M. Tech (Industrial biotechnology)

Shruti

2023

"Exploring plant-based phytocompounds for the treatment of Parkinson's disease"

A Dissertation

Submitted in the Partial Fulfillment of the requirements

For the Award of the Degree of

MASTER OF TECHNOLOGY

IN

INDUSTRIAL BIOTECHNOLOGY

Submitted By

SHRUTI

2K21/IBT/08

Under the Supervision of

DR. NAVNEETA BHARADVAJA

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi-110042

May, 2023

List of contents

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi-110042

CERTIFICATE

I hereby certify that the Project dissertation titled **"Exploring plant-based phytocompounds for the treatment of Parkinson's disease"** submitted by **SHRUTI, 2K21/IBT/08**, Department of biotechnology, Delhi technological university, Delhi in partial fulfillment of the requirements for the award of the degree of Master of Technology, is an authentic record of the work carried out by the student under my guidance. To the best of my knowledge, this work is original and has not been submitted in part or full for any Degree or Diploma to the University or elsewhere.

30/05/2023

Head of department

Department of Biotechnology

Delhi technological university

DR. NAVNEETA BHARADVAJA

Supervisor

Assistant Professor

Department of Biotechnology

Place: **Delhi**

Date: **May 29, 2023**

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Bawana Road, Delhi – 110042

CANDIDATE'S DECLARATION

I, **SHRUTI, 2K21/IBT/08** here by certify that the work which I presented in the major project entitled "**Exploring plant-based phytocompounds for the treatment of Parkinson's disease**" is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirements for the award of the degree of Master of Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any degree, Diploma Associateship, Fellowship or other similar title or recognition.

Shruti

Shruti

2K21/IBT/08

Place: Delhi

Date: May 29, 2023

iv

Acknowledgement

First and foremost, I would like to praise and thank God, the almighty, who has granted countless blessing, knowledge, and opportunity to the writer, so that I have been finally able to accomplish the thesis.

A formal statement of acknowledgement will hardly meet the needs of injustice in the matter of expression of deeply felt sincere and allegiant gratitude to all who encouraged and helped me in many ways throughout the dissertation of masters of technology. It is my privilege to express my profound sense of gratitude and indebtedness to my supervisor **Dr. Navneeta Bharadvaja**, Assistant Professor in the Department of Biotechnology, Delhi technological university for her valuable guidance and consistent encouragement during the progress of the project work. The dissertation wouldn't be completed without her insightful suggestions and support. A special thanks to my seniors (PhD scholars) **Ms. Harshita Singh** & **Mr. Sidharth Sharma** & **Ms. Anuradha**, for their constant support and help during the course of my research work. I am equally grateful and wish to express my wholehearted thanks to respected **Head, Prof. Pravir Kumar** and technical staff including **Mr CB Singh**, **Mr Jitendra** for their kind support. I would also like to acknowledge the Department of biotechnology for their participation and engagement during the study .I would also thank my lab mates for their constant encouragement during the academic year and completion of the thesis. I would like to extend my gratitude to my parents for their timely motivation, blessings in this journey, who dreamt of me and blessed me to cherish them. Finally I wish to thank everyone who directly or indirectly helped me in this endeavor. Thank you to all for your prayers and good wishes. It gave me the strength to persevere and warmed my heart.

Shruti

v

INDEX

ABSTRACT

The food supplement industry is currently conducting research on the use of plant secondary metabolites as a new generation of dietary supplements commonly referred to as "superfoods." Plant secondary metabolites offer a diverse range of nutrients and numerous health benefits, such as their ability to combat microbiological illnesses, hypertension, obesity, and diabetes. This has resulted in a rapidly growing market for plant-derived nutraceuticals. Additionally, the concept of plant prebiotics and their role in regulating gut bacteria has further contributed to this expansion. This project aims to assess the presence and estimate the quantities of various secondary metabolites in plants. The importance of phytocompounds is to increase the overall immune system and improving the nutrition content in the body. Cosmeceutical applications are also showing increased interest in alkaloids, polysaccharides, and other phytocompounds that protect the skin from photodamage. Furthermore, the inclusion of plant-derived secondary metabolites in one's diet aids in the prevention and management of chronic illnesses such as cancer, neurological disorders, and lung and heart disease. For example, Parkinson's disease is condition characterized by memory loss, dopamine deficiency, oxidative stress, and other abnormalities. The DJ-1 protein, acting as a chaperone, is critical in the treatment of this disease. Previous studies have demonstrated that plants can protect neurons and improve motor function in neurodegenerative diseases. The present study deals with different selected phytochemicals of the plant since they have vast medicinal and therapeutic activities via in vitro and in silico processes.

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1- INTRODUCTION

1.1 Background

1.1.1 Neurodegenerative diseases-

Advancements in sequencing technologies have enabled the identification of metabolomic patterns in brain tissues from post-mortem samples, in vitro and in vivo cells, and animal models, providing insight into the underlying neurodegeneration pathways. These investigations have revealed commonalities among different neurodegenerative disorders, including synaptic degradation, and have identified potential therapeutic targets. Despite extensive genetic profiling in patient populations, many cases of neurodegenerative diseases remain of unknown genetic origin. Neurodegenerative diseases have been linked to a variety of factors, including alcohol consumption, social factors, drug abuse, nutritional deficiencies, chemicals, and toxins, all of which contribute to a range of behavioral and pathological impairments. Unfortunately, current drug and therapeutic treatments have been largely ineffective, underscoring the need for new therapies that can slow the progression of these disorders and alleviate symptoms. Neurodegenerative disorders are identified by the loss of neuronal function, and the resulting clinical symptoms can differ significantly depending on the functional systems involved. Misfolded proteins are abnormal proteins that have been deposited due to physicochemical alterations. These proteins often exhibit harmful functionality due to their modified structure, and their accumulation is a common feature in most neurodegenerative disorders. However, while mutations in protein-producing genes are typically the primary cause of protein deposition, this phenomenon has not been observed in spastic paraplegia and spinocerebellar ataxia. Despite the significant progress made in the field of neurodegenerative disease research in recent years, these diseases remain highly debilitating and fatal, and indicating that there is still considerable room for improvement in the development of more effective treatments. Recent developments have shown that epigenetic changes are highly suitable as targets for medicinal intervention. Aberrant epigenetic modifications associated with neurodegenerative disorders are emerging, and there is an increasing demand for the assessment of current progress in the study of these diseases.

1.1.2 Parkinson’s disease-

Parkinson's disease is a highly prevalent neurodegenerative condition and the most frequently encountered movement disorder. It affects around 5% of individuals aged 80 and above and 1% of those aged 60 and above. The hallmark of this disorder is the progressive depletion of dopaminergic neurons in the subcortical basal ganglia, specifically in the SNpc. The depletion of these neurons leads to the pathognomonic triad of resting tremor and rigidity, as this region of the midbrain is critical in enhancing motor pathways and preparing for movement. Lewy bodies (LBs), which are proteinaceous inclusions found inside neurons, are the main pathological characteristic of Parkinson's disease, along with the degeneration of the nigra. The SNCA gene on chromosome 4q21 encodes alpha-synuclein, a protein consisting of 140 amino acids. Alpha-synuclein is abundant in presynaptic terminals and is the main constituent of the inclusions associated with Parkinson's disease. Rare point mutations in the SNCA gene are responsible for the dominant familial forms of Parkinson's disease. LBs found in both sporadic and hereditary forms of the disease contain aggregated forms of alpha-synuclein that accumulate in microtubules.

There is growing evidence suggesting that Parkinson's disease may not be a single disorder. One reason is that Parkinsonism, the clinical syndrome associated with Parkinson's disease, can have various underlying causes that produce similar symptoms. While there are fewer than ten known genes that can definitively cause parkinsonism when mutated, these genetic factors only account for a fraction of cases. Furthermore, Parkinson's disease often exhibits a wide range of symptoms and patterns of progression, even among individuals with a known cause. For example, individuals exposed to the neurotoxin may develop parkinsonian symptoms, but the presentation can vary significantly among affected individuals. In summary, the existence of different underlying causes of Parkinsonism and the variability in symptom presentation and progression suggest that Parkinson's disease may not be a single homogeneous disease entity. Another aspect supporting the notion that Parkinson's disease may not be a singular condition is the fact that each individual with the disease has unique priorities, needs, and desires. For instance, a laborer accustomed to lifting heavy objects might not consider a noticeable resting tremor to be significant, whereas a calligrapher could find a tremor of the same intensity to be debilitating. Consequently, the experience of Parkinson's disease differs among individuals,

highlighting the distinct nature of each person's illness. Taking into consideration all these factors, an extreme assertion could suggest that there are over 6 million distinct forms of Parkinson's disease worldwide.

1.1.3 Phytochemicals-

Phytochemicals are naturally occurring compounds found in plants that have various health benefits. Among these compounds, polyphenols, which include flavonoids and phenolic acids, are known to have potent antioxidant properties. Free radicals can lead to cellular harm through oxidative stress and result into the emergence of various diseases like several different cancers. To combat these harmful free radicals, the body requires antioxidants. Flavonoids, the largest class of polyphenols, possess a range of biological properties that have been shown to improve cardiovascular health, reduce the risk of chronic diseases, and exhibit anti-inflammatory, anti-cancer, and antioxidant effects. Phenolic acids, another class of polyphenols, have also demonstrated anti-inflammatory, antioxidant, and potential anti-cancer properties. A diverse category of plant-based foods material can offer a wealth of phytochemicals such as polyphenols that aid in maintaining good health and preventing illnesses. Therefore, incorporating these foods into your diet can provide a significant source of essential nutrients that promote well-being. Antioxidants must be used in balance with oxidation because it is a natural process that sustains health in the body. While the body has defenses against oxidative stress, research suggests that these defences become less effective as we age, leading to an increase in oxidative stress. The resulting free radicals may contribute to various aging-related degenerative disorders, including cancer, cardiovascular disease, cognitive decline, Alzheimer's disease, immunological failure, cataracts, and macular degeneration.

Many medicinal compounds are derived from plants for treating various health problems indicating the clear significance of plants as a source of medicine. The human species has been dependant on conventional medicinal plants from prehistoric times. The majority of plants produce a vast number of organic molecules that don't directly contribute to their own growth and development but are essential for interactions with other plants, environment, or for their defense. These substances are known to be secondary metabolites. Secondary metabolites find various applications such as in agrochemicals, medicines, flavorings, odors, preservatives, and pesticides. SMs can be categorized based on their chemical structure, solvents and different pathways e.g.

terpenes, phenolic compounds and nitrogen-containing compounds. Terpenes are classified based on their isoprene units and biosynthesis take place via mevalonic pathway (MVA) and methylerythritol phosphate pathway (MEP). The ubiquity and diversity of phenols characterize this class of secondary metabolites in plants. They have one or more phenolic groups as their basic chemical constituents, which are aromatic rings with six carbons (6-C), such as the benzene ring, and one or more hydroxyl functional groups. As a result, they are collectively referred to as polyphenols or phenolic compounds such as flavonols, quinones, tannins, anthocyanins etc. They are synthesized via shikimate/chorismate (succinylbenzoate) pathway, acetate/mevalonate pathway and acetate-malonate or polyketide pathway. Several secondary metabolites originated from plants has the presence of nitrogen within their chemical composition, these are; alkaloids, several glycosides, purines, amino acids, glucosinolates etc. This class is called nitrogen-containing compounds. These classes are synthesized via various pathways, including the shikimate, mevalonate, and non-mevalonate pathways. Medicinal plants, which have been utilized for generations to treat a variety of ailments, are a source of traditional remedies. The increasing demand for plant-based medications has led to the overexploitation of medicinal plant species, causing a significant number of them to become endangered or even go extinct. The pharmaceutical sector, which extensively relies on natural resources for drug discovery and development, is the main cause of over exploitation. In addition, other sectors like the food, beverage, and cosmetics industries also contribute to the overuse of medicinal plants. The demand for plant-derived products in food, cosmetics, and other fields is constantly increasing, but organ culture and suspension culture are insufficient to meet this demand for secondary metabolites. However, it has been observed that certain types of cultures, such as hairy and shoot cultures, can generate considerable quantities of alkaloids and monoterpenes, and also enhance the production of secondary metabolites. Plant cell species, tissue cultures, and organ cultures have become popular alternatives to field cultivation for the production of secondary metabolites due to drawbacks such as lower yield and concentration, as well as unfavorable environmental conditions.

1.2 Justification of study

This study's findings will add to the expanding body of knowledge on medicinal plants and support the promotion of safe and effective use of traditional herbal remedies. The aim of this study is to find possible isolated compounds as a bio-source for future to diagnose Parkinson’s disease via in vitro and in silico processes.

1.3 Objectives of the study

With the growing interest in herbal compounds, it is important to identify and measure the important chemical components in herbal plants to evaluate their potential health advantages.

- To perform qualitative & quantitative test for different constituents in medicinal plants.
- The exploration of phytoconstituents derived from plants through in-silico analysis.
- Comparison between medicinal plants to find the best potent inhibitor against Parkinson’s disease.

CHAPTER 2- LITERATURE REVIEW

2.1 Phytochemicals-

Phytochemicals are chemical compounds derived from plants that may not always be considered nutrients but have the potential to produce health benefits. These organic chemical substances are secondary metabolites found in plants that serve various purposes, including lignification, fruit and leaf colouring, pollination, allelopathy, pathogen and predator resistance, and growth. Phytochemicals, such as lycopene found in tomatoes, isoflavones from soy, and flavonoids from fruits, are well-known for their potential to improve health and prevent diseases, despite not being essential nutrients. Many of the 5000 plant phenolics identified have demonstrated antioxidant effects in model studies. These phytonutrients constitute a significant class that has been extensively studied for their potential to prevent disease. The remarkable property of polyphenols is their ability to inhibit several enzymes that cause inflammation. Polyphenols have the potential to affect various biological processes such as inhibiting platelet aggregation by controlling prostaglandin pathways, inhibiting certain gene expression, deactivating cancer genes, and activating enzymatic systems that detoxify xenobiotics. Although certain polyphenols have demonstrated mutagenic properties in microbiological tests and can serve as co-carcinogens or promoters of skin carcinogenesis when combined with other carcinogens, this fact is not widely recognized. Further research into this possibility is required. In vitro model systems, polyphenols have been observed to bind with non-heme iron (such as that from plant sources), potentially limiting its absorption.

Research has shown that consuming a diet rich in Phytochemicals have the potential to lower the likelihood of developing chronic diseases. For instance, carotenoids included in colorful fruits and vegetables have been demonstrated to lessen the risk of specific types of cancer, whereas flavonoids present in green veggies have been related to a lower risk of heart disease. It has been demonstrated that other phytochemicals, such curcumin, which is found in turmeric, and resveratrol, which is found in grapes and red wine, have anti-inflammatory and anti-cancer characteristics. Alkaloids like caffeine, which are included in coffee and tea, have also been demonstrated to enhance cognitive function and have other positive health effects. Since they may have positive effects on health, phytochemicals have attracted more

and more attention in recent years. Natural compounds are derived from this medicinal plants associated with certain therapeutic activities like anticancer effects. The word "phytochemicals" means large range of substances, such as carotenoids, flavonoids, phenolic acids, alkaloids, and terpenoids. Beta-carotene and lycopene are two examples of carotenoids that are well-known for their antioxidant properties and their contribution to preserving eye health. It has been established that the flavonoids, which are present in many fruits and herbs, have anti-oxidant and anticancer activities. Fruits, vegetables, and cereals are rich in phenolic acids, e.g. caffeic acid and ferulic acid, which have been discovered to have antioxidant properties. Alkaloids are well known for their stimulating effects on the body, including coffee and nicotine. However, some alkaloids have been proven to have anticancer effects and are used in chemotherapy, such as vinblastine and taxol. It has been demonstrated that the terpenoids, which are present in essential oils, have antibacterial, anti-inflammatory, and anticancer activities. The potential of phytochemicals to interact with many cellular pathways, such as those involved in oxidative stress, inflammation, and carcinogenesis, is assumed to be the reason for their beneficial effects on health. They have also been discovered to alter gene expression, which might add to their advantageous effects. Including a range of plant-based foods in people's diet e.g. herbs that are rich in phytochemicals, may reduce the risk of developing chronic illnesses like cancer. However, further investigation is necessary to understand the ways in which phytochemicals function to promote health and to determine the optimal amounts and types of phytochemicals required for disease prevention and treatment.

2.2 Different phytochemicals and their mode of action

Phytochemicals come in a wide variety of forms, such as carotenoids, flavonoids, phenolic acids and stilbenes. Several plant-based foods, such as fruits and vegetables contain these chemicals. Every kind of phytochemical reduces certain health problems such as or good digestion, increase immunity and has certain biological activites like anti inflammation. The phytochemical resveratrol, which is present in red wine and grapes, is one of the most well-known phytochemicals. According to studies, resveratrol may have anti-aging properties and may also lower the chance of developing certain malignancies and cardiac conditions. Lycopene, a different

phytochemical present in tomatoes, has also been associated with a lower risk of prostate cancer.

