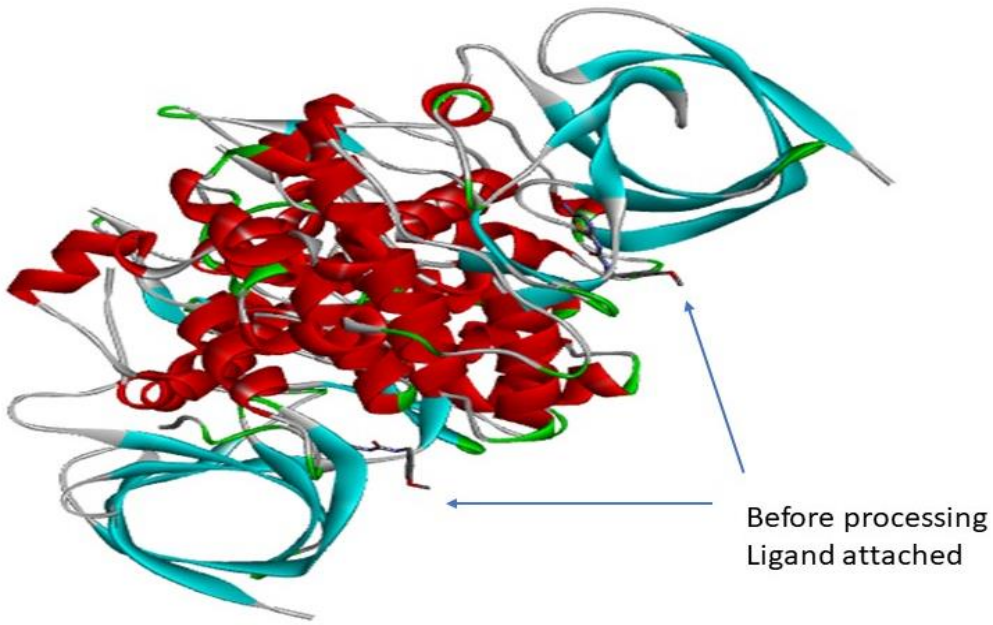

Figure4.2: The 3D structure of crystal structure of Glycogen synthase kinase 3 in complexed with inhibitorMF3958 ligand (PDB ID: 1Q5K)

Preparation of receptor prior docking: The Glycogen synthase kinase 3 protein file was opened in Discovery Studio. Then the pre-attached ligand groups are deleted from the whole structure. Now the new structure containing only the Glycogen synthase kinase 3 protein groups (namely A, B, C chains) only was saved as PDB file by replacing the existing file. [71]

Preparation of ligand prior docking: The phytochemical (SDF file format) 3D structures downloaded from PubChem were opened in Discovery Studio and saved as PDB files.

Figure 4.3: 3D Structure of GSK-3 protein after deletion of pre-existing ligand

After that, ***Autodock tools – 1.5.7*** (Autodock Vina) [14] is opened. Now the protein PDB file was read. Some edits were done like if water molecules existed then those were deleted then only polar hydrogens were added, Kollman charges were added. In a nutshell, introducing hydrogen to the system improved the receptor and ligands. Then charges were applied to the atoms in order to correlate them with partial charges.

Active site selection

The active site of the Glycogen synthase kinase 3 protein (PDB ID: 1Q5K) has been anticipated based on the available receptor cavities. Site 1 was chosen as the active site since the natural inhibitor ligand interacts to it (X=1.2285, Y=-22.0615, Z=27.0265).

Virtual molecular docking

The previously published approach for virtual molecule docking and analysis was used. All of the small compounds were docked to active site 1 (X=1.2285, Y=-22.0615, Z=27.0265) of the Glycogen synthase kinase 3 protein using autodock vina. To confirm our molecular docking methodology, comprehensive redocking was performed.

Chapter Five

RESULTS

[5.1] Phytochemical analysis of Duckweed extracts

Extraction was done using 60% Methanol based on some previous reports.[102]

[5.2] Phytochemical Screening of Duckweed whole plant extract

The presence of phytochemicals like alkaloids, phenols, flavonoids, tannin, and reducing sugar were confirmed by performing the phytochemical screening. It suggests that there may be some kind of medicinal, anticancer activity present in the extract.

Table 5.1: Qualitative Phytochemical Screening of Duckweed Whole plant Extract

Sl No.	Tests	Duckweed whole plant extract
1.	Phenols	+
2.	Flavonoids	+
3.	Alkaloids	+
4.	Tannin	+
5.	Reducing sugars	+

[5.2.1] Total phenol content (TPC)

The Folin–Ciocalteu reagent (FCR) is sensitive to polyphenols. It produces a blue color complex on reaction. The principle of F-C assay is based on the transfer of electrons (which act as reducing equivalents) in an alkaline medium from phenols to phosphomolybdic/ phosphotungstic acid complexes and this is responsible for the formation of blue colored complexes.[56]

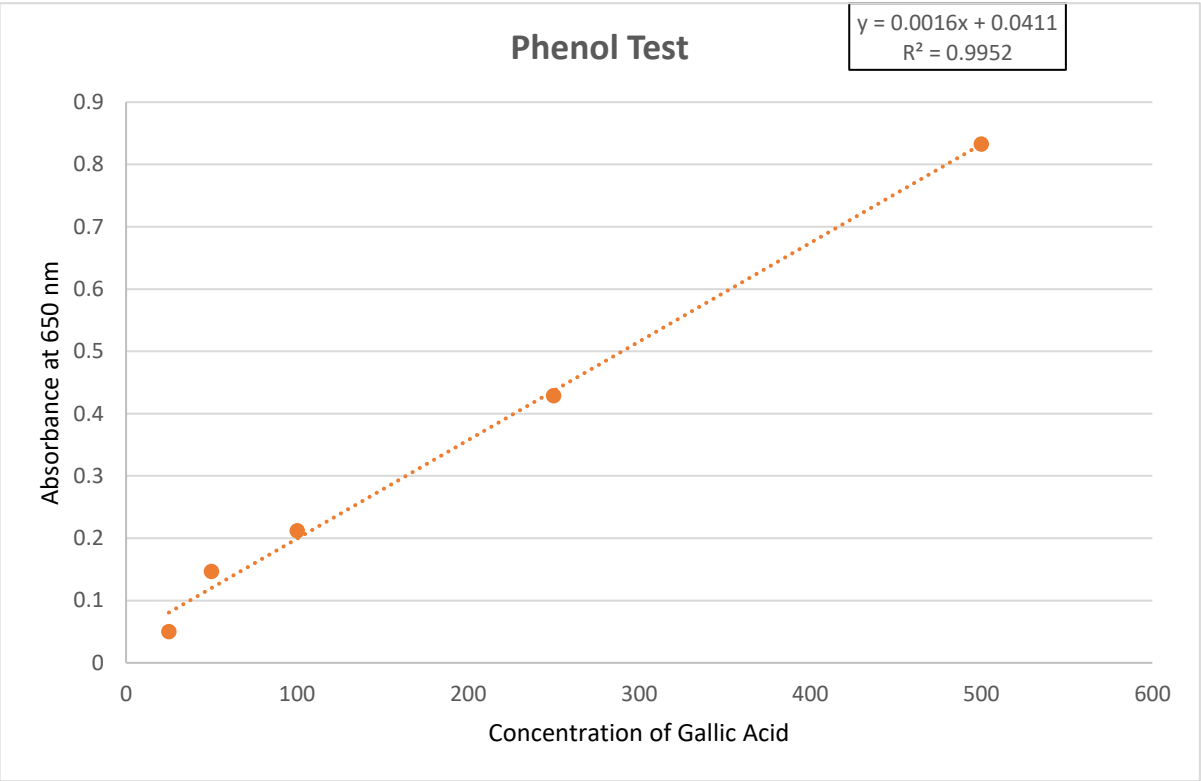

Figure 5.1: Absorbance vs Concentration for standard graph of Gallic acid

Our experimental data showed that the total phenolic content in Duckweed whole plant extract with concentration 500 µg/mL was 210.45 µg/mL gallic acid equivalents (GAE). For this estimation, a standard curve (Fig.5.1.) was used. These phenolic substances have potent anti-Alzheimer activities as well as help to prevent diseases related to oxidative stress [1]. Such pharmacological activities of phenols are mainly for its antioxidant, anti-inflammatory and anti-clastogenic activities [2].

[5.2.2] Total flavonoid content (TFC)

Our experimental data showed that the total flavonoid content in Duckweed whole plant extract having concentration 500 µg/mL was 170.44 µg/mL. For this estimation standard curve of quercetin (Fig. 5.2.) was used. It suggested that Duckweed whole leaf extract, a rich source of flavonoids can play a significance role to block neurodegeneration [57], inhibit angioge nesis [58] or disrupt mitotic spindle formation [59] which makes it a promising substance in Alzheimer research.