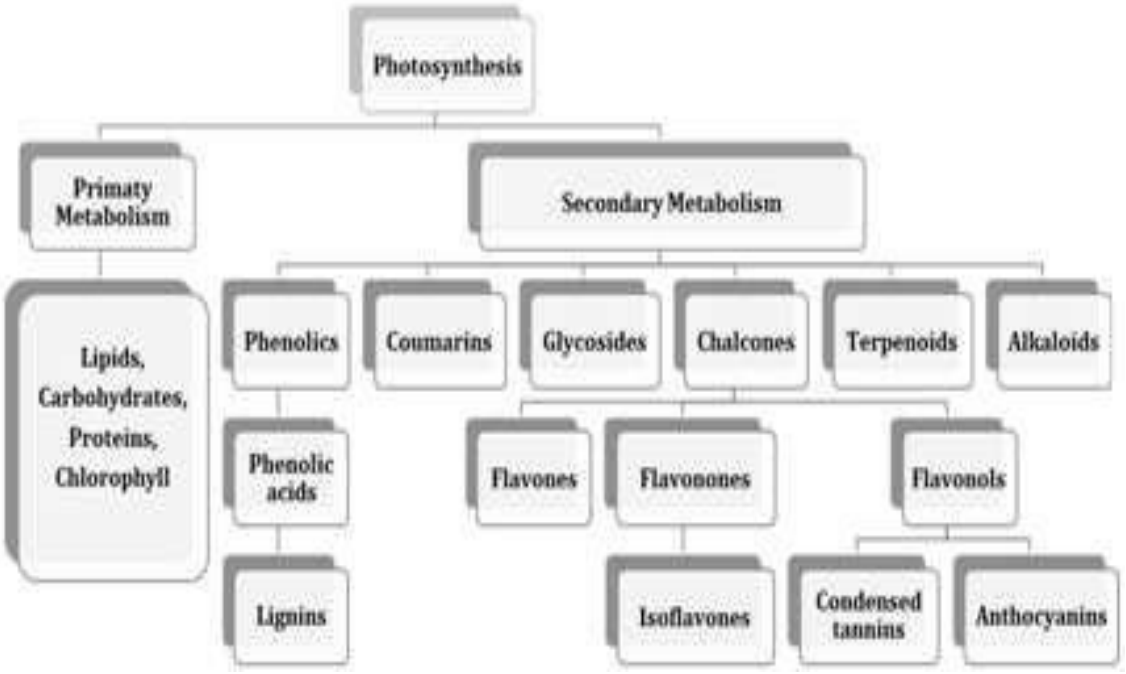


Fig. 2.1 Classification of secondary metabolites

2.2.1 Alkaloids-

Alkaloids are a significant category of natural plant products and encompass a wide variety of chemical compounds. The fundamental feature of the alkaloid group is the presence of a nitrogen atom within the molecule, which may be located in any position. However, the nitrogen atom in question is not involved in any amide or peptide bond. W. Meisner discovered the term "alkaloid" from the opium poppy, Papaver somniferum, and subsequently applied it to various medications that contained alkaloid compounds (Kaur and Arora, 2015). Apart from carbon, nitrogen, and hydrogen, many alkaloids also include oxygen in their molecular structure. Alkaloids, a group of natural compounds in plants, have diverse chemical structures and are known for their many beneficial properties. For example, benzylisoquinoline and indoquinoline, two alkaloids derived from plants, have been found to have antimicrobial activity against certain gram-negative bacteria and can even inhibit viral replication in humans. Quinine,

another popular alkaloid, is known for its efficacy in fighting malaria. Alkaloids also play a crucial role in plant defense against disease. Currently, there are approximately 11,000 alkaloids used in the pharmaceutical industry.

2.2.2 Phenols-

Medicinal and aromatic plants are known to produce phenolic compounds that have diverse health benefits. These compounds are mainly generated through two pathways: acetic acid and shikimic acid pathways. When quantitatively measured, dark vegetables such as black beans, kidney beans, and black gramme are found to have a higher total phenolic content. Phenolic acid is composed of a benzene ring, one or more hydroxyl groups, and a carboxylic group (Pinto et al., 2021). Common phenols such as hydroxybenzenes are not present in higher plants. Quinol and catechol are other common phenols, with quinol being more prevalent in the plant kingdom than catechol (Pinto et al., 2021).

Polyphenols can be classified based on their phenolic and the structural components attached through the ring. Five main categories of polyphenols, includes phenolic acids, flavonoids, stilbenes, tannins, and lignans. Flavonoids are less abundant than polyphenols, with approximately half as many flavonoids as there are polyphenols.

2.3 Medicinal plants-

2.3.1 Botanical name: *Moringa oleifera*

Kingdom: Plantae

Common plant name- Drumstick tree

Family: Moringaceae

Group: - Angiosperms

Order: Capparales

Genus: Moringa

- **Description**- This is a description of a deciduous tree that is indigenous to the Indian subcontinent but is cultivated worldwide. The tree grows quickly and doesn't surpass a height of approx. 8-9 meters. Alternate leaf structure, generally

thrice pinnate, 30-55 cm long, and consist of 4-8 thin, ovate-to-elliptic-shaped leaflets measuring 1-2 cm. The flowers emit an aromatic fragrance and have five uneven, thinly veined, petals have yellowish-white. The pod is pendulous, measuring 15 to 30 cm long, and is three-angled with nine ribs. It contains seeds that are also three-angled.

Fig. 2.2 Images showing *Moringa oliefera*

- **Action and uses-** The tree is consumed as a nutritious veggie in various nations extensively. Additionally, the plant is used to treat phlegmatic conditions such as asthma, arthritis, backaches, paralysis etc. Every part of the plant, including its flowers, leaves, pods, and gum, is utilized. The leaves have analgesic and anti-inflammatory characteristics when used externally. This plant is readily available in all of Nigeria's ecological regions and is employed to manage various ailments such as body aches, fever, weakness, wounds, haemorrhaging, anaemia,. Moreover, it is utilized to manage terminal diseases like cancer and HIV/AIDS. In Ghana, it is used to treat heart problems, inflammations, dyspepsia, and eye conditions and also aids lactation in mothers of premature infants. The leaves of this plant are utilized in traditional medicine in Cameroon to treat a variety of conditions such as diarrhoea, cancer, HIV/AIDS etc. In Benin, the leaves are employed as nutritional supplements for HIV patients.

Indian medicinal plant	Plant part	Therapeutic use	Therapeutic use identifiers	References
Moringa oleifera	aerial part	Antioxidants	MESH:D000975, UMLS:C0003402	ISBN:9770972795006
Moringa oleifera	aerial part	Antiviral agents	MESH:D000998, UMLS:C0003451	ISBN:9770972795006
Moringa oleifera	aerial part	Diabetes mellitus	MESH:D003920, UMLS:C0011849, DOID:9351, ICD-11:5A14	ISBN:9770972795006
Moringa oleifera	bark	Abdominal pain	MESH:D015746, UMLS:C0000737, SYMP:0000457, ICD-11:MD81.4	ISBN:9770972795006
Moringa oleifera	bark	Abortifacient agents	MESH:D000019, UMLS:C0000782, ICD-11:JA00.1	ISBN:9770972795006, ISBN:9788172361266, ISBN:9788172363130, ISBN:9788173717055
Moringa oleifera	bark	Abscess	MESH:D000038, UMLS:C0000833, SYMP:0000672, ICD-11:1B75.3	ISBN:9770972795006
Moringa oleifera	bark	Amenorrhea	MESH:D000568, UMLS:C0002453, DOID:13938, ICD-11:GA20.0	ISBN:9770972795006

Fig.2.3 Therapeutic uses of *Moringa* extracted from database

- **Pharmacology-** The aqueous extract of the leaves was observed to significantly decrease FBG and oral glucose challenge, while improving antioxidant enzyme activities and GSH levels in both normal and diabetic rats. On the other hand, the methanol extract of the leaves greatly enhanced insulin levels and prevented weight loss in diabetic rats. In diabetic rats, the methanol extract of the pods not only substantially lowered serum glucose levels but also increased antioxidant levels in pancreatic tissue. In addition, rats with acute and persistent induced insulin were protected by the ethanol extract of the bark. Additionally, a hot water infusion of seeds exhibited diuretic effects on rats and significantly decreased the constriction of the isolated duodenum induced by ACh and paw edema caused by carrageenan. In rats, a four-week treatment with an aqueous extract improved the animals' energy reserves and tissue antioxidant capacity while also preventing arsenic-induced oxidative stress. When administered to stressed animals, the hydroethanolic leaf extract increased testosterone levels, inhibited PDE-5 activity, reduced blood corticosterone levels, and protected the rats against toxicity of testes induced by chromium. It also decreased LPO and boosted the activity of antioxidant enzymes. Similarly, the oil protected the testicles against the harmful effects of mercury chloride.

- **Mechanism of action**- The mechanism of action of M. oleifera extract involves inhibiting HMG-CoA reductase, a process that is comparable to pravastatin and may lead to a decrease in cholesterol production. Its observed pharmacological benefits are largely attributed to its antioxidant properties.

  Studies have shown that all components of this commonly found tropical tree have positive pharmacological effects, and consuming it regularly as a vegetable may be beneficial for maintaining good health. However, in order to officially establish its effectiveness in treating various illnesses, significant controlled clinical research would be required before it can be regarded as a medicinal plant.

2.3.2 **Botanical Name: -** *Nyctanthes arbor-tritis*

Kingdom: - Plantae

Common name: - Har Singar

Family: - Oleaceae

Group: - Angiosperms

Order: - Lamiales

Genus: - Nyctanthes

- **Description-** *Nyctanthes arbor-tritis* is commonly named "tree of sorrow" cause its petals lose their shine and glow during the day time. It means "sad tree" in Greek. The flowers are also known as Parijat in the nearby Indian state of West Bengal, and are the official flower of the province. Other common names for *Nyctanthes arbor-tristis* include coral jasmine and night-blooming jasmine.

  The plant can grow up to 9 meters tall and takes the form of a large shrub or small tree. The trunk's bark is gray in color and has a flaky, tough texture. The leaves are simple, opposite, green, and have hairy edges, measuring 5-14 cm in length and 2 to 6.5 cm in breath. The flowers are white, fragrant, and have 5 to 8 lobed corollas with an orange to crimson center, usually appearing in groups of two to seven. They bloom heavily at night, losing their scent and

color at daybreak and disappearing in the morning. The fruit is a brown, flat, heart-shaped structure with two sections, each containing one seed. It thrives in the dry deciduous woodlands and on rocky terrain in dry slopes, usually as an undergrowth plant.

Fig.2.4 Images of *Nyctanthes arbor-tritis*

- **Action and uses**- Saxena et al. (1984) found that the plant's leaf extract has anti-inflammatory properties, while Puri et al. (1994) demonstrated that its flowers, leaves, and seeds contain compounds that boost the immune system. It was also discovered that it possesses antiviral, antifungal, and anti-protozoan effects, as well as the ability to protect liver cells. The flower and leaf extracts also contain substances that are larvicidal to mosquitoes. Leaves has Antibacterial, Antifungal, Anti-inflammatory, Antipyretic, Antioxidant properties- Sciatica, Rheumatismand various painful conditions, Ringworm.

Indian medicinal plant	Plant part	Therapeutic use	Therapeutic use identifiers	References
Nyctanthes arbor-tristis	aerial part	Malaria	MESH:D008288, UMLS:C0024530, DOID:12365, ICD-11:1F4Z	ISBN:9788172362461
Nyctanthes arbor-tristis	bark	Analgesics	MESH:D000700, UMLS:C0002771, ICD-11:XM49F7	ISBN:9770972795006
Nyctanthes arbor-tristis	bark	Anti-inflammatory agents	MESH:D000893, UMLS:C0003209, ICD-11:XM7XD1	ISBN:9770972795006
Nyctanthes arbor-tristis	bark	Antipyretics	MESH:D058633, UMLS:C0003419, ICD-11:XM1RS7	ISBN:9770972795006
Nyctanthes arbor-tristis	bark	Antirheumatic agents	MESH:D018501, UMLS:C0003191, ICD-11:XM95N2	ISBN:9770972795006

\mppat/

Fig. 2.5 Therapeutic uses of *Nyctanthes* extracted from database

- **Mechasim of action-** S. Bansal and colleagues successfully regenerated *Nyctanthes arbor-tristis*, a medicinal plant, through in vitro methods using axillary bud explants on MS media. The most abundant shoots were observed when benzylaminopurine was added to the MS media. Half-strength MS supplemented with napthaleneacetic acid (NAA) resulted in the increase of its roots and saplings got petrified. Essential oil was extracted from the leaves and barks of *Nyctanthes arbor-tristis*, and then analyzed using GC-MS. The leaf oil had 26 compounds, while the bark oil had 20 compounds. Hexadecanoic acid and octadecanoic acid were present in similar amounts in both the leaf and bark oils. However, linalool, (E)-phytol, and (3Z) hexenyl benzoate were present in the leaf oil but not in the bark oil. The bark oil had a distinct composition, with -eudesmol and other eudesmol isomers being the primary components. When the oil was tested for antibacterial activity, there was little response from Bacillus cereus and *Aspergillus niger.*

  R.S. Bhadouria and colleagues isolated and characterised the phytoconstituents of *Nyctanthes arbor-tristis* leaves using an ethanolic extract. Two alkaloid compounds were fractionated and extracted from the extract using column chromatography, and their structures were determined based on spectral and chemical analyses.

2.4 Parkinson’ disease-

Paralysis agitans, originally identified by James Parkinson in 1817, is a neurological disorder that primarily affects the elderly and is now commonly referred to as Parkinson's disease. Later, Jean-Martin identified the differences between Parkinson's disease and other tremor-related illnesses and named it accordingly. The prevalence of Parkinson's disease varies with age and is estimated to be between 1% and 2% in the population of that particular age group. The accuracy of epidemiological statistics for Parkinson's disease, however, is dependent on the population studied, the methodology, and the diagnostic standards applied by various researches.

2.4.1 Symptoms-

Understanding the distinctive characteristics of Parkinson's disease (PD), which can be difficult due to the subtlety of its symptoms and indications, is the first step in making a diagnosis. This is where the acronym TRAP, which stands for the four main features that set Parkinson's disease apart, can be useful. Tremor, stiffness, akinesia, and postural instability make up this list. 70% to 100% of people with Parkinson's disease who are 16 years of age or older experience tremors, some of whom have postural tremors or gradual vertical jaw or tongue movements. More than 90% of patients have tight muscles.

Parkinson's disease (PD) is characterised by a decline in voluntary muscle movement, or akinesia. Postural instability might not be noticed in the early stages of the sickness, but as it worsens, it might become more obvious. The two primary types of PD symptoms are:

- Motor and
- Non-motor signs.

Physical symptoms include rigidity, rest tremor, bradykinesia, postural and gait irregularities, and other movement-related problems are referred to as motor symptoms. Parkinsonism is the term used to characterise the PD-related movement symptoms. Other motor symptoms of Parkinson's disease (PD)

may include decreased arm swing, trouble sitting or sleeping, dysphagia, dystonia, and a decreased capacity to carry out daily tasks with ease.

Since they are simpler to recognise than non-motor symptoms, which necessitate a doctor's skill for diagnosis, motor signs are given more consideration while examining PD symptoms. However, there has been an increasing focus on researching the non-motor symptoms of Parkinson's disease in recent years. These non-motor symptoms, which include hallucinations, illusions, anxiety, insomnia, constipation, restlessness, excessive sweating, and urinary and sexual dysfunction, are of great interest to researchers.

2.4.2 Causes-

Lewy bodies, which are fibrillar aggregates and contain the protein alpha-synuclein, are a defining characteristic of Parkinson's disease (PD). The primary anatomy & ecology of PD is the degeneration of pigmented nerves in the brainstem caused by the accumulation of Lewy bodies. Damaged substantia nigra pars compacta neurons can be seen using a microscope. Additionally, 18 specific chromosomal regions, referred to as chromosomal loci and named PARK1, PARK2, PARK3, and so on in sequential order, have been identified thus far.

Various medications are used to treat Parkinson's disease (PD), including levodopa (known as L-Dopa), COMT inhibitors, and anticholinergic agents. Levodopa is considered the most effective treatment for PD since it can replace dopamine deficits and improve quality of life. However, levodopa use can cause adverse effects such as inconsistent plasma levels, unpredictable absorption, and long-term treatment issues like levodopa-induced dyskinesia (LID) and motor fluctuations. Several drugs like Sinemet (carbidopa-levodopa), Parcopa (a mouthwash), Duopa (an enteral suspension), and Rytary (a carbidopa extended-release medicine) are used to treat Parkinson's disease.

2.5 DJ-1 protein-

DJ-1 is a homodimeric peptidase belonging to the C56 family, comprising 189 amino acids organized into nine helices and seven strands. It is found in

various cellular compartments, including neurons, glial cells, cytoplasm, nuclei, and mitochondria. A distinctive feature of DJ-1 is an additional helix in its C-terminal region, believed to cover the catalytic site. In neurodegenerative disorders, DJ-1 is observed to be overexpressed in reactive astrocytes when subjected to oxidative stress. Notably, within DJ-1, the cysteine residues at positions C46, C56, and C106 play important roles. Among them, C106 is particularly vulnerable to oxidative stress and rapidly converts to sulfinic acid, leading to complete inactivation of DJ-1 due to mutations or oxidation.

Pharmacological treatments are available to manage the symptoms of these diseases but there is currently no cure. Medications like Memantine, Donepezil, Galantamine, and Rivastigmine are used to alleviate or delay AD symptoms, while Levodopa, Carbidopa, Bromocriptine, Pramipexole, and Ropinirole are used for PD. However, only Rivastigmine is approved for treating PD-related dementia, which has led to the exploration of alternative therapies. Computational drug repurposing is an alternative approach that utilizes in-silico studies to repurpose already established conventional medications as well as drugs still in the preclinical and clinical stages for potential use as disease-modifying therapies. On the other hand, drug repositioning or de novo drug discovery are alternative strategies.