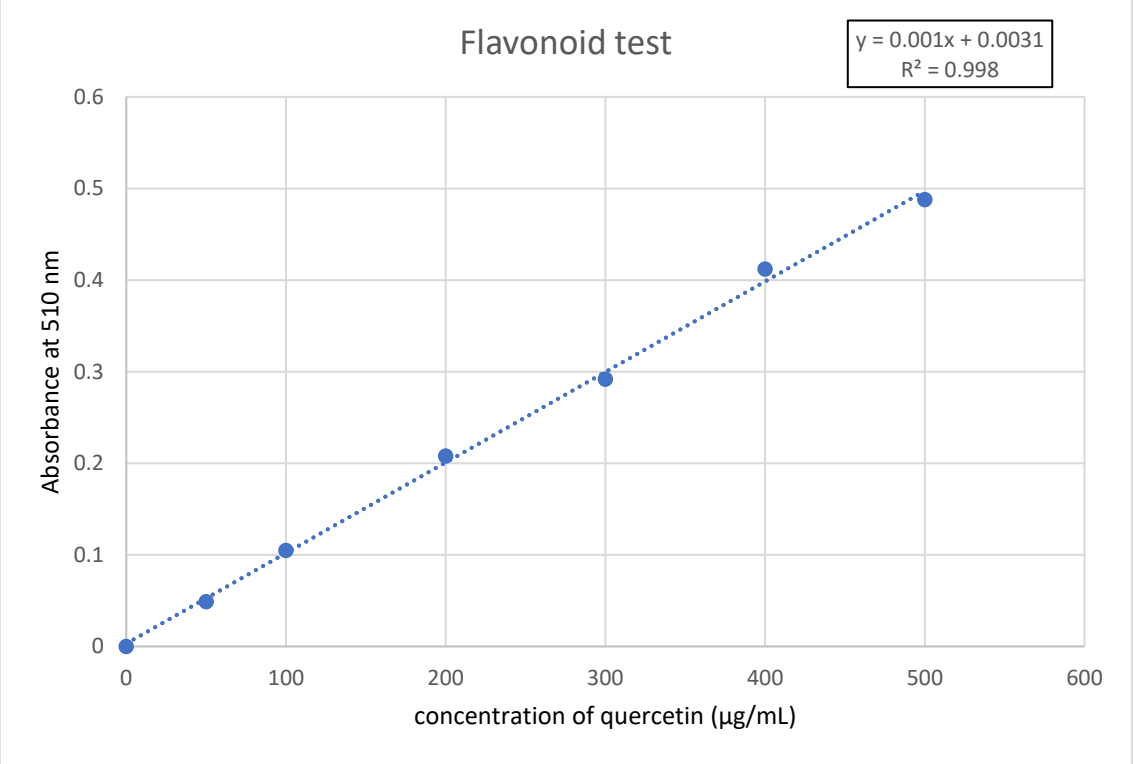

Figure 5.2: Absorbance vs Concentration for standard graph of Quercetin

So, the experimental results of total phytochemical contents (total phenolic content & total flavonoid content) of Duckweed whole plant extracts of concentration 500 µg/mL are presented in the following table 5.2. –

Phytochemical	**Concentration (µg/mL)**
Phenols	210.45
Flavonoids	170.44

Table 5.2. Quantitative Evaluation of Phytochemicals

[5.3] Antibacterial activity Evaluation:

Methanolic extract exhibited the most antibacterial action. It was discovered that the total concentration of phenols and flavonoids combined had a direct impact on the percentage inhibition, i.e., the higher the value of the sum of TPC and TFC, the larger the zone of inhibition. [45]

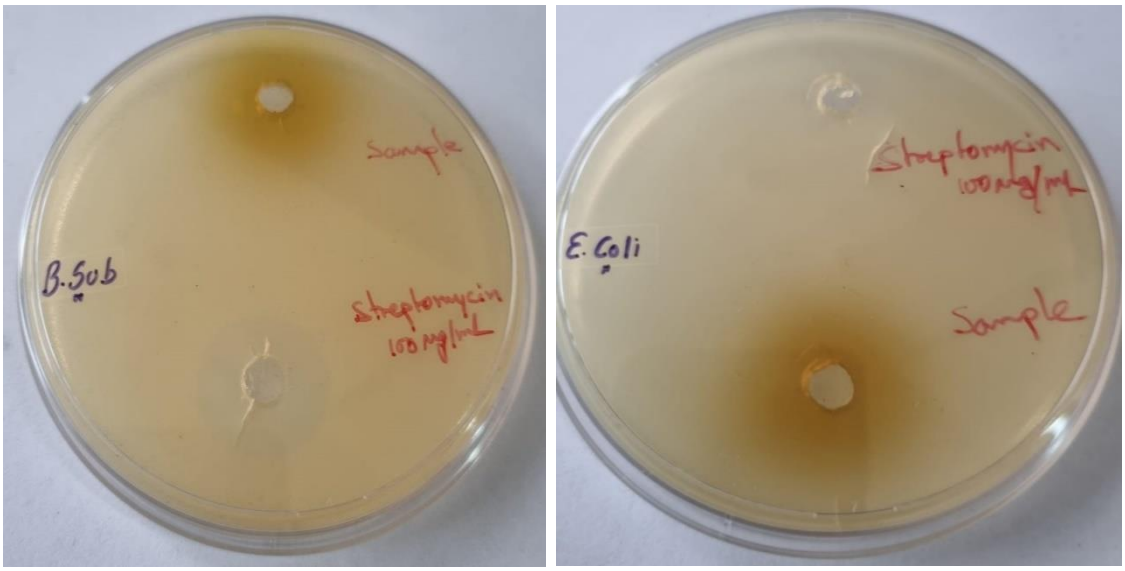

Figure 5.3: Antibacterial activity of turmeric extracts showing zone of inhibition against B. sub and E. coli respectively

Zone of inhibition in *B. subtilis* and *E.coli* for the standard drug (i.e. streptomycin at 100 μg/mL) is 14±0.24 mm and 12±0.18 mm respectively, while that of the methanolic plant extract (at a concentration of 50 μg/mL) is 8±0.19 mm and 9±0.26 mm respectively, indicating the antibacterial activity of the prepared extract against both gram-positive and gram-negative bacteria.

[5.4] Antioxidant activity – by DPPH assay:

Free radical scavenging activity is measured using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) as a free radical. Our data (Fig.5.4) showed that ascorbic acid contained 69.429 % to 88.567 % DPPH free radical scavenging activity at the treatment concentration of 25μg/mL

to 100µg/mL. Percentage free radical scavenging activity is also compared with the methanolic extract of Duckweed plant at same concentrations.

This result suggests that antioxidant activity of Duckweed whole plant extract is quite comparable with the standard ascorbic acid used in the assay. The % free radical scavenging activity for each concentration is calculated and the dose that cause 50% inhibition i.e. the IC50 value is estimated.