CHAPTER 3- METHODOLOGY

3.1 Plant extracts preparation-

3.1.1 Aqueous extract of shade dried leaves-

To prepare *Moringa oleifera* and *Nyctanthes arbor-tritis* leaves for use, which was collected from university itself. The petioles were cut down and leaves were washed thoroughly with the help of milli-Q water to eliminate any remaining dust particles. Subsequently, the leaves were thoroughly washed with tap water and arranged individually on a clean surface, then stored in a shaded area for five days until completely dry. After the drying process, the leaves were weighed and grinded into fine leaf powder using mixer grinder.

To produce plant extract, 1 gram of leaf powder was added in 100 ml of milli-Q water in a flask. This solution was then heated using a heating mantle for 5 minutes at 50 °C, followed by 10 minutes of boiling. Once the flask contents had cooled to room temperature, they were poured into a 50ml falcon tube and centrifugation was done at 3000 rpm for five minutes. Lastly the supernatant was separated using Whatman filter paper.

3.1.2 Aqueous Extract of Sun Dried Leaves-

To prepare the *Moringa oleifera* and *Nyctanthes arbor-tritis* leaves, the petioles were removed, and the leaves were rinsed twice with milli-Q water to eliminate any remaining dust particles. The leaves were then thoroughly washed with running tap water and arranged individually on a clean surface, where they were exposed to light for three days until completely dry. After the drying process, the leaves were weighed and grinded into a fine powder using a mixer grinder

To produce plant extract, 1 gram of leaf powder was added within 100 ml of milli-Q water in a 200 ml beaker. The mixture was then heated using a heating mantle for 5 minutes at 50 °C, followed by 10 minutes of boiling. After cooling the flask's contents to room temperature, they were poured into a 50ml falcon tube and centrifugation was done at 3000 rpm for 5-10 minutes. The resulting supernatant was separated from the leaf pellets using a Whatman

filter paper. The collected supernatant is the aqueous extract obtained from the *Moringa oleifera* leaves.

3.1.3 Aqueous extract of fresh leaves-

The petioles of *Moringa oleifera* and *Nyctanthes arbor-tritis* leaves were shed, and the leaves were washed heavily with distilled water to eliminate any remaining dust particles before being thoroughly cleaned with running tap water. The leaves were then ground into a fine paste using a mixer grinder. To prepare the plant extract, 100 ml distilled water and 2-5 g of the leaves paste were mixed in a 200 ml flask, heated for 5 minutes at 50 ℃ for 10 minutes using a heating mantle, and then allowed to cool at room temperature. The flask's contents were then transferred to a 50ml falcon tube, and the extract was centrifuged at 3000 rpm for 5-10 min for the pellet to get separated.

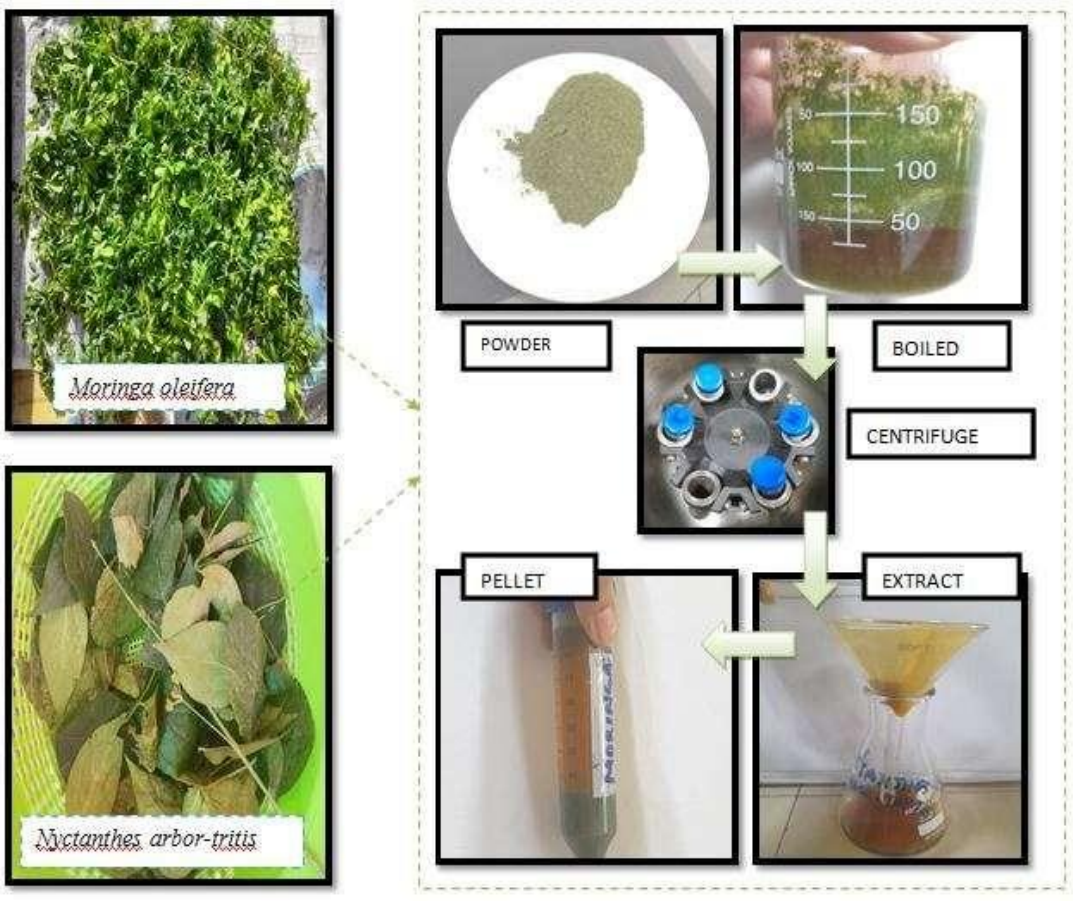


Fig. 3.1 Extraction process flow diagram

3.2 Qualitative test:

3.2.1 Test for alkaloid-

To conduct the qualitative test for alkaloids, Wagner's Test was employed. Wagner's reagent was prepared by measuring 2.25 grams of iodine and 12.5 grams of KI using a weighing balance. Then, iodine and potassium iodide were added to 250 ml of distilled water. Subsequently, 1 ml of prepared Wagner's Reagent and 1 ml of prepared plant extract was pipette into the solution.

3.2.2 Test for Phenol-

The presence of phenol in the plant was determined using the FeCl test. A solution of 5% ferric chloride was prepared by measuring 1gm of ferric chloride salt and transferring it to a flask. Then, 20ml of milli-Q water was added to the flask. Following that, 1ml of the prepared plant extract was pipette, and 1ml of the ferric chloride solution was added to it.

3.2.3 Test for carbohydrate-

The presence of carbohydrate in the plant was determined using Starch test. To prepare the test solution, 5mL of 5% KOH solution was added to the aqueous extract.

3.2.4 Test for Anthraquinones-

The presence of glycosides in the plant was determined using Borntrager's test. The hydrolysate filtrate (2mL) was combined with 3mL of chloroform and thoroughly shaken. Subsequently, the extra layer was separated, and then ammonia solution was added.

3.2.5 Test for Proteins and Amino acids-

The presence of protein and amino acid in the plant was determined using Biuret test. To test for the presence of reducing sugars, 2mL of the filtrate was mixed copper sulphate solution followed by 1mL of 95% ethanol and KOH pellets.

3.2.6 Test for flavonoids-

The presence of flavonoids in the plant was determined using con. H2SO4 test. To the 1mL of extract, few drops of con. H2SO4 was added.

3.2.7 Test for Tannins-

The presence of tannins in the plant was determined using gelatin test. In 5mL of distilled water, plant extract was added and combined with a 1% gelatin solution and 10% NaCl.

3.2.8 Test for Saponins-

The presence of saponins in the plant was determined using foam test. The finw powder was mixed with 2mL of water and vigorously shaken.

3.2.9 Test for Triterpinoides -

The presence of Triterpinoides in the plant was determined using Salkowski's test. After adding a few drops of concentrated H2SO4, the filtrate was shaken well and allowed to stand.

3.2.10 Test for Lignin-

The presence of lignin in the plant was determined using Labet test. Few drops of Gallic acid were added to the solution of the plant extract.

3.2.11 Test for Quinones-

The presence of quinone in the plant was determined using Sulfuric test. 10 mg of extract can be dissolved in isopropyl alcohol with the addition of a single drop of concentrated sulfuric acid.

3.2.12 Test for Glycosides-

The presence of glycosides in the plant was determined using concentrated H2SO4 test. To prepare the solution, 5ml of plant extract is mixed with 2mL of glacial acetic acid, and ferric chloride solution is added. Finally, few drops of concentrated sulfuric acid is added to the solution.

S.No	Tests	Expected Observations
1	Test for alkaloid	Reddish Brown Precipitate
2	Test for phenol	Bluish Black ring
3	Test for carbohydrate	A cinary color
4	Test for Anthraquinones	Pink, violet or red solution
5	Test for Proteins and Amino acids	Pink color solution
6	Test for flavonoids	Orange color
7	Test for tannins	White ppt.
8	Test for saponins	Foam
9	Test for Triterpinoides	Golden yellow color at bottom
10	Test for lignin	Olive green color
11	Test for quinones	Red color
12	Test for glycosides	Brown ring

Table 3.2 Different Phytochemical test for qualitative tests

3.3 Quantitative tests

3.3.1 Thin layer chromatography (semi-quantitative method)

The development of a TLC (Thin Layer Chromatography) plate involves the separation of compounds on the plate based on their differential migration rates. Here is a general overview of how a TLC plate is developed:

- **Prepare the TLC plate**: Start by ensuring that the TLC plate is clean and free from any contaminants. The plate typically is made up of a thin layer of adsorbent material, such as silica gel or cellulose, coated onto a solid support (e.g., a glass or aluminum plate). If necessary, activate or precondition the plate by heating it in an oven or allowing it to equilibrate in a controlled environment.

- **Apply the sample:** Using a capillary tube or microsyringe, carefully apply small spots or bands of the sample to the starting line or a specific location on the TLC plate. Ensure that the spots are small and concentrated to avoid overlapping or excessive spreading.

  - Carefully place a small spot, approximately 1-2 cm from the edge of the plate, using a pencil.
  - Take caution not to apply excessive pressure that may damage the plate's surface.
  - Clean the capillary tube with acetone after each use.
  - Gently touch the capillary tube to a piece of paper towel to remove excess solvent.
  - Aim to create small sample spots, preferably less than 3mm in diameter.
  - Lightly touch the plate with the capillary tube and quickly release it to apply the sample.

- **Prepare the developing chamber:** Choose a suitable solvent system for your sample and pour it into a developing chamber. The solvent system should be selected based on the solubility and expected mobility of the compounds of interest. The solvent level should be below the starting line on the TLC plate.

- **Place the TLC plate in the chamber**: To ensure accurate development, carefully insert the TLC (thin-layer chromatography) plate into the developing chamber. After the development process is complete, remove the plate, ensuring that the plate is vertical and the spots are above the solvent level. The chamber should be covered to prevent evaporation and ensure a controlled environment.

- **Allow the plate to develop:** As the solvent in the chamber rises through capillary action, it carries the compounds on the TLC plate. The compounds start to separate based on their affinity for the adsorbent material and their solubility in the solvent system. The plate is allowed to develop until the solvent front is near the top of the plate, but not reaching it entirely.

- **Remove the plate and visualize the separation**: Once the desired development is achieved, carefully remove the TLC plate from the chamber and solvent is marked at the front. Let it air dry or use a fume hood or gentle airflow to speed up the drying process. The separated compounds will appear as spots or bands on the TLC plate.

- **Visualize the spots:** Use appropriate visualization techniques to observe the separated spots. This can include exposing the plate to UV light, spraying with specific reagents, or using a staining agent to enhance the visibility of the compounds. Take note of the location, size, and intensity of the spots for further analysis.

  It's important to note that the development process may change according to certain factors such as the nature of the sample, the choice of solvent system, and the experimental conditions. Adjustments may be needed to optimize the separation and obtain clear spots on the TLC plate.

  The formula to calculate the retention factor (Rf) is given by:

  $$\mathbf{Rf} = \frac{\text{Distance covered by the substance spot from the initial line}}{\text{Distance covered by the solvent front from the initial line}}$$

  To determine the Rf value, you need to measure and divide the distance covered by the substance spot from the initial line and the distance covered by the solvent front from the initial line.

3.3.2 Quantitative estimation of alkaloids –

The procedure involves measuring 5 grams of the sample material and placing it in a 100 mL flask. Next, 50 ml of a 10% acetic acid solution in ethanol is added to the beaker, which is then covered and left undisturbed for duration of 4 hours. The resulting mixture is separated, and the concentrated solution is placed in a water bath to reduce its volume to one-fourth from the initial. Gradually, con. NH4OH is drop by drop added within the concentrated solution until complete ppt^{n} occurs. Once the entire mixture has settled, the precipitate is collected. The remaining accumulation, which contains the alkaloid, is then dried and weighed.

$$\text{Alkaloid content (\%)} = \frac{\text{Weight of extracted material}}{\text{Weight of sample}} \times 100$$

3.3.3 Quantitative estimation of phenols –

10 ml of 80% methanol solution was utilized to extract 2 grams of the plant sample. The solution was subsequently kept at room temperature for a period of four hours. Afterward, the entire mixture was filtered through filter paper. The obtained filtrate was allowed to dry overnight and then weighed.

$$\text{Flavonoid content (\%)} = \frac{\text{Weight of extracted material}}{\text{Weight of sample}} \times 100$$

3.4 Web databases and tools based on bioinformatics

3.4.1 Protein Data Bank

The Protein Data Bank (PDB) is a freely available database designed to store the structural data of big macromolecules discovered through experimental research. It acts as a thorough database that the general public and researchers can use to obtain and examine the structural information of many biomolecules.

3.4.2 Chimera 1.13.1

A flexible piece of software called Chimera 1.13.1 was created to make interactive visualisation and study of molecular structures and the data they are accompanied by possible. It provides a flexible framework for visualising density maps, supramolecular assemblies, sequence alignments, docking findings, trajectories, and conformational ensembles in addition to supporting PDB structures. Chimera 1.13.1's extensive toolkit enables researchers to study and analyse various facets of molecular structures and related data.

3.4.3 Uniprot

Uniprot https://www.uniprot.org/, a top-notch and thorough protein database that provides free, open access to useful data on protein activities and sequencing. This database is available to researchers who want to learn more about diverse proteins and develop understanding of its biological characteristics and functional capabilities.

3.4.4 Marvin Sketch (tool)

Users of MarvinSketch can define the R-groups that make up a molecule, which enables the representation of a user-defined collection of structures. These R-groups can be utilised in R-group searches because they serve as variables. Researchers can effectively search for a variety of hits relating to various substructures by including R-groups into the query structure. This function offers a simple method for exploring and locating different molecular fragments and substructural patterns inside a molecule.

3.4.5 CASTp server

CASTp http://sts.bioe.uic.edu/, a technique for determining functional protein locations is known as (Computed Atlas of Surface Topography of Proteins). CASTp forecasts the presence of active site residues within a particular protein molecule using the 3D protein structure as input. This makes it possible to locate and examine parts of the protein that are essential to the protein's operation.

3.4.6 Open Babel GUI

Molecules and chemical reactions can be converted between different file formats with the use of the obabel command-line tool. It gives an option to using the Open Babel GUI, which has functions that are equal. Researchers may effectively alter and modify chemical data via the command line while still having access to all of Open Babel's features without relying on the GUI.

3.4.7 Discovery studio visualizer

It is the software widely used for studying protein-ligand interactions. It serves as an advanced molecular modeling tool, offering capabilities for visualizing and analyzing data related to different proteins and other small molecules as well. The BIOVIA Discovery Studio Visualizer, which is available for free, enables experts and their colleagues to efficiently exchange research findings without any loss of time or scientific knowledge. This software provides a valuable platform for collaborative exploration and understanding of molecular interactions.

3.4.8 Lipinski's Rule of Five

Lipinski's Rule of Five is a guideline used to assess the drug-like properties of a compound, specifically its similarity to orally administered medications. It can aid in determining whether a chemical has potential as a drug. The molecular docking procedure holds significant. This field shows great potential for advancements in drug discovery as it predicts the conformation and orientation of ligands within their specific binding sites.

According to Lipinski, if a compound exceeds certain thresholds, such as a molecular weight over 500, an octanol/water partition coefficient over 5, more than 10 hydrogen-bond acceptors (N and O atoms), and more than 5 hydrogen-bond donors (N and O atoms), it is more likely to exhibit poor absorption and permeation. These guidelines were established in 2000 by Lipinski and provide valuable insights for drug development and optimization.

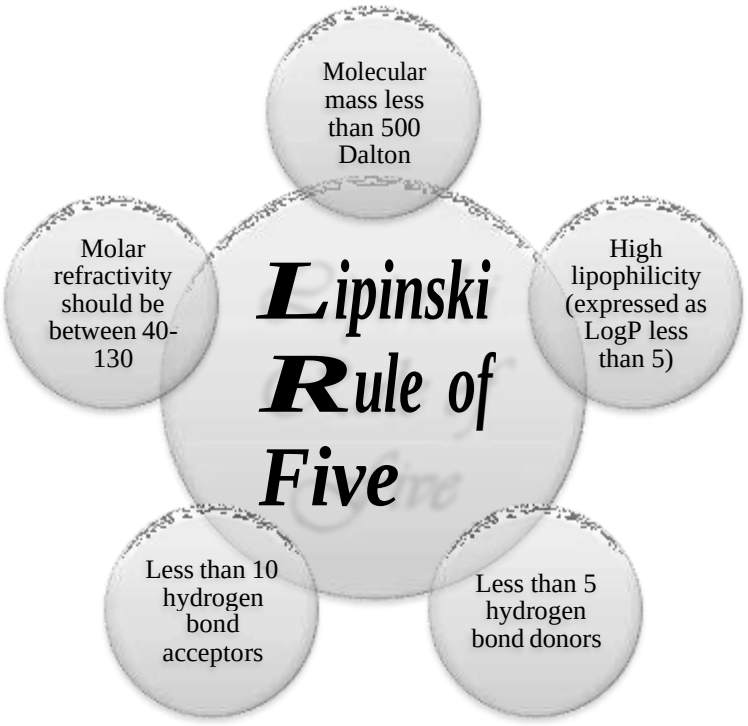


Fig. 3.2 Lipinski's Rule of Five Model

3.4.9 Multiple sequence alignment

Multiple sequence alignment https://www.ebi.ac.uk/Tools/msa/, also known as MSA, refers to the process of aligning three or more biological sequences, such as proteins or nucleic acids that are of comparable length. This alignment provides valuable insights into the evolutionary connections among the sequences and allows for the inference of homology. By studying the results of multiple sequence alignment, researchers can gain a deeper understanding of the relationships and similarities between different sequences, aiding in various biological and evolutionary analyses.

- **Clustal omega**

  Clustal Omega is an innovative tool designed for multiple sequence alignment (MSA), which enables the alignment of three or more sequences. It achieves this by utilizing HMM profile-profile algorithms and seeded guide trees. This advanced approach allows Clustal Omega to generate accurate alignments, considering both sequence similarities and evolutionary relationships. By employing these sophisticated techniques, Clustal Omega enhances the precision

and efficiency of multiple sequence alignment, providing researchers with a valuable resource for studying biological sequences and inferring their homology.

3.4.10 IMPPAT

IMPPAT 2.0 abbreviated as Indian Medicinal Plants, Phytochemistry And Therapeutics 2.0 is a meticulously curated database that has been developed by digitizing information from over 100 books on traditional Indian medicine, as well as more than 7,000 published research publications and other existing resources. This comprehensive database represents a significant enhancement and expansion over its predecessor, IMPPAT 1.0. Currently, IMPPAT 2.0 holds the distinction of being the largest digital library dedicated to the phytochemicals of Indian medicinal plants. It serves as a valuable resource for researchers and enthusiasts seeking detailed information on the phytochemistry and therapeutic properties of Indian medicinal plants.

3.4.11 Autodock 4.2

It is a freely accessible docking program utilized for investigating protein-ligand interactions. The binding efficacy of the docked complex, which is employed to assess the stability of the resulting complex, can be estimated using various techniques. This docking program serves as a valuable tool for researchers to explore and analyze the interactions between proteins and ligands, aiding in the understanding of molecular recognition and drug discovery processes.

3.4.12 PatchDock

PatchDock is a molecular docking algorithm employed for the purpose of docking different types of molecules, such as proteins, DNA, peptides, and drugs. It takes two molecules as input and generates a list of potential complex formations, arranged according to criteria that assess how well their shapes complement each other. The algorithm employs shape matching techniques to align and superimpose the identified patches. This allows for the exploration of potential binding modes and the prediction of molecular interactions between the docked molecules. PatchDock provides researchers

with a valuable tool for studying and understanding protein-ligand interactions and other molecular docking scenarios.

3.4.13 SwissADME

Swiss ADME http://www.swissadme.ch/, a freely available online program, was utilized to obtain valuable information regarding the physicochemical properties of drugs. This includes characteristics related to absorption, distribution, metabolism, excretion/elimination, and toxicity. By utilizing Swiss ADME, researchers can access and analyze important aspects of drug molecules that influence their behavior within the body. The program offers a convenient platform to investigate and understand the pharmacokinetic and toxicological properties of potential drug compounds.

3.4.14 Different filters used for screening

Five filters were implemented -- lipinski, Ghose, Veber, Egan and Muegge for showing Drug likeliness properties of the compounds used.

- ✓ Lipinski filter: MW ≤ 500, MLOG ≤ 4.15, N/O ≤ 10, NH/OH ≤ 5.
- ✓ Ghose filter: 160 ≤ MW ≤ 480, -0.4 ≤ WLOGP ≤ 5.6, 40 ≤ MR ≤ 130, 20 ≤ atoms ≤ 70.
- ✓ Veber filter: TPSA ≤ 140, Rotatable bonds ≤ 10.
- ✓ Egan filter: WLOGP ≤ 5.88, TPSA ≤ 131.6.
- ✓ Muegge filter: 200 ≤ MW ≤ 600, -2 ≤ XLOGP ≤ 5, TPSA ≤ 150, Rings ≤ 7, C > 4, Heteroatom > 1, Rotatable bonds ≤ 15, H-bond acc. ≤ 10, H-bond don ≤ 5.

Please note that MW stands for molecular weight, MLOGP/WLOGP represents LogP values, N/O denotes the number of Nitrogen or Oxygen atoms, NH/OH refers to the number of NH or OH groups, MR represents molecular refractivity, atoms indicates the number of atoms, TPSA stands for Topological Polar Surface Area, Rings refers to the number of rings, C denotes the number of carbon atoms, and Heteroatom represents the number of heteroatoms.

Fig. 3.3 Drug likeliness filters used in screening purposes of the compound

CHAPTER 4 RESULTS & DISCUSSION

4.1 Qualitative test

4.1.1 Aqueous extract of shade dried leaves-

S.No	**Tests**	***Moringa oleifera***	***Nyctanthes arbor-tritis***
1	Alkaloid	++	+
2	Phenol	++	+
3	Carbohydrate	+	+
4	Anthraquinones	-	-
5	Proteins and Amino acids	+	+
6	Flavonoids	+	+
7	Tannins	+	-
8	Saponins	++	++
9	Triterpinoides	+	+
10	Lignin	+	+
11	Quinones	++	+
12	Glycosides	++	++

Table 4.1 Table showing results of shade dried leaves

4.1.2 Aqueous extract of fresh leaves-

S.No	**Tests**	***Moringa oleifera***	***Nyctanthes arbor-tritis***
1	Alkaloid	+++	+++
2	Phenol	+++	++
3	Carbohydrate	++	+
4	Anthraquinones	+	+
5	Proteins and Amino acids	+	+
6	Flavonoids	+++	+
7	Tannins	+	-
8	Saponins	+++	+++
9	Triterpinoides	++	++
10	Lignin	+	++
11	Quinones	+	+
12	Glycosides	+++	+++

Table 4.2 Table showing results of fresh leaves

4.1.3 Aqueous extract of sun dried leaves-

S.No	**Tests**	***Moringa oleifera***	***Nyctanthes arbor-tritis***
1	Alkaloid	+	+
2	Phenol	+	+
3	Carbohydrate	+	+
4	Anthraquinones	-	-
5	Proteins and Amino acids	+	+
6	Flavonoids	+	-
7	Tannins	-	-

8	Saponins	+	+
9	Triterpinoides	-	+
10	Lignin	+	+
11	Quinones	+	-
12	Glycosides	+	+

Table 4.3 Table showing results of sun dried leaves

S. No	**Tests**	***Moringa oleifera***	***Nyctanthes arbor-tritis***
1	Test for alkaloid		
2	Test for phenol		

3	Test for carbohydrate		
4	Test for Anthraquinones		
5	Test for Proteins and Amino acids		

6	Test for flavonoids		
7	Test for tannins		
8	Test for saponins		

9	Test for Triterpinoides		
10	Test for lignin		
11	Test for quinones		

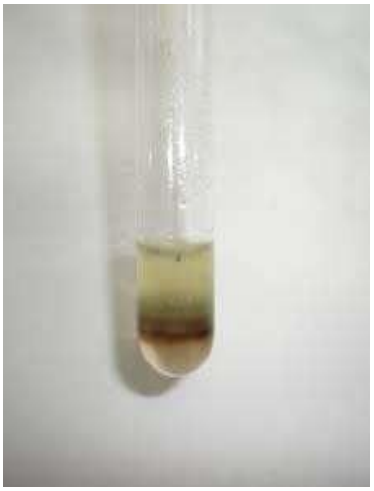
12	Test for glycosides		

Fig. 4.1 Images showing different phytochemical tests for medicinal plants

4.2 Quantitative tests

4.2.2 Thin layer chromatography

In TLC, an aqueous extract and an ethanol extract of *Moringa oleifera* and *Nyctanthes arbor-tritis* were taken, and the solvent system used was a mixture of isopropyl alcohol and chloroform in a ratio of 9:1 (v/v). However, it was observed that the ethanol extract provided a better and more distinct separation on the TLC plate. This is likely because the ethanol extract effectively extracted and released all the constituents present in the extracts due to its stronger solubility properties in ethanol.

- When analyzing the ethanol extract of *Moringa oleifera* leaves using TLC, two distinct bands were observed on the TLC plate with corresponding Rf values of 0.78 and 0.81. However, in the case of the aqueous extract, no bands were visualized or detected on the TLC plate.
- During the TLC analysis of the ethanol extract from *Nyctanthes arbor-tritis*, two bands were observed on the TLC plate, with Rf values of 0.62 and 0.9. On the other hand, the aqueous extract did not show any distinct spots on the TLC plate, indicating the absence of clear separable compounds in the aqueous extract of clove buds.

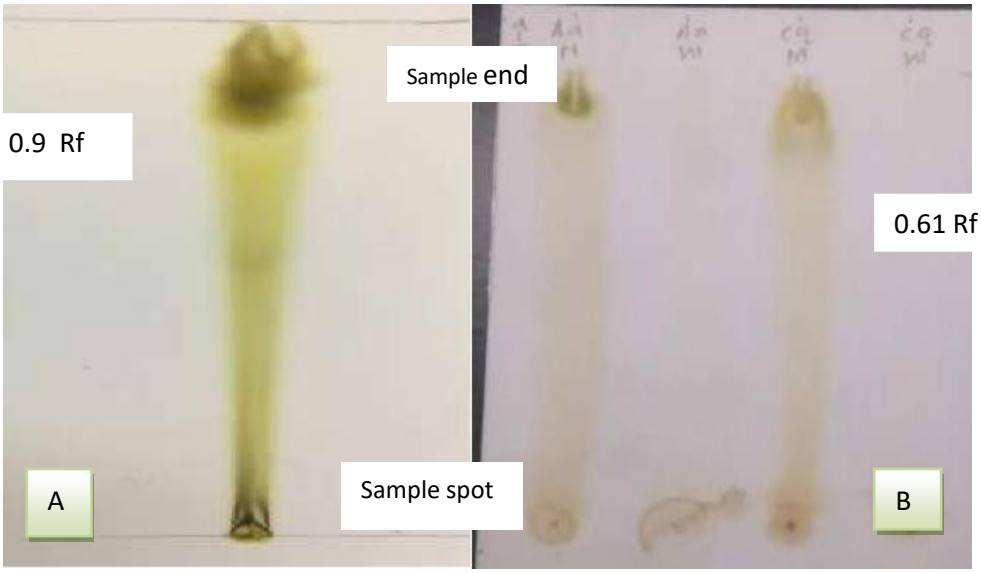


Fig. 4.2 (a, b) Plant extracts of Moringa and *Nyctanthes arbor-tritis* thonolic extracts respectively

4.3 Quantitative estimation of alkaloid content-

- The analysis indicated the presence of alkaloid content in the leaf extract of *Nyctanthes arbor-tristis* plant. The percentage of alkaloids found in the water extraction from leaves was 8%, while the solvent extraction yielded 0.6% alkaloid content.
- The analysis indicated the presence of alkaloid content in the leaf extract of *Moringa oleifera* plant. The percentage of alkaloids found in the water extraction from leaves was 9.56%, while the solvent extraction yielded 0.82% alkaloid content.

4.4 Quantitative estimation of Flavonoid content-

- The leaf extract of *Nyctanthes arbor-tristis* plant contained flavonoid content. The water extraction from the leaves exhibited a percentage of 3.12% flavonoid content, while the solvent extraction yielded 2.5% flavonoid content.
- The leaf extract of *Moringa oleifera* plant contained flavonoid content. The water extraction from the leaves exhibited a percentage of 5.83% flavonoid content, while the solvent extraction yielded 2.87% flavonoid content.

Fig. 4.3 (A) Water extraction of the plant leaves for the quantification

Fig. 4.3 (B) Solvent extraction of the plant leaves for the quantification

4.5 Sequence analysis of the protein

4.5.1 Protein data bank

The sequence of aa of the DJ-1 was retrieved from the Uniprot database, specifically using the Uniprot. The 3-D structure of DJ-1, obtained from the Protein Data Bank (PDB) having PDB ID 4ZGG. To visualize the structure, the Pymol was used.

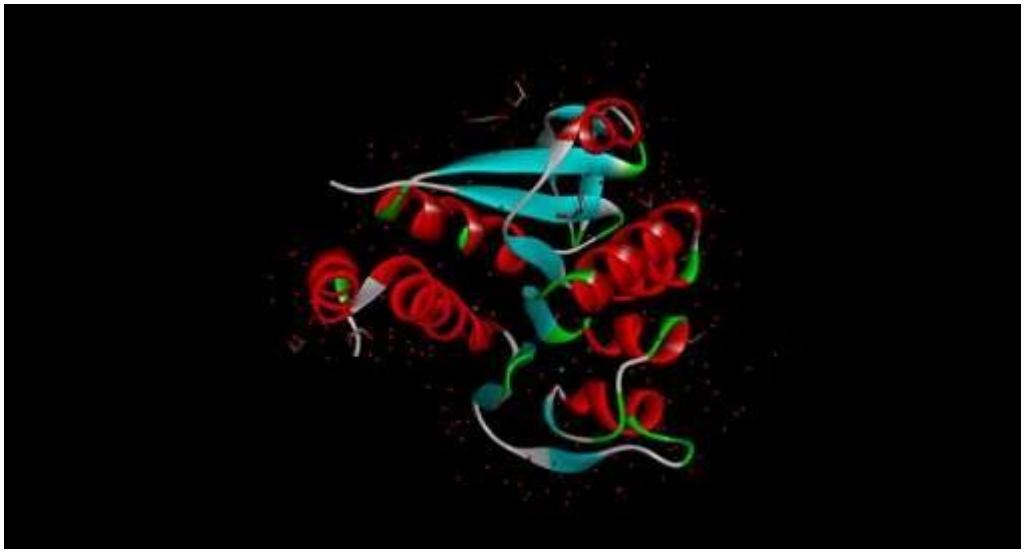


Fig. 4.4 3D structure of target protein DJ 1 (4ZGG)

4.5.2 Macromolecule visualization

The protein was visualized using Chimera. In the protein, the Gasteiger partial charges were assigned to carbon atoms connected to hydrogen, while polar hydrogens were considered and non-polar hydrogens were disregarded. By utilizing default settings and a grid-based method, the protein's binding pocket was established. A grid map with dimensions of 34×34×34 points was created, where each point was spaced 0.375 units apart, effectively covering the active site as well as a substantial area of the surrounding surface.

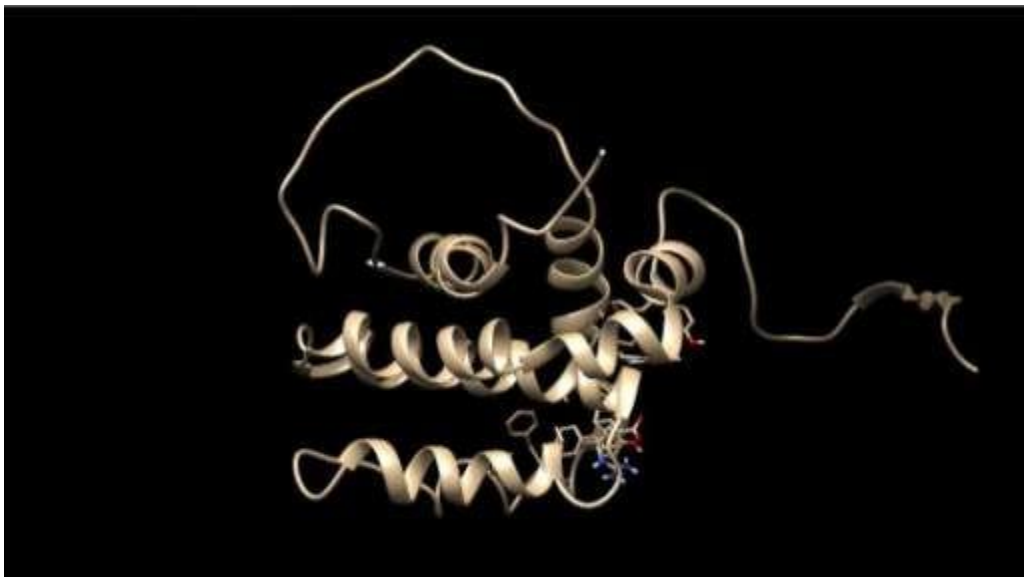


Fig. 4.5 Structural visualization in Chimera

4.5.3 BLAST result of the target protein

Using BLASTP, DJ 1 protein with Uniprot ID- ACZ52888.1 shows 100 % query coverage and 90-97% identity results.