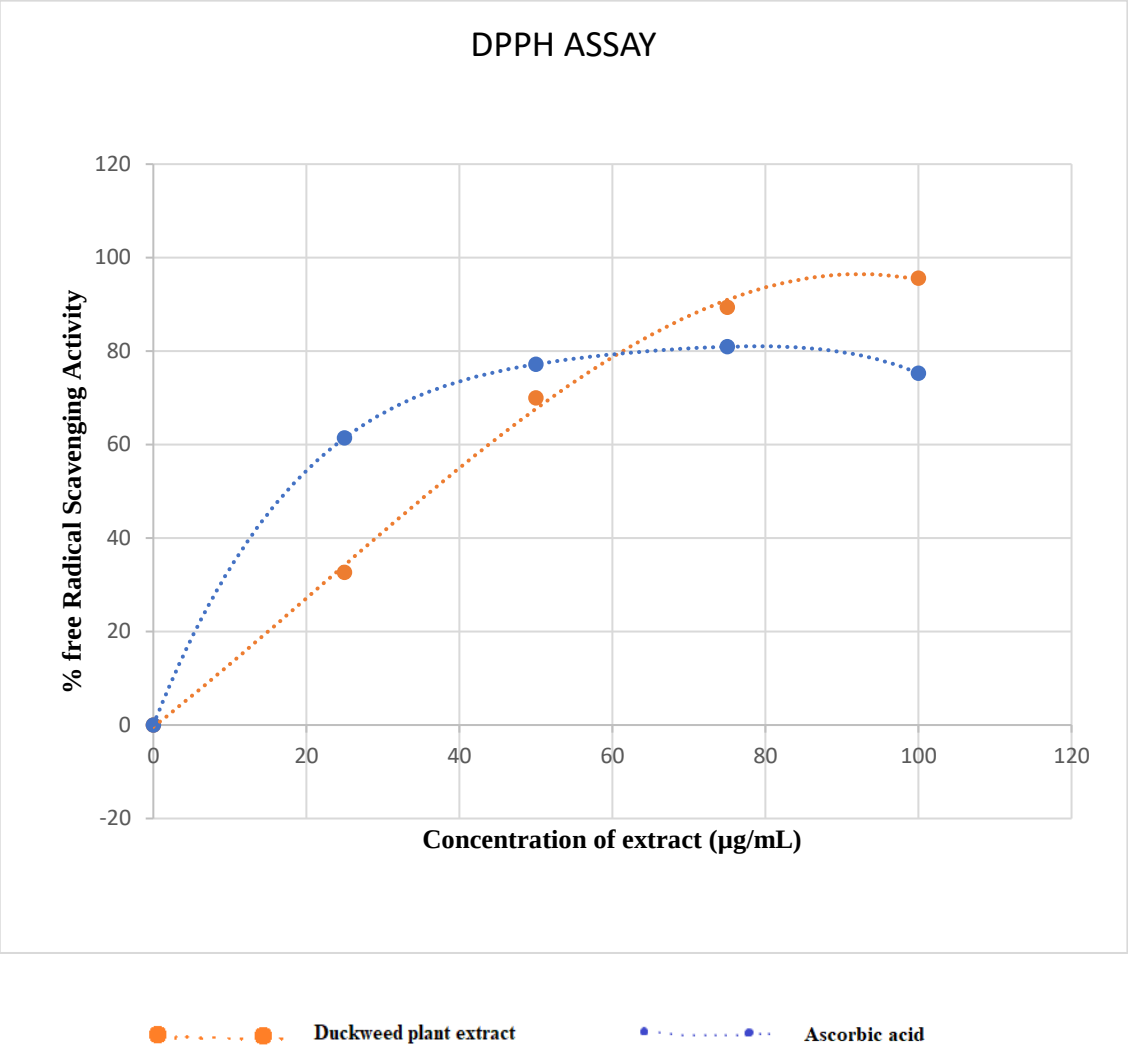

Figure 5.4: Antioxidant Activity of Duckweed whole plant Extract

The IC_{50} value of ascorbic acid is estimated to be 14 µg/mL, while that of the plant extract is found to be 38 µg/mL.

[5.5] Cytotoxic test – MTT assay

MTT test was used to determine the dose-dependent cytotoxicity of Duckweed whole plant extract against normal non-cancerous mouse fibroblast cell line L929.

After 24 hours of treatment with varied doses of Duckweed whole plant extract on L929 cells in 96- well plate, the MTT test was conducted. Our results demonstrated that different concentrations of Duckweed whole plant extract stimulated considerable proliferation of fibroblast (L929) cells (Fig. 5.5). According to the graphical depiction of the experimental results (fig. 5.5), the maximum cell proliferation occurred at a concentration of 25µg/mL of Duckweed whole plant extract. The lowest percentage of cell viability was seen at a concentration of 500µg/mL. Wells without any treatment (i.e. consisting complete medium only) was considered to 100 % viable and the percentage cell viability of cells in the treated wells was calculated with respect to the untreated wells (i.e. control sample). It is observed that the concentration of Duckweed whole plant extract is reduced to 25µg/mL, the percentage viability of L929 cells increases to 107 %.

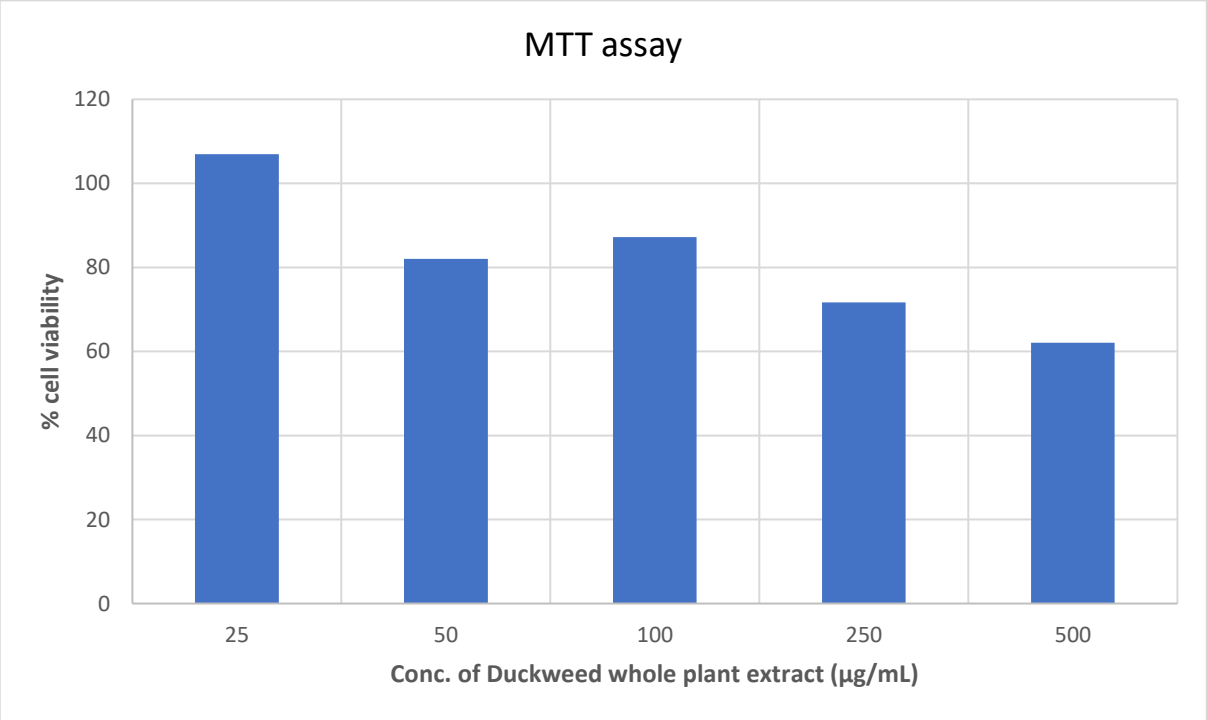

Figure5.5: Cytotoxic effect of different concentrations of Duckweed whole plant extract against L929 Cell Line

[5.6] Bovine Serum Albumin (BSA) denaturation Assay

Protein denaturation is one of the primary causes of inflammation. The results demonstrated that varied doses of Duckweed whole plant extract protected Bovine Serum Albumin (BSA) against heat-induced denaturation (fig. 5.7).

The percentage anti-denaturation activity at 25 mg/mL concentration is 78.0, which is the highest score here. If we look at the curve in fig. 5.5, we can see that as the concentration of leaf extract increases, the percentage of anti-denaturation activity decreases. As the content of Duckweed extract was reduced, the anti-denaturation activity of the leaf extract against heat-induced BSA denaturation. Therefore, it can be seen that the Duckweed whole plant extract indeed possesses a very good anti-denaturation property in a concentration-dependent manner from 25 to 250 ug/ml concentration range.

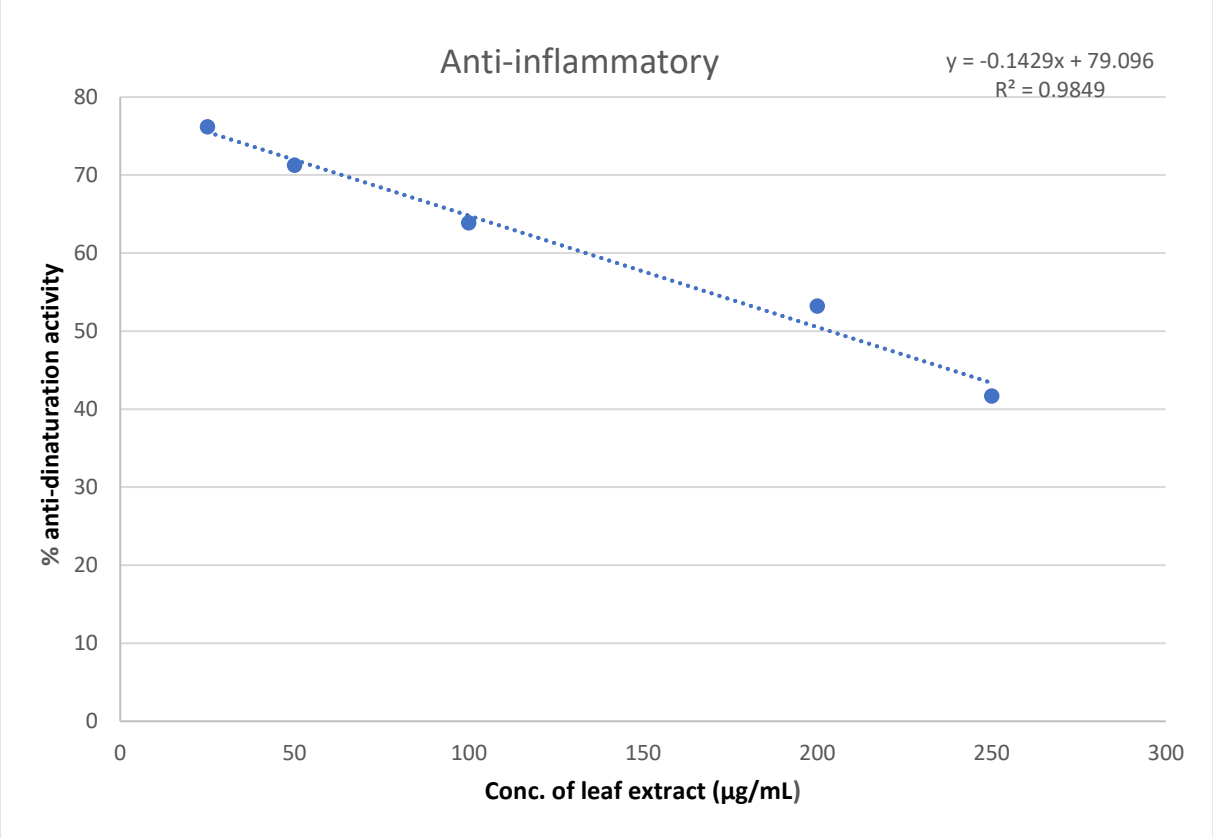

Figure 5.6: Anti-denaturation activity of Duckweed Whole plant Extract against heat-induced BSA denaturation.

[5.7] VIRTUAL MOLECULAR DOCKING:

The protein PDB file of Glycogen synthase kinase 3 prepared early was opened with Autodock Vina software. After that polar only H – bond added. Then kollman charge was added to followed by checking total charge on residues.

Afterwards, PDB file of a phytochemical was opened along with the receptor protein. Then through Torsion Tree path option, root was detected and number of torsions for the ligand was set accordingly. The outcome result was saved in PDBQT file format.

In next step opened start up directory and set the file location. Now, the protein molecule was selected as macromolecule; here the colour of the protein molecule faded and the output file was saved as protein PDBQT file format.

From Grid option, Grid Box of the following specifications was constructed-
size_x = 126, size_y = 126, size_z = 126; center_x = 34.9, center_y = 76.7, center_z = 89.5

Now the output grid dimension file was saved as a TXT file format.
Using that grid dimension TXT file as reference, a new file named Configuration file (txt format; created using notepad application) was created specifying the following details-
receptor = protein.pdbqt; ligand = ligand.pdbqt; size_x = 126; size_y = 82; size_z = 126; center_x = 34.9; center_y = 76.7; center_z = 89.5; energy_range = 4; exhaustiveness = 8

Then Command Prompt was opened and the specific command was put in it to run the docking process of receptor with the selected ligand. After some times when the whole program was done running, an output file of PDBQT format containing the docked result was produced.
Then, Discovery Studio was opened and that output PDBQT file and protein PDBQT file were dragged and dropped respectively inside the open window.
After selecting Receptor-Ligand-Interaction option, the protein file was defined as receptor and Hetatm was defined as ligand.

Now the end result for docking i.e., the detailed 2D diagram of receptor-ligand complex structure could be seen in the Discovery Studio window along with its 3D format.

Docking Scores of standard drugs for Alzheimer's disease with Glycogen synthase kinase 3 Receptor are given below -

Sl. No.	Name of Drugs	Affinity to GSK-3
1	Donepezil	-8.3
2	Galantamine	-7.5
3	Rivastigmine	-7.7

Table 5.2: Affinity of Marketed Drugs with GSK-3 inhibitor

Donepezil drug is most used Alzheimer medicine and it is also used for every stage of Alzheimer's disease condition.

Galantamine and Rivastigmine is approved medicine used for mild to moderate Alzheimer disease treatment. In case of severe condition skin patch of Rivastigmine is used.

As the docking score of Donepezil, Galantamine and Rivastigmine with GSK-3 was found respectively -8.3, -7.5 and -7.7 kcal/mol.

Score beyond -8.1 (less than -8.1) was clearly regarded as even better score than the standard drugs. The scores greater than -7.6 was regarded as poor docking score indicating less affinity with the receptor.

Sr. No.	Phytochemicals	Docking score	No of hydrogen bonds	Accepted/ Rejected
1	Nicotinamide	-4.8	2	Rejected
2	Leucine/Isoleucine	-4.5	1	Rejected
3	Valine	-4	2	Rejected
4	Tyrosine	-5.8	5	Rejected

5	Aspartic acid	-4.1	1	Rejected
6	Alanine	-3.2	2	Rejected
7	Tryptophan	-6.5	2	Rejected
8	Phenylalanine	-5.7	1	Rejected
9	Umbelliferone	-6.4	0	Rejected
10	Quercetin	-8.3	1	**Accepted**
11	Tricin	-8	1	**Accepted**

Table - 5.3. Docking Scores of Duckweed phytochemicals with GSK-3 Receptor

From Table 5.3. it could be easily seen that 2 out of the 11 phytochemicals (ligands) might be accepted as potential substitution of the standard drugs for GSK-3 targeted Alzheimer disease.

Among those 2 ligands Tricin (score -8) and Quercetin (score -8.3), these 2 ligands had shown even better affinity with GSK-3 than the standard drugs. Among these 2 ligands, Quercetin has the highest affinity with GSK-3 protein receptor. So, this can be considered as the best among the lot.

Therefore, Quercetin might be considered as a natural substitution of Donepezil, Galantamine and Rivastigmine, the standard drugs for GSK-3 based Alzheimer's disease treatment.

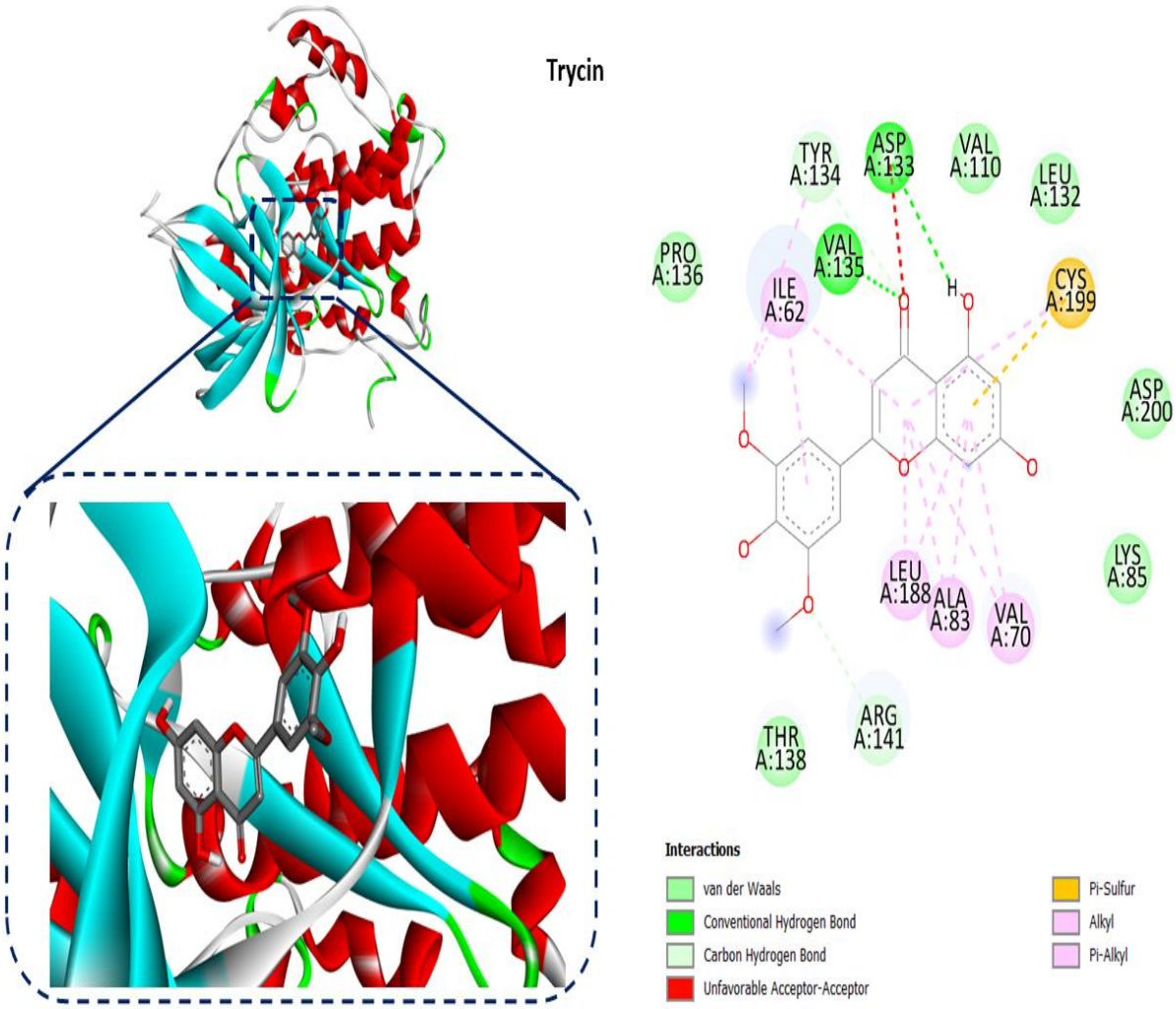

Figure 5.7 Tricin ligand interaction with Glycogen synthase kinase 3 (GSK-3) protein.