S.NO	ACCESSION ID	MAX/TOTAL SCORE	QUERY COVERAGE	E-VALUE	IDENTITY
1.	ACZ52888.1	5202	100	0	97
2.	NP_009193.2	5039	100	0	91
3.	NP_001264182.1	4757	100	0	91
4.	NP_001005938.1	4754	100	0	91
5.	XP_028533864.1	4710	99	0	90
6.	OTN64797.1	4614	100	0	90

Table. 4.4 Blast result of DJ1 using protein blast

4.5.4 Multiple sequence alignment of the target protein

Using the Clustal omega tool, the fasta sequences of the DJ1 protein from different organisms were aligned to assess their similarity. Upon analyzing the multiple sequence alignment, it is evident that most of the sequences exhibit a high degree of alignment with only a few gaps, with the accession number ACZ52888.1.

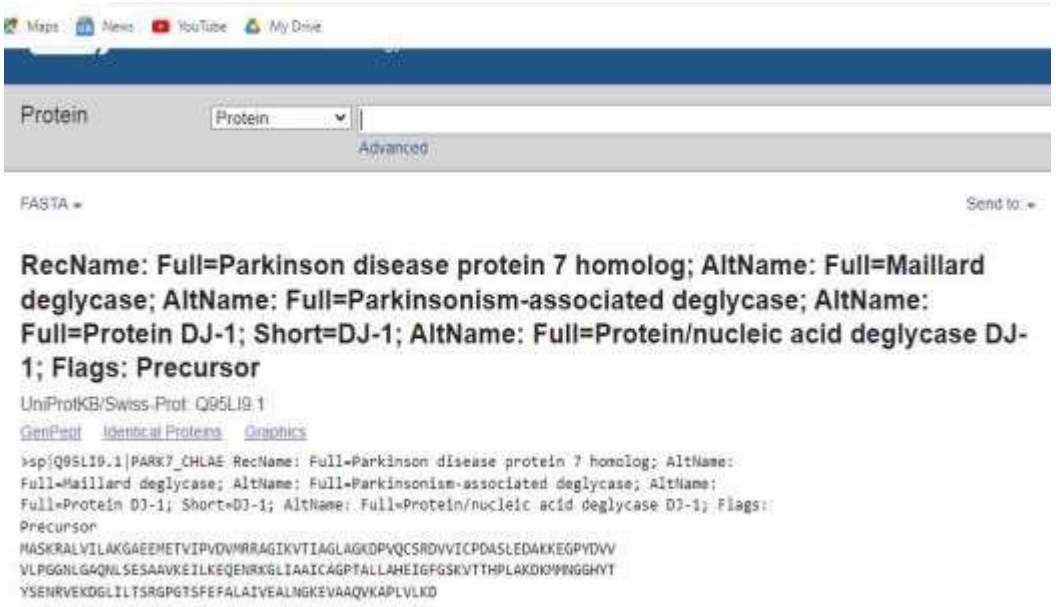


Fig. 4.6 FASTA sequence of the protein

```
CLUSTAL O(1.2.4) multiple sequence alignment

ACZ52888.1      MVKETKLYDLLGVSTDASQDAIKK-GYRKCALKWHPDKNKDNPDAAEKFKECSQAYEILS   59
NP_009193.2     MASKRALV-ILAKGAEEMETVIPVDVMRRAGIKVTVA-----GLAGKDPVQCSRDVVICP   54
                *..:  *  :*. .::  : .*     *:..:*          *.:.  :**:   *

ACZ52888.1      -----DPEKRKIYDQFGLEFLLRGGAPPPEGGAGAAGGNPFADAGGMPGGFSGFDFGNMG   114
NP_009193.2     DASLEDAKKEGPYDVV----------------------------VLPGG----------   75
                     * :*.  ** .                             :***

ACZ52888.1      GGPGGARTFHFSTGGGGPSGFNPSNPQSIFETFMRSGGAGMGGDDDDDMADLFAQFGGGA   174
NP_009193.2     --NLGAQNLS--------------ESAAVKE-ILKE---------QENRKGLIAAICAG-   108
                   **:.:               :  :: * :::.         :::  .*:* : .*

ACZ52888.1      GGGGRPRTRVRTGFGDPAGRSARQHTPEVTTVERPLPVSLEDMFQGAQKKMKIKCKLFDE   234
NP_009193.2     ---PTALLAHEIGFGSKV---------------TTHPLAKDKMMN---------------   135
                         . ***. .                *:: :.*::

ACZ52888.1      NGKRTTTEKVLDVPIKAGLKKGSKIRFEGVGDQEEGGQQDLCFVVEEKPHILYTRDGDDL   294
NP_009193.2     ----------------------------------GGHYTYSENRVEKDGLILTSRGPGT   160
                                                 **:   .    **  :: *  * .

ACZ52888.1      SMTVDLDLKEALTGWKRTVSTIDGKQIALEKAGPTQPGSQDVYPNQGMPISKKPGQRGNF   354
NP_009193.2     SFEFALAIVEALNG----------KEVAAQVKA---------------------------   183
                *: . * : ***.*          *::* :  .

ACZ52888.1      IIKYNVKFPTSLTAQQKQQLKEIL       378
NP_009193.2     --------PLVLKD----------       189
                        *  *.
```

Fig. 4.7 Multiple sequence alignment of DJ 1 protein using clustal Ω

4.5.5 Multiple sequence alignment using Clustal W

Similar to other tools in the Clustal family, ClustalW is utilized for the rapid alignment of multiple protein or nucleotide sequences. It employs a method

called progressive alignment, where sequences that exhibit the highest similarity are aligned initially, and then the less similar sequences are aligned gradually, ultimately resulting in a global alignment. This process enables the comparison and identification of conserved regions among the sequences, aiding in the analysis of evolutionary relationships and functional similarities.

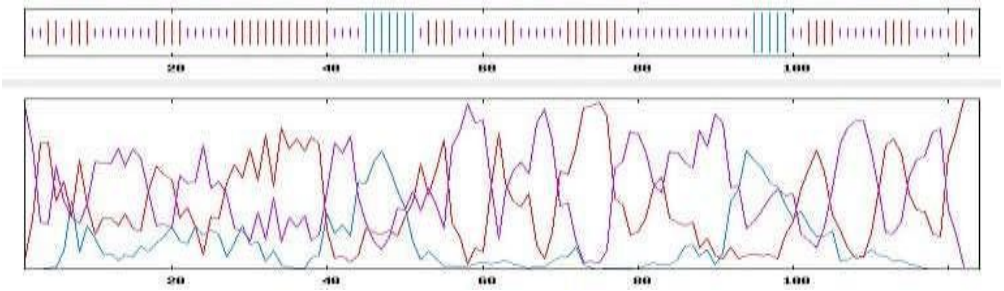


Fig. 4.8 GOR method for the prediction of secondary structure protein

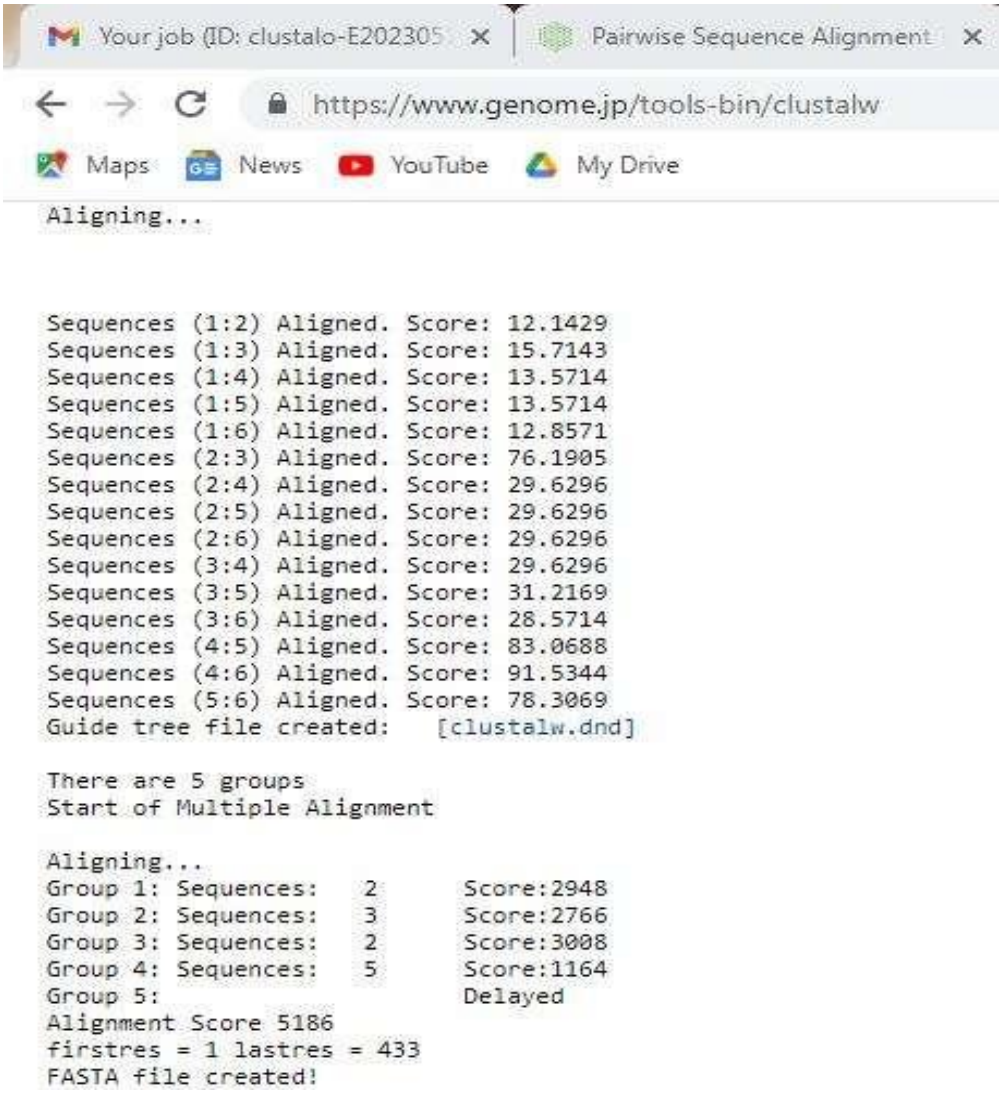


Fig. 4.9 Multiple sequence alignment of DJ 1 protein using Clustal W

4.5.6 Phylogenetic study of the targeted protein

1. A phylogenetic tree is a pictorial illustration that showcases ancestral lineages among different organisms. Rather than being definitive facts, phylogenetic trees represent hypotheses based on scientific theories. The Phylogenetic tree have branched structure which reflects the diversification of species or other groups, demonstrating the emergence and development of multiple lineages from shared ancestors. Through the examination of these branching patterns, scientists gain insights into the evolutionary history and interconnectedness of various creatures.

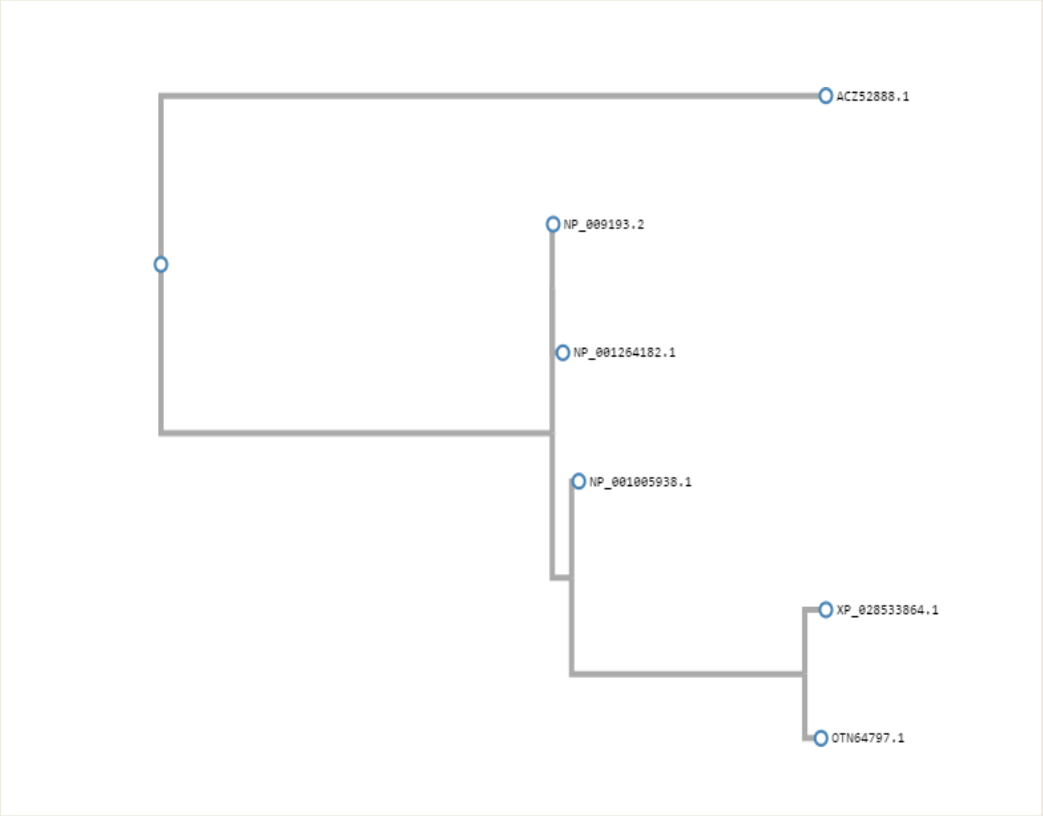


Fig. 4.10 Phylogenetic tree analysis of DJ 1protein

4.6 Structural analysis of the targeted protein

4.6.1 Ramachandran plot

We utilized PDBsum database to assess the suitability of the DJ 1protein as a model for docking experiments. The analysis of the DJ 1 protein structure's Ramachandran plot indicates that it is indeed a favorable choice and possesses excellent quality for docking with ligands. A significant percentage of its

residues, specifically 68.75%, are located in the most favored regions, suggesting a high-quality target protein structure. Moreover, only a small proportion, approximately 3.13%, of residues reside in forbidden regions, indicating that the protein structure is well-behaved and suitable for further docking investigations.

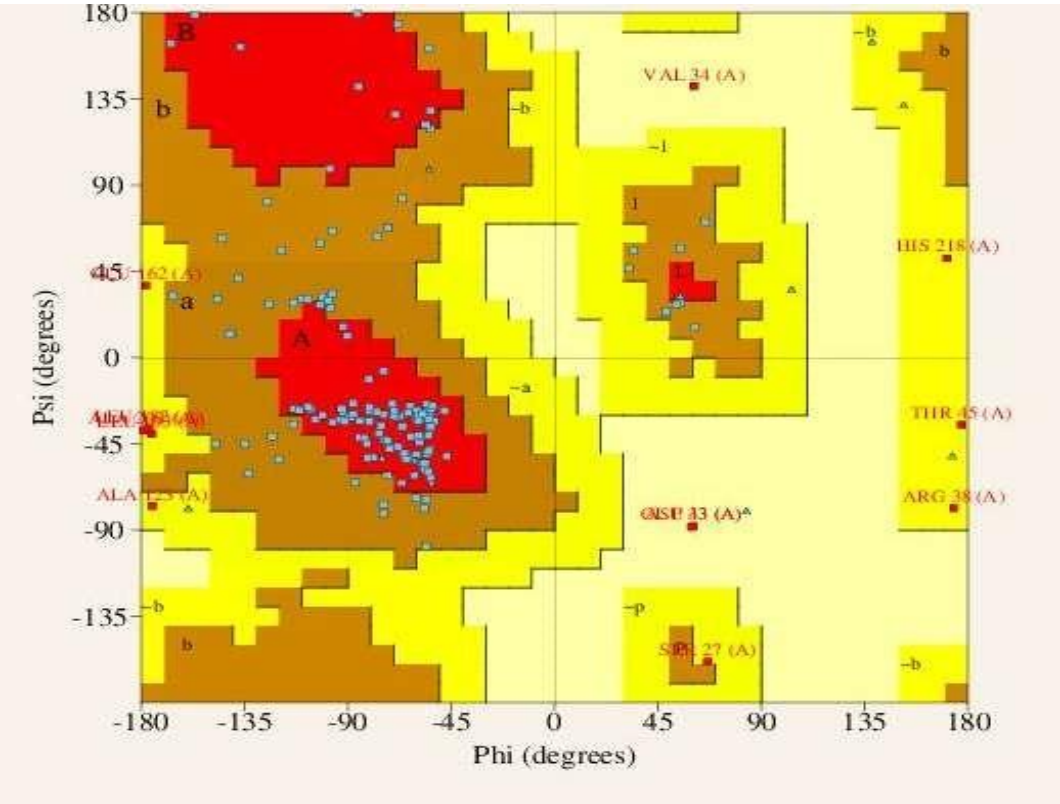


Fig. 4.11 Ramachandran plot of DJ 1 protein

PROCHECK statistics

1. Ramachandran Plot statistics

		No. of residues	%-tage
Most favoured regions	[A,B,L]	105	64.0%**
Additional allowed regions	[a,b,l,p]	47	28.7%
Generously allowed regions	[~a,~b,~l,~p]	9	5.5%
Disallowed regions	[XX]	3	1.8%*
Non-glycine and non-proline residues		164	100.0%
End-residues (excl. Gly and Pro)		2	
Glycine residues		12	
Proline residues		3	
Total number of residues		181	

Based on an analysis of **118** structures of resolution of at least **2.0** Angstroms and *R*-factor no greater than **20.0** a good quality model would be expected to have over **90%** in the most favoured regions [A,B,L].

Fig. 4.12 Percentage of Allowed and disallowed region in DJ 1 protein

4.6.2 Active site prediction

Using the CASTp server, we identified a total of 45 pockets within the LRRK2 protein. Among these pockets, pocket number 45 stood out with the largest surface area and volume. This indicates that pocket number 45 possesses the most spacious and significant cavity within the protein structure.

S.No	Pocket No	Area ($Å^2$)	Volume ($Å^3$)
1	45	130.741	100.940
2	44	134.158	76.472
3	43	67.342	37.522
4	42	65.466	26.856
5	41	59.591	20.252
6	40	25.252	19.428
7	39	20.351	3.034
8	38	7.630	1.727
9	37	9.461	1.294
10	36	7.249	0.773

Table. 4.5 Various pockets in DJ 1 protein using CASTp server

4.7 Ligand preparation and preparation

The compounds' structures were initially created in 2D using Marvin Sketch 15.1.19 in .mol format. Subsequently, the 2D structures were converted into 3D structures using Chimera 1.13.1. Chimera takes the .mol file as input and generates the molecule's 3D structure. You can retrieve the optimized ligands of the molecule in a PDB format, which is compatible with Autodock4.2 Tools. This process is illustrated in the accompanying figures.

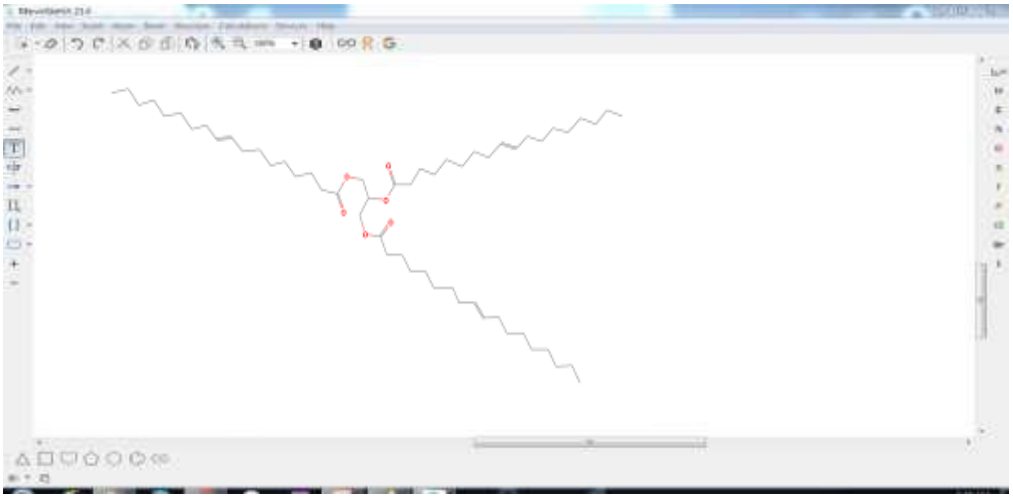


Fig.4.13 Manual representation of the compound in Marvin sketch window

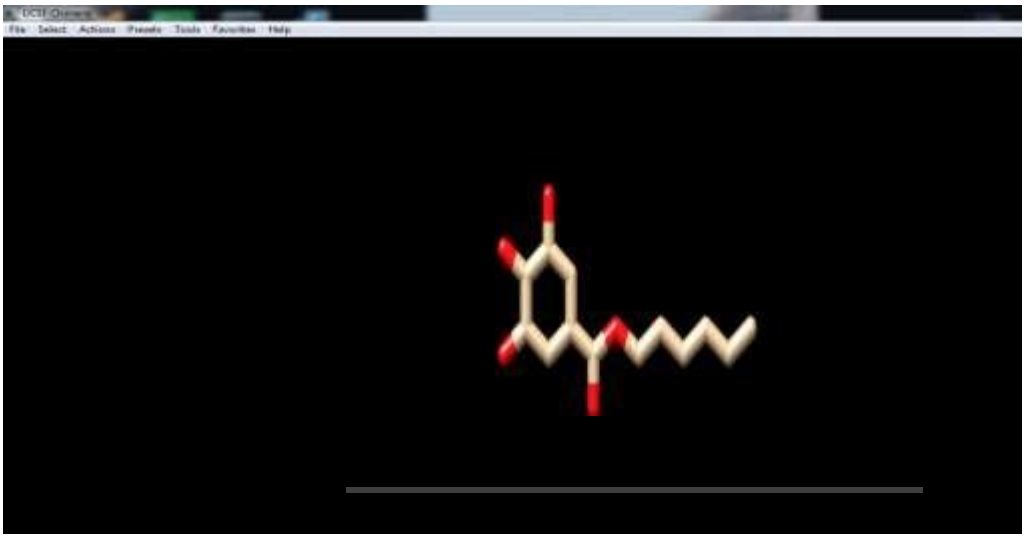
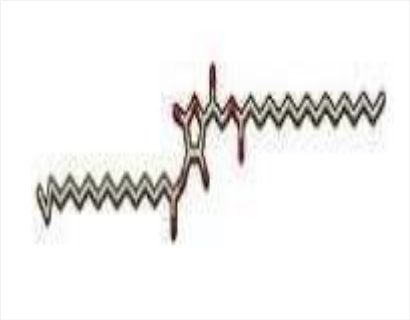


Fig. 4.14 Eg: 3D structure of the compound in PDB format

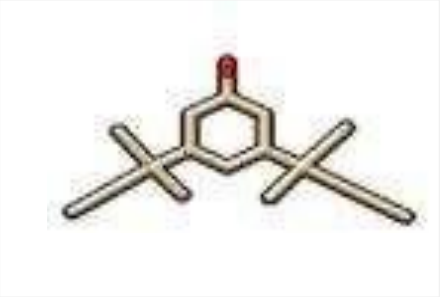
4.7.1 Ligand selected for *Moringa oliefera*

(A) 9-Octadecenoic acid

propanetrieyl **(B) 3-Ethyl-2,4-dimethyl-pentane**



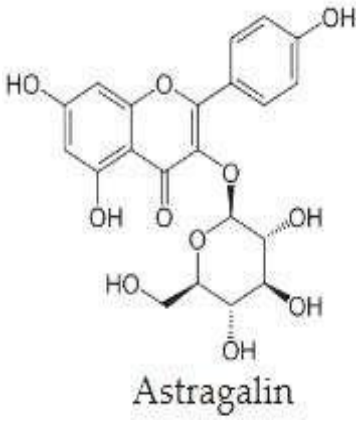
(C) - (+)-Ascorbic acid 2,6dihexa-decanoate **(D) 1-Hexadecanol**



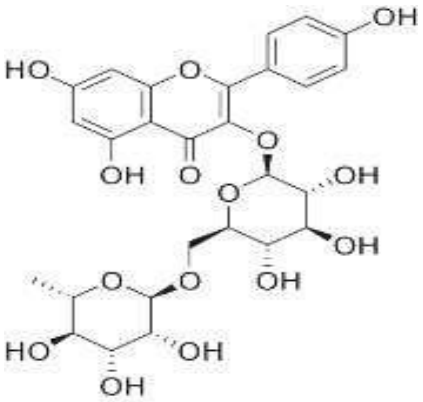
(E) 3, 5-bis (1,1-dimethylethyl)-phenol

Fig. 4.15 Figures showing compounds with their IUPAC names

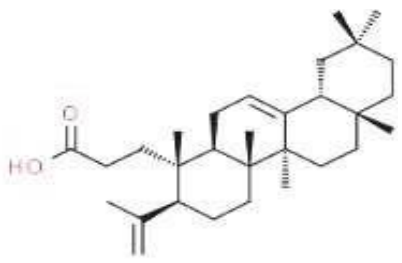
4.7.2 Ligand selected for *Nyctanthes arbor-tristis*



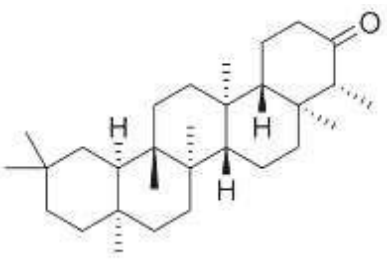
Astragalin



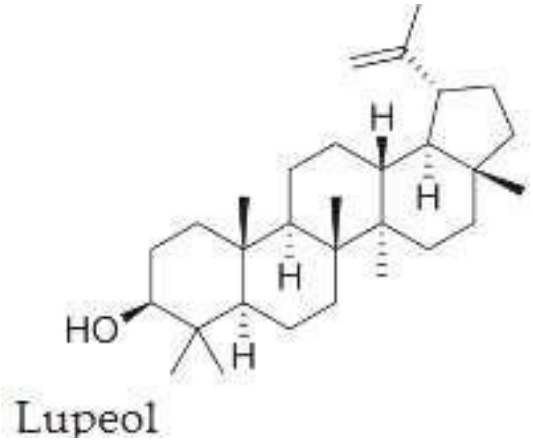
Nicotiflorin



Nyctanthic acid



Friedeline



Lupeol

Fig. 4.16 Figures showing compounds with their IUPAC names

4.8 Results of ADMET analysis

The drug likeliness properties of the specific compounds were also analyzed using Swiss ADME.

In order to ensure saturation, the molecule should have a Fraction Csp3 value of at least 0.25, indicating a sufficient number of sp hybridized carbons. It is also important for the molecule's molecular weight to fall within the range of 150 to 500 g/mol, which determines its overall size. The TPSA (Topological Polar Surface Area) should be within the range of 20 to 130, indicating the desired polarity of the molecule. For solubility, the Log S value should not exceed 6. Regarding lipophilicity, the XLOGP3 value should range from 0.7 to +6.0, reflecting the desired level of hydrophobicity. Additionally, it is preferable for the molecule to have no more than 9 rotatable bonds, ensuring a certain degree of flexibility while maintaining a manageable complexity.

4.8.1 ADMET analysis of *Moringa oliefera* compounds

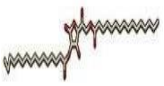
S.No	COMPOUND & THEIR MOLECULAR FORMULA	ADME PROPERTIES							
		M.Wt ≤500 Da	**Log P ≤5**	**H-bond Acceptor ≤10**	**H-bond Donor ≤5**	**TPSA ≤130Å**	**Log S <6 (ESOL)**	**Rotatable bonds ≤ 9**	**violations**
1.	 C57H10O6	883.42 g/mol	4.69	8	3	114.68 Å	-2.15	8	0
2.	 C 6H12O	128.26 g/mol	2.79	7	3	105.45 Å	-2.43	8	0
3.	 C38H68O	652.94 g/mol	4.58	8	2	112.68 Å	-2.15	7	0
4.	 C 16H34O	240.42	4.23	6	1	105.45 Å	-2.43	5	0
5.	 C 14H22O	206.32	2.86	7	1	97.99 Å	-1.64	6	0

Table 4.6 Various parameters showing ADMET analysis of the plant - *Moringa oliefera*

4.8.2 ADMET analysis of *Nyctanthes arbor-tristis* compounds

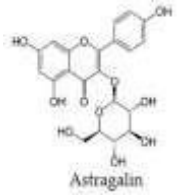
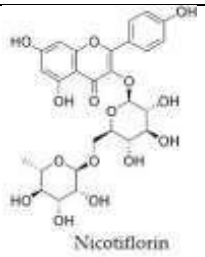
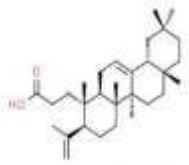
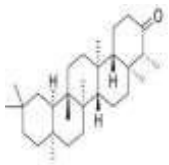
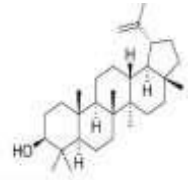
S.No	COMPOUND & THEIR NAME	ADME PROPERTIES							
		M.Wt ≤500 Da	Log P ≤5	H-bond Acceptor ≤10	H-bond Donor ≤5	TPSA ≤130Å	Log S <6 (ESOL)	Rotatable bonds ≤ 9	violations
1.	 Astragalin	448.4 g/mol	2.3	4	1	104.68 Å	-2.15	4	0
2.	 Nicotiflorin	594.5 g/mol	1.8	2	0	115.05 Å	-2.43	6	0
3.	 Nyctanthic acid	440.7 g/mol	3.4	3	2	111.60 Å	-2.15	5	0
4.	 Friedeline	426.7	1.7	6	0	189.45 Å	-2.43	7	0
5.	 Lupeol	426.7	3.1	3	1	123.99 Å	-1.64	7	0

Table 4.7 Various parameters showing ADMET analysis of the plant - *Nyctanthes arbor-tristis*

4.9 Docking analysis

Methodical approach to determine the interactive nature between protein and molecule; we adopted the following pipeline that is discussed in detail below.

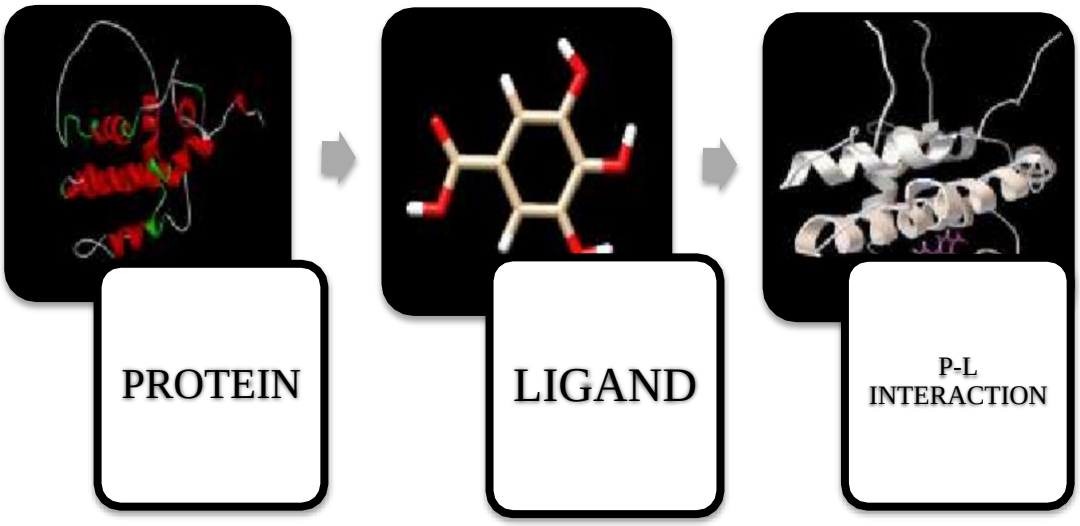


Fig. 4.17 Process diagram showing interaction of protein and ligand

Due to the proven effectiveness in swiftly and accurately predicting the binding energies and bound conformations of ligands with macromolecular targets, we have opted to utilize the Autodock4.2 tool for our molecular docking experiments. Autodock4.2 is widely recognized as a valuable tool in the field, providing reliable results and aiding in the exploration of ligand-target interactions.

Requirements for performing molecular Docking

- Windows version
- MGL tools
- Open Babel GUI
- Auto dock 4.2
- Autodock4 file

- Autogrid4 file
- ADT files

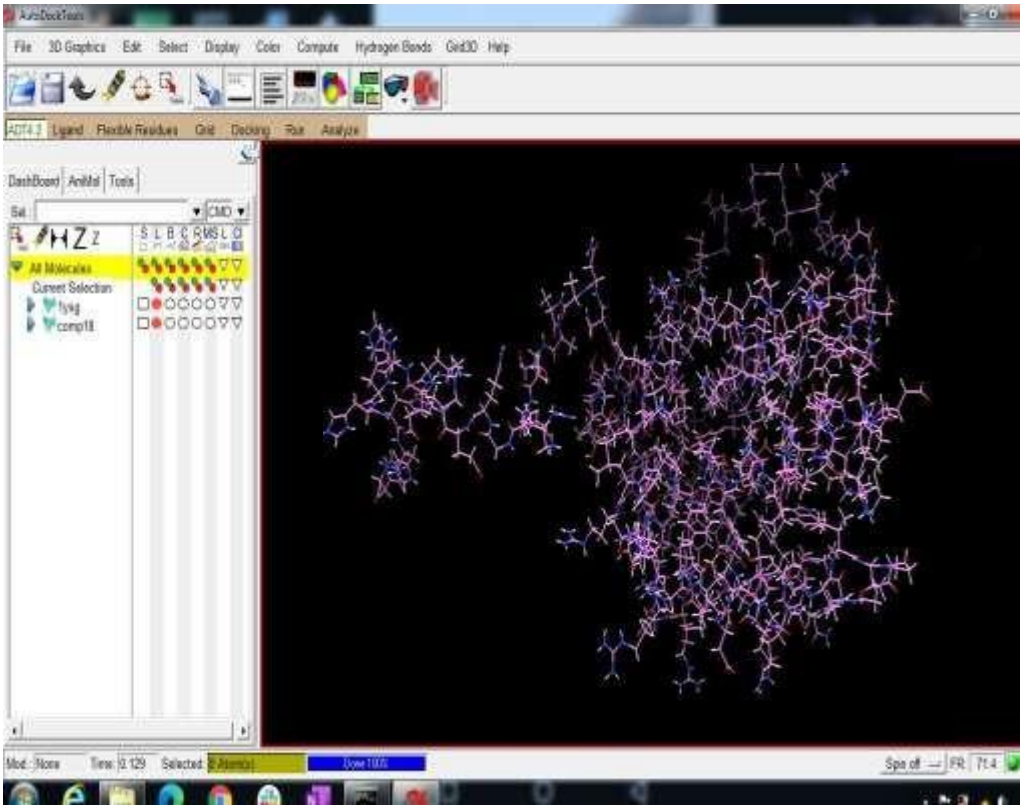


Fig. 4.18 Autodock interface. The current version of AutoDock 4.2

The Lamarckian genetic method was employed to investigate the pattern of protein-target interactions. In each docking procedure, 10 separate genetic algorithms were executed, utilizing a population size of 150 individuals. The execution of the Lamarckian genetic algorithm (LGA) was limited to a maximum of 2,500,000 energy assessments and 27,000 generations. Subsequently, autogrid and autodock were employed to generate the GLG and DLG files, respectively. These files contain crucial information pertaining to the ligand-protein docking process and are instrumental in further analyses and investigations.