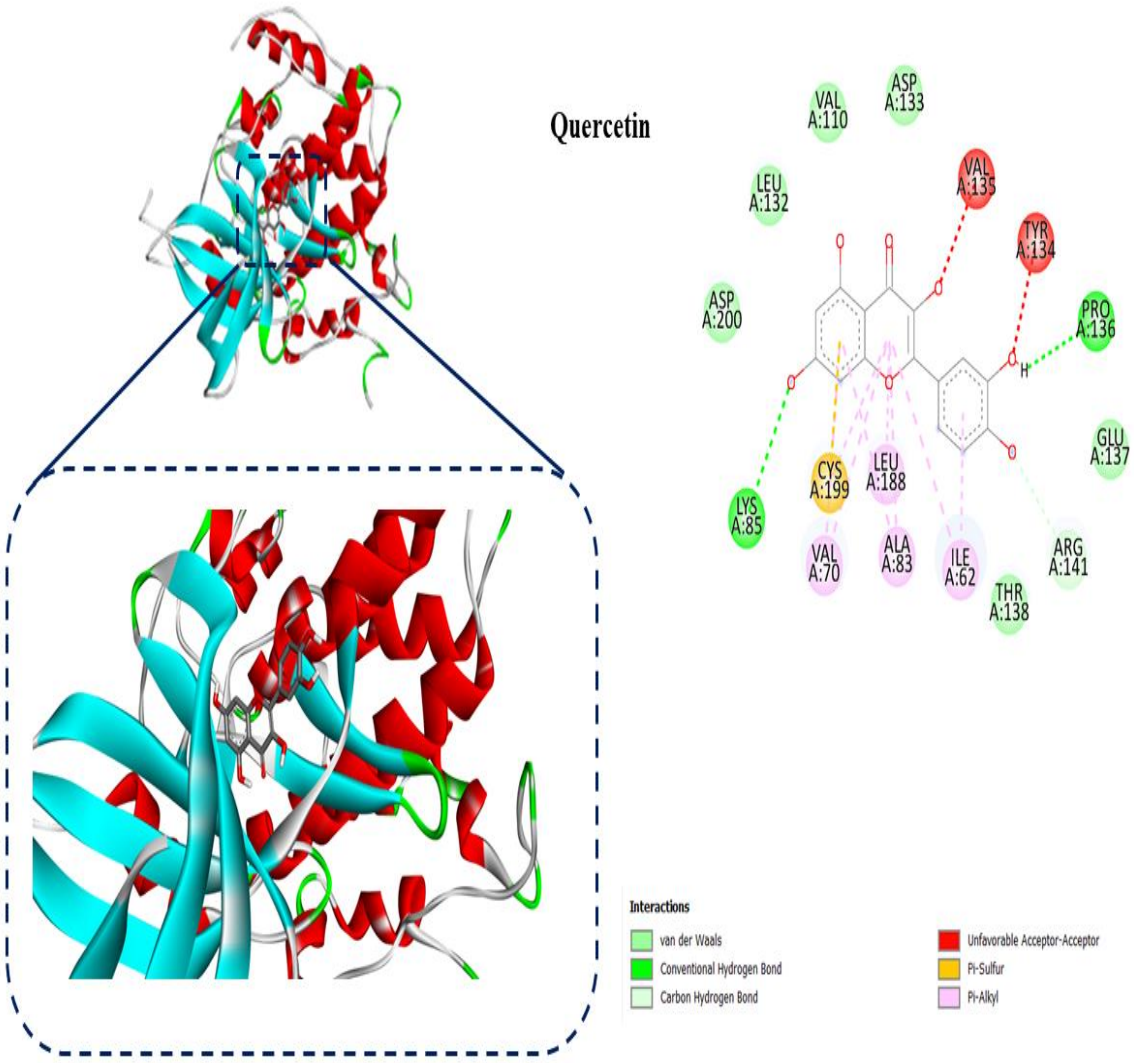

Figure 5.8: Quercetin ligand interaction with Glycogen synthase kinase 3 (GSK-3) protein

CHAPTER SIX

CONCLUTION AND FUTURE PLAN OF WORK

[6.1] CONCLUTION:

Duckweed has shown significant content of phenols and flavonoids which can be claimed to be responsible for its biological activities. Our study described that Duckweed whole plant extract has a significant amount of antioxidant and anti-inflammatory properties. Such pharmacological activity of Duckweed extract may be due to the presence of secondary metabolites like alkaloids, phenolic compounds, flavonoids. As Duckweed is rich in phenols, lots of micronutrients are present, it helps to prevent our brain from neurodegeneration.

Crude Duckweed whole plant extract showed small zone of inhibition against both gram positive and gram-negative bacteria proving it possesses antibacterial property.

From in silico molecular docking study, we get that specific phytochemical compounds of Duckweed plant can inhibit Alzheimer responsible protein (Glycogen Synthase Kinase 3 is selected here as a receptor protein). Thus, it may prevent neurodegeneration process.

Therefore, considering both *in vitro* and *in silic*o study of duckweed whole plant phytochemicals, we can conclude that this duckweed extract can inhibit the neurodegeneration process of brain cells as well as it provide micronutrients to the normal cells. So, we can say that if it is possible to design drug from duckweed phytochemicals, they can be very effective in Alzheimer's disease as well as cost effective.

Still much more future studies and researches in this regard are necessary to actualize the idea of manufacturing a drug from Duckweed phytochemicals, which will effectively fight against Alzheimer.

[6.2] FUTURE PLAN OF WORK

- Isolation and purification of the antitumor bioactive compounds from Duckweed whole plant extract.
- *In vitro* anti-Alzheimer study of the different concentration of Duckweed sample was cultivated against different Alzheimer cell lines.
- *In silico* docking of Duckweed phytochemicals against beta-amyloid and cholinesterase receptor proteins.
- Green Synthesis of biogenic silver nanoparticles (AgNPs) from Duckweed whole plant extract.
- Evaluation of cytotoxic effect of AgNPs against Alzheimer cell lines.
- Calculation of IC_{50} and LD_{50}.
- Investigation of the detailed mechanism of action of bioactive agents against Alzheimer disease.
- Performing *in-vivo* experiment with nude mice model.
- Investigating the effect of Duckweed extract on the immune system.
- Development of a non-invasive, patient-friendly drug delivery system for administrating bioactive agents targeting Alzheimer disease.

REFERENCES:

1. Bog, M., Appenroth, K. J., & Sree, K. S. (2019). Duckweed (Lemnaceae): its molecular taxonomy. *Frontiers in Sustainable Food Systems*, *3*, 117.

2. Baek, G., Saeed, M., & Choi, H. K. (2021). Duckweeds: their utilization, metabolites and cultivation. *Applied Biological Chemistry*, *64*(1), 1-15.
3. Sankhalkar, S., & Vernekar, V. (2016). Quantitative and Qualitative analysis of Phenolic and Flavonoid content in Moringa oleifera Lam and Ocimum tenuiflorum L. *Pharmacognosy research*, *8*(1), 16.

4. Skillicorn, P., Spira, W., & Journey, W. (1993). *Duckweed aquaculture: a new aquatic farming system for developing countries*. World Bank.

5. Zayed, A., Gowthaman, S., & Terry, N. (1998). *Phytoaccumulation of trace elements by wetland plants: I. Duckweed* (Vol. 27, No. 3, pp. 715-721). American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America.

6. Thakur, A. K., Kamboj, P., Goswami, K., & Ahuja, K. (2018). Pathophysiology and management of Alzheimer's disease: An overview. *J. Anal. Pharm. Res*, *7*(1).

7. Adhikary T, Hossain CM, Mallick S, Paul S, Banerjee N, Basak P. Revisiting Therapeutic Potentials of Ethanolic Extract of Curcuma longa L. rhizomes to Evaluate wound Healing Progression upon Topical Application of its Ointment. Indian J of Pharmaceutical Education and Research. 2021;55(1):174-83.

8. Longhe, Z. (2020). Alzheimer's disease facts and figures. *Alzheimer's Dement*, *16*, 391-460.

9. Sperling, R. A., Aisen, P. S., Beckett, L. A., Bennett, D. A., Craft, S., Fagan, A. M., ... & Phelps, C. H. (2011). Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia*, *7*(3), 280-292.

10. Albert, M. S., DeKosky, S. T., Dickson, D., Dubois, B., Feldman, H. H., Fox, N. C., ... & Phelps, C. H. (2013). The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Focus*, *11*(1), 96-106.

11. McKhann, G. M., Knopman, D. S., Chertkow, H., Hyman, B. T., Jack Jr, C. R., Kawas, C. H., ... & Phelps, C. H. (2011). The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia*, *7*(3), 263-269.

12. Jack Jr, C. R., Albert, M. S., Knopman, D. S., McKhann, G. M., Sperling, R. A., Carrillo, M. C., ... & Phelps, C. H. (2011). Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia*, *7*(3), 257-262.

13. Bennett, D. A., Schneider, J. A., Arvanitakis, Z., Kelly, J. F., Aggarwal, N. T., Shah, R. C., & Wilson, R. (2006). Neuropathology of older persons without cognitive impairment from two community-based studies. *Neurology*, *66*(12), 1837-1844.

14. Knopman, D. S., Parisi, J. E., Salviati, A., Floriach-Robert, M., Boeve, B. F., Ivnik, R. J., ... & Petersen, R. (2003). Neuropathology of cognitively normal elderly. *Journal of Neuropathology & Experimental Neurology*, *62*(11), 1087-1095.

15. Sharma, O. P., & Bhat, T. K. (2009). DPPH antioxidant assay revisited. *Food chemistry*, *113*(4), 1202-1205.

16. Das, P., Majumder, R., Mandal, M., & Basak, P. (2021). In-Silico approach for identification of effective and stable inhibitors for COVID-19 main protease (Mpro) from flavonoid based phytochemical constituents of Calendula officinalis. *Journal of Biomolecular Structure and Dynamics*, *39*(16), 6265-6280.

17. Friedman, M. (2007). Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. Molecular nutrition & food research, 51(1), 116-134.

18. Hebert, L. E., Weuve, J., Scherr, P. A., & Evans, D. A. (2013). Alzheimer disease in the United States (2010–2050) estimated using the 2010 census. *Neurology*, *80*(19), 1778-1783.

19. D. International, ADI-Dementia Statistics, Alzheimer's Disease International (ADI), London, UK, 2015, https://www. alzint.org/about/dementia-facts-figures/dementia-statistics/. [11]

20. Daisy, P., & Saipriya, K. (2012). Biochemical analysis of Cassia fistula aqueous extract and phytochemically synthesized gold nanoparticles as hypoglycemic treatment for diabetes mellitus. *International journal of nanomedicine*, *7*, 1189.

21. Rao, P. V., Sujana, P., Vijayakanth, T., & Naidu, M. D. (2012). Rhinacanthus nasutus–Its protective role in oxidative stress and antioxidant status in streptozotocin induced diabetic rats. *Asian Pacific Journal of Tropical Disease*, *2*(4), 327-330.

22. Haskell, C. F., Kennedy, D. O., Wesnes, K. A., & Scholey, A. B. (2005). Cognitive and mood improvements of caffeine in habitual consumers and habitual non-consumers of caffeine. *Psychopharmacology*, *179*(4), 813-825.

23. Moss M, Scholey A, Wesnes K (1998) Oxygen administration selectively enhances cognitive performance in healthy young adults: a placebo-controlled double-blind crossover study. Psychopharmacology (Berl) 138, 27–33.

24. Scholey A, Moss M, Neave N et al. (1999) Cognitive performance, hyperoxia, and heart rate following oxygen administration in healthy young adults. Physiol Behav 67, 783–789.

25. Scholey A, Laing S, Kennedy D (2006) Blood glucose changes and memory: effects of manipulating emotionality and mental effort. Biol Psychol 71, 12–19.

26. Kennedy D, Stevenson E, Jackson P et al. (2016) Multivitamins and minerals modulate whole-body energy metabolism and cerebral blood-flow during cognitive task performance: a double-blind, randomised, placebocontrolled trial. Nutr Metab 13,1.

27. Kennedy D, Wightman EL, Reay JL et al. (2010) Effects of resveratrol on cerebral blood flow variables and cognitive performance in humans: a double-blind, placebo-controlled, crossover investigation. Am J Clin Nutr 91, 1590–1597.

28. Wightman E, Reay J, Haskell C et al. (2014) Effects of resveratrol alone or in combination with piperine on cerebral blood flow parameters and cognitive performance in humans: a randomised, double-blind, placebo-controlled, crossover investigation. Br J Nutr 112, 203–213.

29. Wightman E, Haskell-Ramsay C, Reay J et al. (2015) The effects of chronic trans-resveratrol supplementation on aspects of cognitive function, mood, sleep, health and cerebral blood flow in healthy, young humans. Br J Nutr 114, 1427–1437.

30. Rönnlund M, Nyberg L, Bäckman L et al. (2005) Stability, growth, and decline in adult life span development of declarative memory: cross-sectional and longitudinal data from a population-based study. Psychol Aging 20, 3–18.

31. Association As (2016) 2016 Alzheimer's disease facts and figures. Alzheimer's Demen 12, 459–509.

32. International AsD (2015) World Alzheimer Report 2015: The Global Impact of Dementia. An Analysis of Prevalence, Incidence, Cost and Trends. Available at www.alz.co.uk/ research/world-report-2015 (Accessed February 2016).

33. Karch CM, Goate AM (2015) Alzheimer's disease risk genes and mechanisms of disease pathogenesis. Biol Psychiatry 77, 43–51.

34. Luchsinger JA, Mayeux R (2004) Dietary factors and Alzheimer's disease. Lancet Neurol 3, 579–587.

35. Lee P (1994) Smoking and Alzheimer's disease: a review of the epidemiological evidence. Neuroepidemiology 13, 131–144.

36. Durazzo TC, Mattsson N, Weiner MW et al. (2014) Smoking and increased Alzheimer's disease risk: a review of potential mechanisms. Alzheimer's Demen 10, S122–S145.

37. Mukamal KJ, Kuller LH, Fitzpatrick AL et al. (2003) Prospective study of alcohol consumption and risk of dementia in older adults. JAMA 289, 1405–1413.

38. Markesbery WR (1997) Oxidative stress hypothesis in Alzheimer's disease. Free Radic Biol Med 23, 134–147.

39. Vignini A, Giulietti A, Nanetti L et al. (2013) Alzheimer's disease and diabetes: new insights and unifying therapies. Curr Diab Rev 9, 218–227.

40. Kalaria RN (2000) The role of cerebral ischemia in Alzheimer's disease. Neurobiol Aging 21, 321–330.

41. Matrone C, Djelloul M, Taglialatela G et al. (2015) Inflammatory risk factors and pathologies promoting Alzheimer's disease progression: is RAGE the key. Histol Histopathol 30, 125–139.

42. Adlard PA, Bush AI (2006) Metals and Alzheimer's disease. J Alzheimer's Dis 10, 145–163.

43. Vest RS, Pike CJ (2013) Gender, sex steroid hormones, and Alzheimer's disease. Horm Behav 63, 301–307.

44. Sattler C, Toro P, Schönknecht P et al. (2012) Cognitive activity, education and socioeconomic status as preventive factors for mild cognitive impairment and Alzheimer's disease. Psychiatry Res 196, 90–95.

45. Abraham, G. (2013). Evaluation of antimicrobial activity of methanolic extracts of Azolla microphylla. Vegetos 26, 200–204.

46. Amjad Masood, Nisar Ahmad Shah, Mohd. Zeeshan, G., & Abraham. (2005). Differential response of antioxidant enzymes to salinity stress in two varieties of Azolla (Azolla pinnata and Azolla filiculoides), Environmental and Experimental Botany 58, 216–222.

47. Carrapiço, F. (2010). Azolla as a superorganism. Its implication in symbiotic studies. In: Seckbach J, Grube M (eds) Symbioses and stress: Joint ventures in biology, cellular origin, life in extreme habitats and astrobiology. Springer Science + Business Media B.V, Amesterdam, pp. 225–241.

48. Kareti, S. R., & Subash, P. (2020). In Silico Molecular Docking Analysis of Potential Anti-Alzheimer's Compounds Present in Chloroform Extract of Carissa

carandas Leaf Using Gas Chromatography MS/MS. *Current Therapeutic Research*, *93*, 100615.

49. Johnson, M., Babu, A., Irudayaraj, V. (2011). Antibacterial studies on in vitro derived Calli of Ocimum basilicumL. J. Chem. Pharm. Res. 3(1), 715-720.

50. Mangesh Kumar, Tamanna Talreja, Dinesh Jain, Dhuria, R.K., Asha Goswami & Tribhuwan Sharma, (2016). Comparative evaluation of in vitro antibacterial activity of several extracts of Achyranthes aspera, Azolla pinnata, Cissus quadrangularis and Tinospora cordifolia. International Journal of Chemical Studies. 5(1), 154- 157

51. Piccardi, R., Frosini, A., Tredici, M.R., Margheri, M.C. (2000). Bioactivity in free-living and symbiotic cyanobacteria of the genus Nostoc. J. Appl. Phycol. 12, 543–547.

52. Subash, P., & Rao, K. S. (2021). In silico molecular docking analysis for potential anti-Alzheimer's compounds from the methanolic leaf extract of Erythroxylum monogynum using Gas chromatography–mass spectrometry. *Journal of Saudi Chemical Society*, *25*(8), 101285.