4.9.1 Docking analysis of *Moringa oleifera* plant

S.No	Compounds of *Moringa oleifera*	Docking Results			
		Binding energy (kcal/mol)	**Inhibition constant (Ki)**	**No of H bonds**	**Amino acid Residues**
1.	9-Octadecenoic acid-1,2,3-propanetrieyl ester	-11.19	6.32	3	ARG143, GLU133,TYR105
2.	3-Ethyl-2,4-dimethyl-pentane	-10.96	19.77	3	ARG143, TYR105,GLY142
3.	-(+)-Ascorbic acid 2,6dihexa-decanoate	-10.12	38.39	1	ARG143
4.	1-Hexadecanol	-9.91	54.50	2	ASN140, ARG143
5.	3,5-bis(1,1-dimethylethyl)-phenol	-4.82	294.26	2	ASN140, TYR105

Table 4.8 Docking interactive studies of *Moringa oleifera* being displayed in the form of table having binding energy, no of H bonds & KI value

4.9.2 Docking analysis of *Nyctanthes arbor-tristis* plant

S.No	Compounds of *Nyctanthes arbor-tristis*	Docking Results			
		Binding energy (kcal/mol)	**Inhibition constant (Ki)**	**No of H bonds**	**Amino acid Residues**
1.	Astragalin	-9.46	16.02	∞	ARG143, GLU133,TYR105
2.	Nicotiflorin	-9.23	13.89	∞	ARG143, TYR105,GLY142
3.	Nyctanthic acid	-7.12	67.29	∞	ARG143,TYR115
4.	Friedeline	-5.11	34.55	∞	ASN140, ARG143..
5.	Lupeol	-4.07	99.20	∞	ASN140, TYR105…

Table 4.9 Docking interactive studies of *Nyctanthes arbor-tristis* being displayed in the form of table having binding energy, no of H bonds & KI value

Best compound which can be used as a natural drug after comparing the target protein with the 2 plant species

After the comparison, it is clear that ***Moringa oleifera*** has shown the better results in both in-vitro and in-silico study. The binding energy of **-11.19 kcal/mol** was exhibited by **9-Octadecenoic acid-1, 2, 3-propanetrieyl ester**. This Compound have better ΔG value than the compounds of *Nyctanthes arbor-tristis*, This result showed that 9-Octadecenoic acid-1, 2, 3-propanetrieyl ester is a potential inhibitor for DJ 1 protein in treating Parkinson's disease. The decreasing value of the free binding energy is utilized to represent the energy levels of the docked complex formed by the target protein and ligand molecule in the above 2 tables. The docked complex that possesses the lowest free energy is considered the most stable, thereby making it the optimal choice for a potential medication.

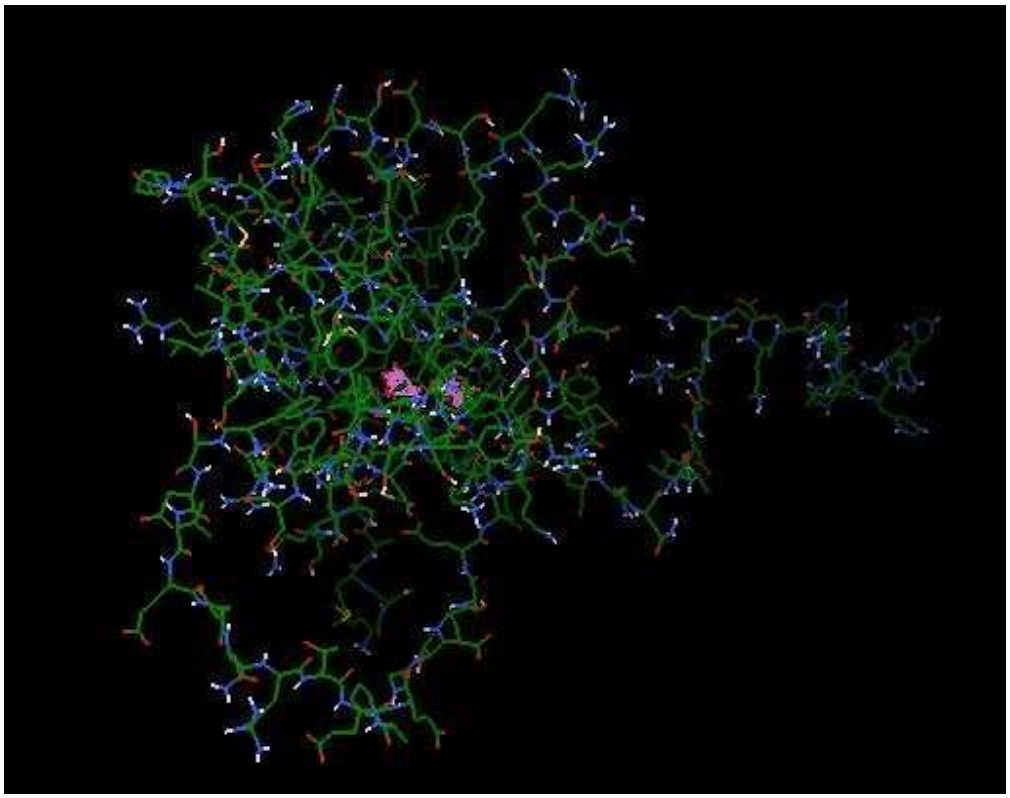


Fig. 4.19 Best docked compound of Moringa oleifera - 9-Octadecenoic acid-1, 2, 3-propanetrieyl ester.

4.10 Visualization of the results

To assess the interactive relationship between a protein and a molecule in a three-dimensional view, a systematic methodology is employed. This methodology utilizes the following equation, which is elaborated below.

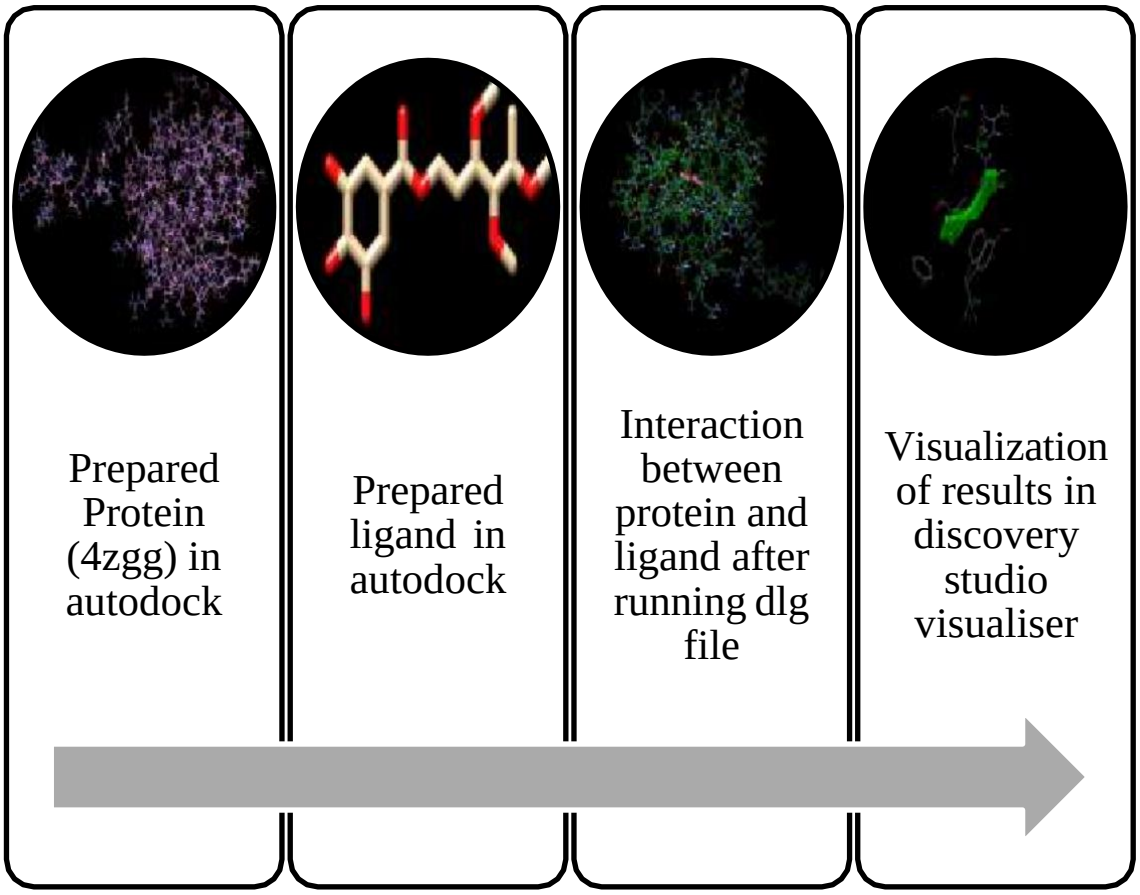
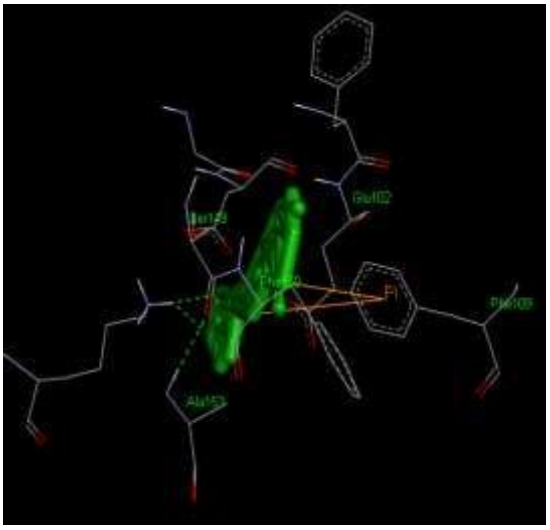
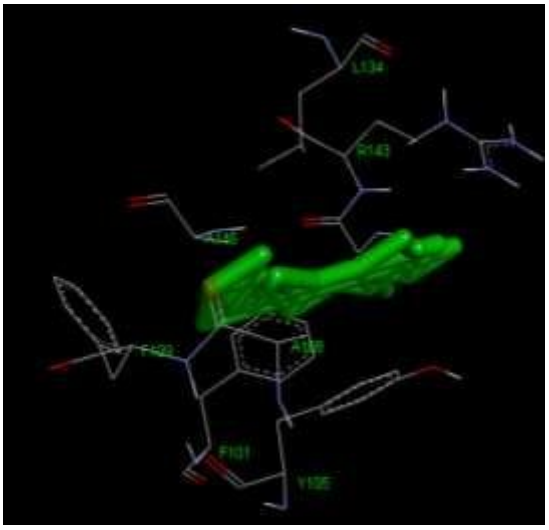


Fig. 4.20 Pipeline formed to display the procedure of visualization of macromolecule

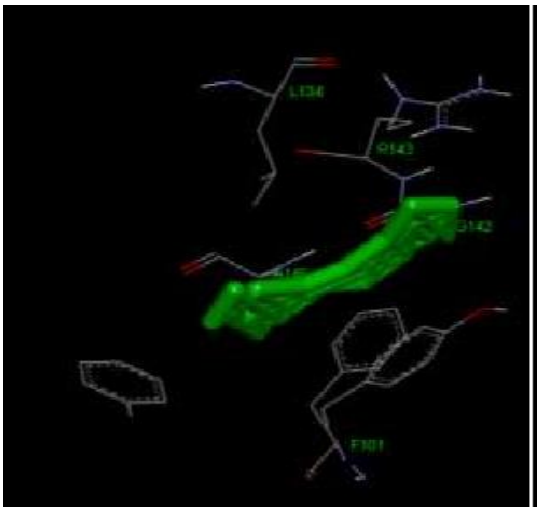
The software Discovery Studio Visualizer, a widely utilized tool for studying protein-ligand interactions, was employed to visualize the obtained results. This free and comprehensive molecular modeling tool allows for the viewing, sharing, and analysis of protein and small molecule data. It enables experts and their peers to efficiently exchange results, thereby facilitating the preservation of scientific knowledge and saving time.



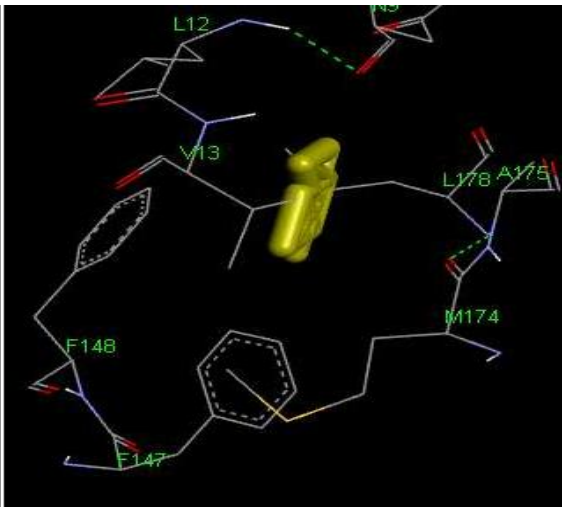
(A) 9-Octadecenoic acid-1,2,3-propanetrieyl ester



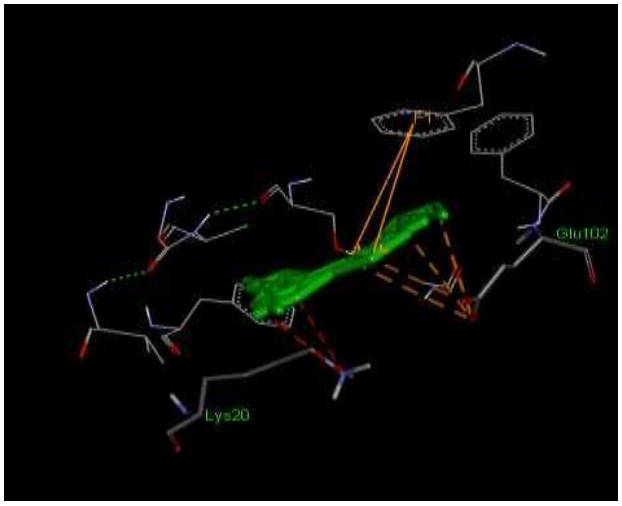
(B) 3-Ethyl-2,4-dimethyl-pentane



(C) - (+)-Ascorbic acid 2,6dihexa-decanoate

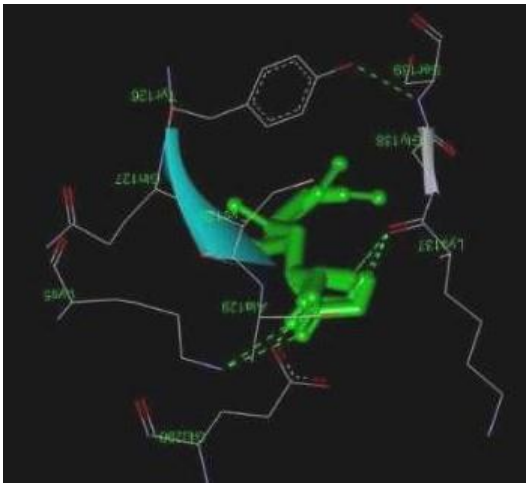


(D) 1-Hexadecanol

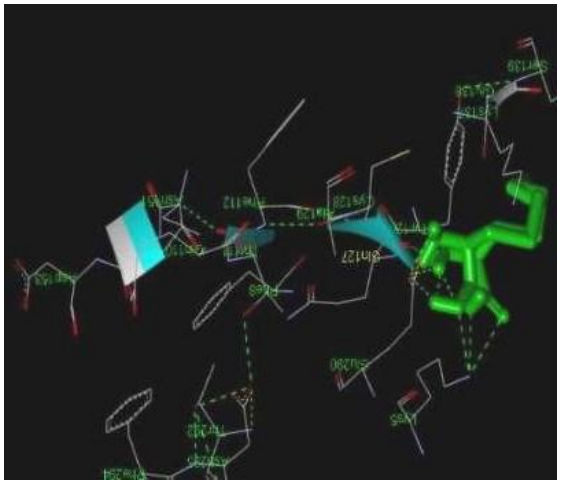


(E) 3, 5-bis (1, 1-dimethylethyl)-phenol

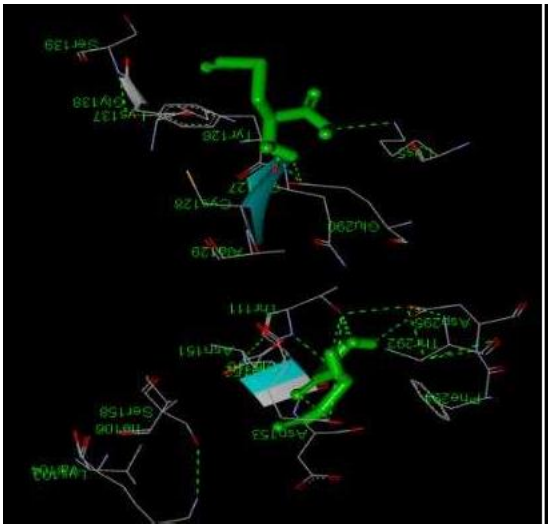
Fig. 4.21 (A, B, C, D, and E) Docking studies of *Moringa oleifera* compounds