53. Sathammaipriya, N., Thamilmaraiselvi, B., Steffi, P.F., Sangeetha, K. (2018). Investigation of phytochemical constituents in Azolla microphylla for antibacterial activity National Journal of Physiology, Pharmacy and Pharmacology.

54. Scalbert, A., Manach, C., Morand, C., Remesy, C., Jimenez, L. (2005). Dietary poly phenols and the prevention of diseases. Crit. Rev. Food Sci. Nutr. 45, 287-306.

55. Mufson EJ, Counts SE, Perez SE et al. (2008) Cholinergic system during the progression of Alzheimer’s disease: therapeutic implications. Expert Rev Neurother 8, 1703–1718.

56. NICE (2009) Donepezil, Galantamine, Rivastigmine (review) and Memantine for the Treatment of Alzheimer’s Disease. UK: National Institute of Clinical Excellence.

57. Birks J, Craig D (2006) No Consistent Evidence of Efficacy of Galantamine in Vascular Cognitive Impairment. Health. Cochrane library. Available at onlinelibrary.wiley.com/doi/10. 1002/14651858.CD004746.pub2full (Accessed February 2016).

58. Society As (2016) Treating behavioural and psychological symptoms of dementia. https://www.alzheimers.org.uk/ site/scripts/documents_info.php? documentID=1191&pageNumber=2 (accessed May 2016).

59. Kennedy D, Wightman E (2011) Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. Adv Nutr: Int Rev J 2, 32–50.

60. Fehske CJ, Leuner K, Müller WE (2009) Ginkgo biloba extract (EGb761®) influences monoaminergic neurotransmission via inhibition of NE uptake, but not MAO activity after chronic treatment. Pharmacol Res 60, 68–73.

61. Agarwal, O. P., & Raju, P. S. (2006). Opportunities for India. Traditional Systems of Medicine, 5, 5–10.

62. Al-Khafaji, K., Al-DuhaidahawiL, D., & Taskin Tok, T. (2020). Using integrated computational approaches to identify safe and rapid treatment for SARS-CoV-2. Journal of Biomolecular Structure and Dynamics, 1–11.

63. Anand, K., Ziebuhr, J., Wadhwani, P., Mesters, J. R., & Hilgenfeld, R. (2003). Coronavirus main proteinase (3CLpro) structure: Basis for design of anti-SARS drugs. Science (New York, N.Y.).), 300(5626), 1763–1767. https://doi.org/10.1126/science.1085658

64. AshwlayanVD, K. A., & Verma, M. (2018). Therapeutic potential of Calendula officinalis. Pharmacy and Pharmacology International Journal, 6(2), 149–155.

65. Berendsen, H. J. C., van der Spoel, D., & van Drunen, R. (1995). GROMACS: A message-passing parallel molecular dynamics implementation. Computer Physics Communications, 91(1–3), 43–56. https://doi.org/10.1016/0010-4655(95)00042-E

66. Bhatnagar, S. S., & Sastri, B. N. (1960). The wealth of India raw materials (A Dictionary of Indian Raw Materials and Industrial Products). New Delhi, India, 10, 64–68.

67. Bickerton, G. R., Paolini, G. V., Besnard, J., Muresan, S., & Hopkins, A. L. (2012). Quantifying the chemical beauty of drugs. Nature Chemistry, 4(2), 90–98. https://doi.org/10.1038/nchem.1243

68. Boopathi, S., Poma, A. B., & Kolandaivel, P. (2020). Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment. Journal of Biomolecular Structure and Dynamics, 1–14.

69. Chakraborthy, G. S. (2010). Phytochemical screening of Calendula officinalis Linn leaf extract by TLC. International Journal of Research in Ayurveda and Pharmacy (IJRAP), 1(1), 131–134.

70. Chen, Y. W., Yiu, C.-P B., & Wong, K.-Y. (2020). Prediction of the SARSCoV- 2 (2019-nCoV) 3C-like protease (3CLpro) structure: Virtual screening reveals velpatasvir, ledipasvir, and other drug repurposing candidates.

71. Abraham, M. J., Murtola, T., Schulz, R., Páll, S., Smith, J. C., Hess, B., & Lindah, E. (2015). Gromacs: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX*, *1–2*, 19–25. https://doi.org/10.1016/j.softx.2015.06.001

72. Broekman, F. (2011). Tyrosine kinase inhibitors: Multi-targeted or single-targeted? *World Journal of Clinical Oncology*, *2*(2), 80. https://doi.org/10.5306/wjco.v2.i2.80

73. Chandrashekar, D. S., Bashel, B., Balasubramanya, S. A. H., Creighton, C. J., Ponce-Rodriguez, I., Chakravarthi, B. V. S. K., & Varambally, S. (2017). UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia (United States)*, *19*(8), 649–658. https://doi.org/10.1016/j.neo.2017.05.002

74. Cross, Sarah J. Linker, Kay E. Leslie, F. M. (2016). 乳鼠心肌提取 HHS Public Access. *Physiology & Behavior*, *176*(1), 100–106. https://doi.org/10.1016/j.canlet.2008.05.045.The

75. Daina, A., Michielin, O., & Zoete, V. (2014). Article pubs.acs.org/jcim iLOGP: A Simple, Robust, and Efficient Description of n-Octanol/ Water Partition Coefficient for Drug Design Using the GB/SA Approach. *Journal of Chemical Information and Modeling*. https://doi.org/10.1021/ci500467k

76. Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, *7*(October 2016), 1–13. https://doi.org/10.1038/srep42717

77. Dannenberg J. J. (1998). *Book re v iews Book re v iews*. *123*(39), 1009–1011.

78. Eldehna, W. M., Al-Rashood, S. T., Al-Warhi, T., Eskandrani, R. O., Alharbi, A., & El Kerdawy, A. M. (2021). Novel oxindole/benzofuran hybrids as potential dual CDK2/GSK-3β inhibitors targeting breast cancer: design, synthesis, biological evaluation, and in silico studies. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *36*(1), 270–285. https://doi.org/10.1080/14756366.2020.1862101

79. Giordano, S., & Petrelli, A. (2008). From Single- to Multi-Target Drugs in Cancer Therapy: When Aspecificity Becomes an Advantage. *Current Medicinal Chemistry*, *15*(5), 422–432. https://doi.org/10.2174/092986708783503212

80. Bickerton G R, Paolini G V, Besnard J, et al. Quantifying the chemical beauty of drugs[J]. Nat Chem, 2012, 4(2): 90-8.

81. Ertl P, Schuffenhauer A. Estimation of synthetic accessibility score of drug-like molecules based on molecular complexity and fragment contributions[J]. J Cheminform, 2009, 1(1): 8.

82. Lovering F, Bikker J, Humblet C. Escape from flatland: increasing saturation as an approach to improving clinical success[J]. J Med Chem, 2009, 52(21): 6752-6.

83. Ivanenkov Y A, Zagribelnyy B A, Aladinskiy V A. Are We Opening the Door to a New Era of Medicinal Chemistry or Being Collapsed to a Chemical Singularity?[J]. J Med Chem, 2019, 62(22): 10026-10043.

84. Ertl P, Roggo S, Schuffenhauer A. Natural product-likeness score and its application for prioritization of compound libraries[J]. J Chem Inf Model, 2008, 48(1): 68-74.

85. Lipinski C A, Lombardo F, Dominy B W, et al. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings[J]. Adv Drug Deliv Rev, 2001, 46(1-3): 3-26.

86. Hughes J D, Blagg J, Price D A, et al. Physiochemical drug properties associated with in vivo toxicological outcomes[J]. Bioorg Med Chem Lett, 2008, 18(17): 4872-5.

87. Gleeson M P. Generation of a set of simple, interpretable ADMET rules of thumb[J]. J Med Chem, 2008, 51(4): 817-34.

88. Johnson T W, Dress K R, Edwards M. Using the Golden Triangle to optimize clearance and oral absorption[J]. Bioorg Med Chem Lett, 2009, 19(19): 5560-4.

89. Baell J B, Holloway G A. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays[J]. J Med Chem, 2010, 53(7): 2719-40.

90. Huth J R, Mendoza R, Olejniczak E T, et al. ALARM NMR: a rapid and robust experimental method to detect reactive false positives in biochemical screens[J]. J Am Chem Soc, 2005, 127(1): 217-24.

91. Pearce B C, Sofia M J, Good A C, et al. An empirical process for the design of high-throughput screening deck filters[J]. J Chem Inf Model, 2006, 46(3): 1060-8.

92. Agrawal A, Johnson S L, Jacobsen J A, et al. Chelator fragment libraries for targeting metalloproteinases[J]. ChemMedChem, 2010, 5(2): 195-9.

93. Amado, R., Muller-Riemeyer, R., & Morti, U. (1980). Protein, neutral sugar content and amino acid composition of Lemnaceae. *Biosystematic investigations in the family of duckweeds (Lemnaceae)*, 102-117.

94. *AOAc 1980. Methods of Analysis. Washington, DC: Association of Official Agricultural*
Chemists.

95. *BERRY, S.K., M.A. AVGCSTli'I and L.K. HE~G.1982. itrate and nitrite content of Malaysian vegetables. Symposium on Vegetables and Ornamentals in the Tropics, Universiti Pertanian*
Malaysia.

96. *CVLLEY, D.D. and R.W. MYERS. 1980. Effect of hanrest rate on duckweed yield and nutrient extraction in dairy waste lagoons. In SA Department of Energy Final Report, School of Forestry and Wildlife Management, ed. D.D. Culley and J.B. Fyre, p 1-6, Louisiana State University Press.*

97. *CULLEY, D.D., J.H. GHOLSON, T.S. CHILSHOD1, L.C. STANDIFER and E.A. Err. 1978. Water quality renovation of animal waste lagoons utilizing aquatic plants, EPA Publication*
o. 600/2-78-153 U.S. Environmental Protection Agency,

98. *CVLLEY, D.D., E. REJMA~HORA, J. KVET and J.B. FRYE. 1981. Production, chemical quality*
and use of duckweeds (Lemnaceae) in aquaculture, waste management and animal feeds, j. World Maricull, Soc., 12(2): 27-49.

99. *HARVEY, R.M. and Fox.J.L. 1973. utrient removal using Lemna minor. J. Water Pollut. Control Fed. 45:1928-1938.*

100. *HILLMAN, W.S. and D.D. CULLEY. 1978. The uses of duckweed. American Scientist 66(4):442-451.*

101. *LAwso:'\, T.B., Hl, BRAVO and F.T. WRATTEN 1984. Method for drying duckweed Lemnaceae.Winter Meeting American Society of Agriculture Engineers, 1984.*

102. *KAKAoE, M.L., Jl RACKlS, J.E. MCGHEE and G. PUSKI 1974. Determination of trypsininhibitor activity ofsoy products: A collaborative analysis of an improved procedure.Cereal Chern. 51: 376-381.*

103. Baeyens, J., Kang, Q., Appels, L., Dewil, R., Lv, Y., & Tan, T. (2015). Challenges and opportunities in improving the production of bio-ethanol. *Progress in Energy and Combustion Science*, *47*, 60-88.

104. Chen, Q., Jin, Y., Zhang, G., Fang, Y., Xiao, Y., & Zhao, H. (2012). Improving production of bioethanol from duckweed (Landoltia punctata) by pectinase pretreatment. *Energies*, *5*(8), 3019-3032.

105. Zhao, X., Moates, G. K., Elliston, A., Wilson, D. R., Coleman, M. J., & Waldron, K. W. (2015). Simultaneous saccharification and fermentation of steam exploded duckweed: improvement of the ethanol yield by increasing yeast titre. *Bioresource Technology*, *194*, 263-269.

106. Fujita, T., Nakao, E., Takeuchi, M., Tanimura, A., Ando, A., Kishino, S., ... & Shimizu, S. (2016). Characterization of starch-accumulating duckweeds, Wolffia globosa, as renewable carbon source for bioethanol production. *Biocatalysis and agricultural biotechnology*, *6*, 123-127.

107. Bayrakci, A. G., & Koçar, G. (2014). Second-generation bioethanol production from water hyacinth and duckweed in Izmir: a case study. *Renewable and Sustainable Energy Reviews*, *30*, 306-316.

108. Chen, G., Zhao, K., Li, W., Yan, B., Yu, Y., Li, J., ... & Fang, Y. (2022). A review on bioenergy production from duckweed. *Biomass and Bioenergy*, *161*, 106468.

109. Pagliuso, D., Grandis, A., Lam, E., & Buckeridge, M. S. (2021). High saccharification, low lignin, and high sustainability potential make duckweeds adequate as bioenergy feedstocks. *BioEnergy Research*, *14*(4), 1082-1092.

110. Srichuwong, S., Arakane, M., Fujiwara, M., Zhang, Z., Takahashi, H., & Tokuyasu, K. (2010). Alkali-aided enzymatic viscosity reduction of sugar beet mash for novel bioethanol production process. *biomass and bioenergy*, *34*(9), 1336-1341.

111. Adhikary, T., Hossain, C. M., Mallick, S., Paul, S., Banerjee, N., & Basak, P. (2021). Revisiting Therapeutic Potentials of Ethanolic Extract of Curcuma longa L. rhizomes to Evaluate wound Healing Progression upon Topical Application of its Ointment. *INDIAN JOURNAL OF PHARMACEUTICAL EDUCATION AND RESEARCH*, *55*(1), 174-183.

112. Mohanarao, G. J., Mukherjee, A., Banerjee, D., Gohain, M., Dass, G., Brahma, B., ... & De, S. (2014). HSP70 family genes and HSP27 expression in response to heat and cold stress in vitro in peripheral blood mononuclear cells of goat (Capra hircus). *Small Ruminant Research*, *116*(2-3), 94-99.

113. Chaudhari, M., & Mengi, S. (2006). Evaluation of phytoconstituents of Terminalia arjuna for wound healing activity in rats. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *20*(9), 799-805.

Report NC

ORIGINALITY REPORT

9%
SIMILARITY INDEX

PRIMARY SOURCES

1	admetmesh.scbdd.com Internet	152 words — 2%
2	Dr. Piyali Basak, Tathagata Adhikary, Pratik Das, Shreya Biswas. "Phytochemical analysis and comparative study of antibacterial effect of turmeric extracts using different solvent.", IOP Conference Series: Materials Science and Engineering, 2018 Crossref	83 words — 1%
3	GahYoung Baek, Maham Saeed, Hyung-Kyoon Choi. "Duckweeds: their utilization, metabolites and cultivation", Applied Biological Chemistry, 2021 Crossref	62 words — 1%
4	" Alzheimer's disease facts and figures ", Alzheimer's & Dementia, 2021 Crossref	53 words — 1%
5	alz-journals.onlinelibrary.wiley.com Internet	52 words — 1%
6	Piyali Basak, Samrat Paul, Ranabir Majumder. "Invitro cytotoxic study of aloe vera whole leaf extract on PBMC and breast cancer cell line", 2017 2nd International Conference for Convergence in Technology (I2CT), 2017 Crossref	35 words — < 1%

7	www.tandfonline.com Internet	35 words — < 1%
8	Srinivasa Rao Kareti, Subash P. "In Silico Molecular Docking Analysis of Potential Anti-Alzheimer's Compounds Present in Chloroform Extract of Carissa carandas Leaf Using Gas Chromatography MS/MS", Current Therapeutic Research, 2020 Crossref	21 words — < 1%
9	Www.dovepress.com Internet	17 words — < 1%
10	s3.amazonaws.com Internet	17 words — < 1%
11	Pratik Das, Ranabir Majumder, Mahitosh Mandal, Piyali Basak. " In-Silico approach for identification of effective and stable inhibitors for COVID-19 main protease (M) from flavonoid based phytochemical constituents of ", Journal of Biomolecular Structure and Dynamics, 2020 Crossref	16 words — < 1%
12	www.biospace.com Internet	16 words — < 1%
13	jlmissouri.com Internet	15 words — < 1%
14	bmccomplementmedtherapies.biomedcentral.com Internet	14 words — < 1%
15	ses.library.usyd.edu.au Internet	14 words — < 1%

16	A.M. Vijesh, Arun M. Isloor, Sandeep Telkar, T. Arulmoli, Hoong-Kun Fun. "Molecular docking studies of some new imidazole derivatives for antimicrobial properties", Arabian Journal of Chemistry, 2013 Crossref	13 words — < 1%
17	notulaebotanicae.ro Internet	13 words — < 1%
18	pubs.rsc.org Internet	13 words — < 1%
19	ijrar.com Internet	12 words — < 1%
20	www.frontiersin.org Internet	12 words — < 1%
21	"2022 Alzheimer's disease facts and figures", Alzheimer's & Dementia, 2022 Crossref	11 words — < 1%
22	De, Subrata. "Pharmacognostic and phytochemical studies on antiinflammatory and antihepatotoxic activity of Ayurvedic drugs.", Proquest, 2015. ProQuest	11 words — < 1%
23	en.wikipedia.org Internet	11 words — < 1%
24	www.tjpr.org Internet	11 words — < 1%
25	G. Abellan van Kan. "Prevention of Alzheimer's disease: implication of nutritional factors", Food for the ageing population, 2009 Crossref	10 words — < 1%

26	doaj.org Internet	10 words — < 1%
27	ir.cftri.com Internet	10 words — < 1%
28	mail.scialert.net Internet	10 words — < 1%
29	web.cgu.edu Internet	10 words — < 1%
30	www.fao.org Internet	10 words — < 1%
31	www.sabiia.cnptia.embrapa.br Internet	10 words — < 1%

EXCLUDE QUOTES	OFF	EXCLUDE SOURCES	OFF
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 10 WORDS