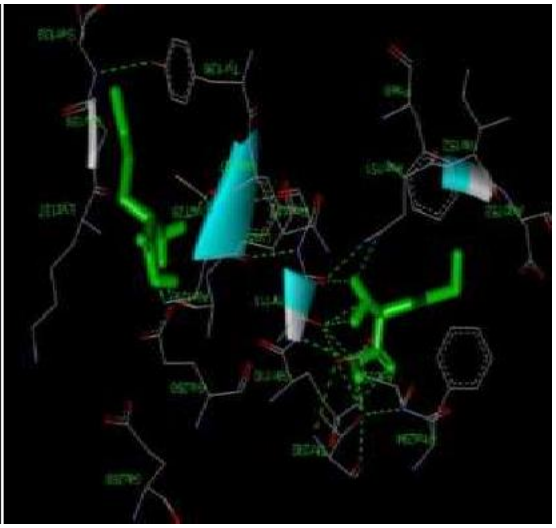
(A) Astragalin



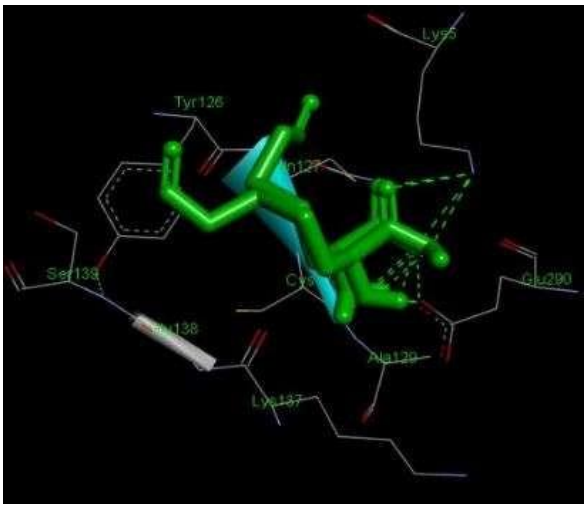
(B) Nicotiflorin



(C) Nyctanthic acid



(D) Friedeline



(E) Lupeol

Fig. 4.22 (A, B, C, D, and E) Docking studies of *Nyctanthes arbor-tristis* compounds

4.11 Validation of results with the help of PatchDock

A methodical approach to determine the accuracy of the Autodock results by validation process; we adopted the following equation that is discussed in detail below.

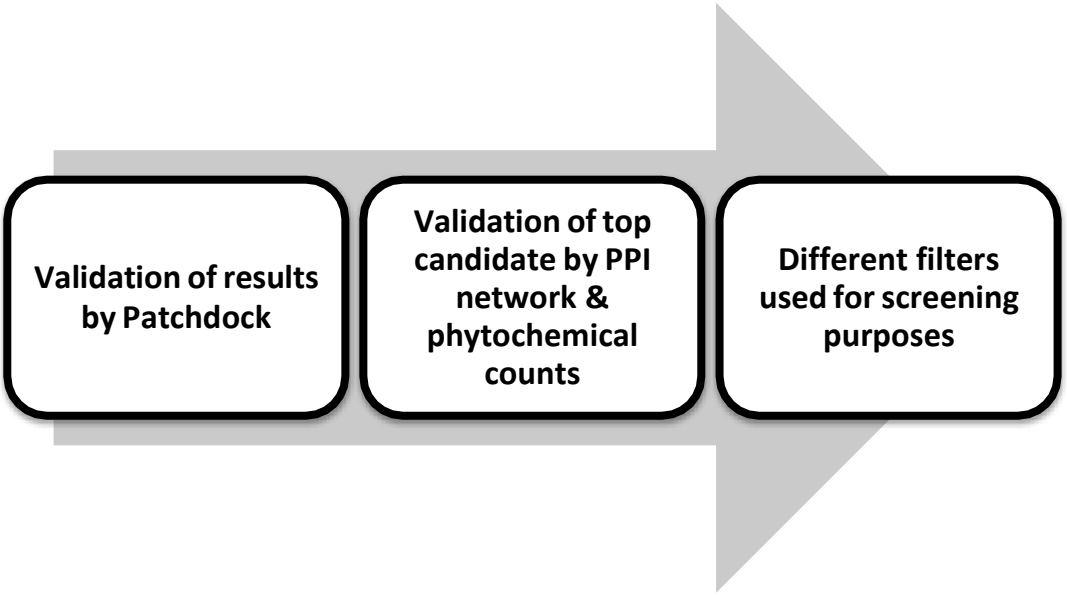


Fig. 4.23 Pipeline designed for validation purposes

The docking results obtained from Autodock 4.2 were validated using this online server. The molecular docking algorithm employed is based on shape complementarity.

Fig. 4.24 Window showing PatchDock results

4.11.1 PatchDock results of *Moringa oleifera*

S.NO	COMPOUND OF *Moringa oleifera*	DOCKING SCORE
1.	9-Octadecenoic acid-1,2,3-propanetrieyl ester	3178
2.	3-Ethyl-2,4-dimethyl-pentane	3170
3.	-(+)-Ascorbic acid 2,6dihexa-decanoate	3026
4.	1-Hexadecanol	2916
5.	3,5-bis(1,1-dimethylethyl)-phenol	2866

Table 4.10 Table Results of Patchdock - *Moringa oleifera*

4.11.2 PatchDock results of *Nyctanthes arbor-tristis*

S.NO	COMPOUND OF *Nyctanthes arbor-tristis*	DOCKING SCORE
1.	Astragalin	2890
2.	Nicotiflorin	2856
3.	Nyctanthic acid	2693
4.	Friedeline	2399
5.	Lupeol	1179

Table 4.11 Table Results of Patchdock - *Nyctanthes arbor-tristis*

Similar results were obtained and 9-Octadecenoic acid-1, 2, 3-propanetrieyl ester have shown the maximum docking score in comparison to other docking compounds.

4.12 Phytochemical counts

After identifying the relevant genes for plant phytochemicals through the IMPPAT database, it is recommended to utilize the canonical SMILES (Simplified Molecular Input Line Entry System) notation. This notation serves as a computer-readable symbolization of chemical structures, enabling efficient processing and analysis.

- *Moringa oleifera* phytochemicals had 22 matched genes.
- *Nyctanthes arbor-tristis* plant phytochemicals had 12 matched genes.

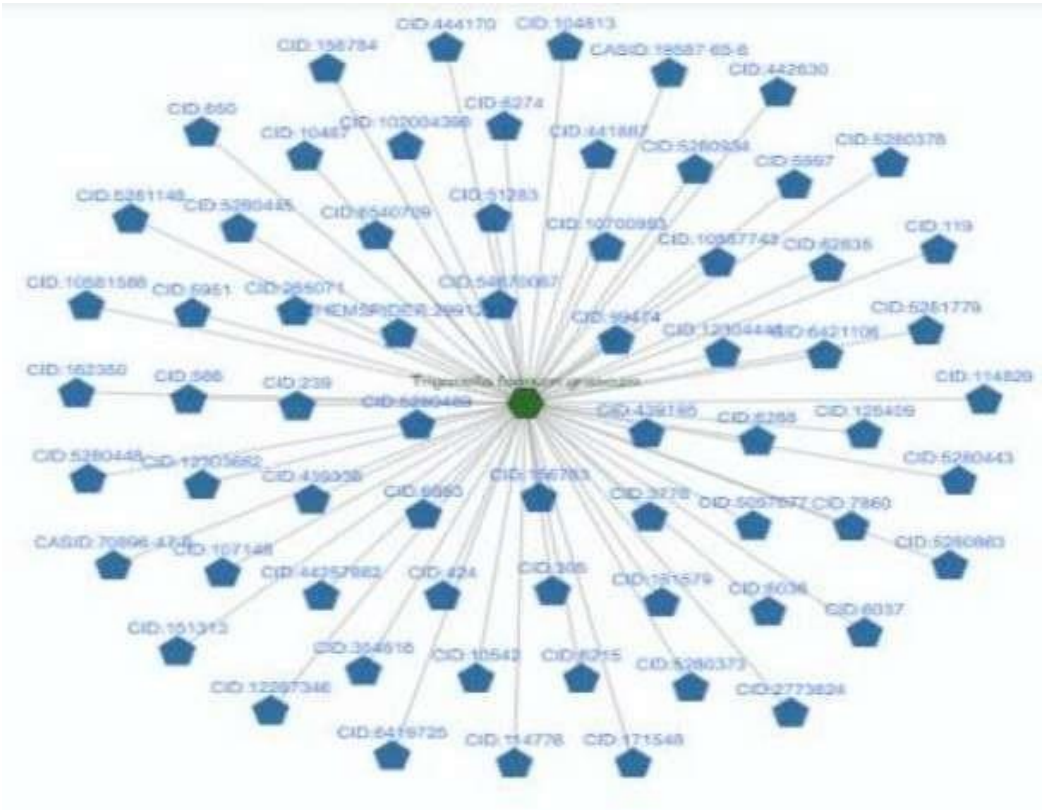


Fig. 4.25 Cytoscape network for medicinal plant (*Moringa oliefera*) and its associated phytochemicals.

4.13 Different filters used for screening purposes

All top 6 compounds there docked were passing through the filters that were taken i.e. lipinski, Ghose, Veber, Egan and Muegge These filters were used for showing Drug likeliness properties of the compounds used and their effectiveness.

4.13.1 Filters used for *Moringa oleifera*

S.No	COMPOUND OF *Moringa oleifera*	Lipinski's filter	Ghose filter	Veber filter	Egan filter	Muegge filter
1.	9-Octadecenoic acid-1,2,3-propanetrieyl ester	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
2.	3-Ethyl-2,4-dimethyl-pentane	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
3.	-(+)-Ascorbic acid 2,6dihexa-decanoate	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
4.	1-Hexadecanol	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
5.	3,5-bis(1,1-dimethylethyl)-phenol	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**

Table 4.12 Compounds of *Moringa oleifera* passing through different drug likeliness filters

4.13.2 Filters used for *Nyctanthes arbor-tristis*

S.No	COMPOUND OF *Nyctanthes arbor-tristis*	Lipinski filter	Ghose filter	Veber filter	Egan filter	Muegge filter
1.	Astragalin	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
2.	Nicotiflorin	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
3.	Nyctanthic acid	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
4.	Friedeline	**Yes**	**Yes**	**Yes**	**Yes**	**No**
5.	Lupeol	**Yes**	**Yes**	**No**	**Yes**	**Yes**

Table 4.13 Compounds of *Nyctanthes arbor-tristis* passing through different drug likeliness filters

CHAPTER 5- CONCLUSION

The identification of *Moringa oleifera* represents a notable breakthrough in the realm of alternative natural medicine, offering potential solutions for numerous diseases caused by pathogens. This is substantiated by the potent antibacterial properties exhibited by the leaf extract, as well as the presence of secondary metabolites. As a result, the plant holds promise for addressing various conditions including typhoid fever, diarrhea, gastric ulcers, tumors, postmenopausal syndrome, arteriosclerosis, blood sugar regulation, gastrointestinal disorders, cancer, diabetes, and more.

In this project, we have concluded that the compound 9-Octadecenoic acid-1, 2, 3-propanetrieyl ester serve as potential inhibitor of DJ-1 protein. In vitro and in silico based approach has proven to be useful in finding potent inhibitor. All the results were also validated and proved that this compound could be a potent candidate to treat Parkinson’s disease.

REFERENCES

[1] A. J. Lees, J. Hardy, and T. Revesz, “Parkinson’s disease,” Lancet, vol. 373, no. 9680, pp. 2055–2066, 2009, doi: 10.1016/S0140-6736(09)60492-X.

[2] M. L. Choi, R. Meleckyte, D. Little, A. Y. Abramov, R. Patani, and S. Gandhi, “P-385- Overproduction of reactive oxygen species is the primary pathological event related to neuronal cell death in iPSC derived neurons from patients with familial Parkinson’s disease,” Free Radic. Biol. Med., vol. 120, p. S162, 2018, doi: https://doi.org/10.1016/j.freeradbiomed.2018.04.532.

[3] P. Piyush and N. P. Jivani, “Effects of hydroalcoholic extract of Parkinsonia aculeate L. seeds and Ananas comosus fruits on rotenone induced Parkinson’s disease in rats,” Int. J. Res. Dev. Pharm. Life Sci., vol. 4, no. 4, pp. 1647–1653, 2015.

[4] S. Piramanayagam and S. Selvaraj, “A review on factors causing parkinson’s syndrome,” MOJ Proteomics Bioinforma., vol. 7, no. 4, pp. 257–261, 2018, doi: 10.15406/mojpb.2018.07.00243.

[5] W. H. Oertel, “Recent advances in treating Parkinson’s disease,” F1000Research, vol. 6, pp. 1–14, 2017, doi: 10.12688/f1000research.10100.1.

[6] K. M. Doherty et al., “Parkin disease: A clinicopathologic entity?,” JAMA Neurol., vol. 70, no. 5, pp. 571–579, 2013, doi: 10.1001/jamaneurol.2013.172.

[7] W. G. Meissner et al., “Priorities in Parkinson’s disease research,” Nat. Rev. Drug Discov., vol. 10, no. 5, pp. 377–393, 2011, doi: 10.1038/nrd3430.

[8] S. Chandrasekaran and D. Bonchev, “A network view on Parkinson’s disease,” Comput. Struct. Biotechnol. J., vol. 7, pp. e201304004–e201304004, Jul. 2013, doi: 10.5936/csbj.201304004.

[9] H. H. Fernandez, “2015 Update on Parkinson disease.,” Cleve. Clin. J. Med., vol. 82, no. 9, pp. 563–568, Sep. 2015, doi: 10.3949/ccjm.82gr.15004.

[10] L. V Kalia and A. E. Lang, “Parkinson’s disease.,” Lancet (London, England), vol. 386, no. 9996, pp. 896–912, Aug. 2015, doi: 10.1016/S0140-6736(14)61393-3.

[11] N. Papagiannakis et al., “Alpha-synuclein dimerization in erythrocytes of patients with genetic and non-genetic forms of Parkinson’s Disease,” Neurosci. Lett., vol. 672, pp. 49145–149, 2018, doi: 10.1016/j.neulet.2017.11.012.

[12] B. R. Bloem, M. S. Okun, and C. Klein, “Parkinson’s disease,” Lancet, May 2021, doi:10.1016/S0140-6736(21)00218-X.

[13] C. M. Lill, “Genetics of Parkinson’s disease.,” Mol. Cell. Probes, vol. 30, no. 6, pp.386–396, Dec. 2016, doi: 10.1016/j.mcp.2016.11.001.

[14] S. K. Das et al., “Epidemiology of Parkinson disease in the city of Kolkata, India: a community-based study.,” Neurology, vol. 75, no. 15, pp. 1362–1369, Oct. 2010, doi:10.1212/WNL.0b013e3181f735a7.

[15] H. Apaydin, J. E. Ahlskog, J. E. Parisi, B. F. Boeve, and D. W. Dickson, “Parkinson disease neuropathology: later-developing dementia and loss of the Levodopa response.,” Arch. Neurol., vol. 59, no. 1, pp. 102–112, Jan. 2002, doi:10.1001/archneur.59.1.102.

[16] J. Jankovic and W. Poewe, “Therapies in Parkinson’s disease.,” Curr. Opin. Neurol., vol. 25, no. 4, pp. 433–447, Aug. 2012, doi: 10.1097/WCO.0b013e3283542fc2.

[17] J. M. Brotchie, J. Lee, and K. Venderova, “Levodopa-induced dyskinesia in Parkinson’s disease,” J. Neural Transm., vol. 112, no. 3, pp. 359–391, 2005, doi:10.1007/s00702-004-0251-7.

[18] T. G. Beach et al., “Multi-organ distribution of phosphorylated α-synuclein histopathology in subjects with Lewy body disorders,” Acta Neuropathol., vol. 119, no. 6, pp. 689–702, 2010, doi: 10.1007/s00401-010-0664-3.

[19] A. Schrag and R. N. Taddei, “Depression and Anxiety in Parkinson’s Disease.,” Int.Rev. Neurobiol., vol. 133, pp. 623–655, 2017, doi: 10.1016/bs.irn.2017.05.024.

[20] J. Pagonabarraga and J. Kulisevsky, “Apathy in Parkinson’s Disease.,” Int. Rev. Neurobiol., vol. 133, pp. 657–678, 2017, doi: 10.1016/bs.irn.2017.05.025.

[21] M. A. Hely, W. G. J. Reid, M. A. Adena, G. M. Halliday, and J. G. L. Morris, “The Sydney multicenter study of Parkinson’s disease: the inevitability of dementia at

20 years.," Mov. Disord., vol. 23, no. 6, pp. 837–844, Apr. 2008, doi:10.1002/mds.21956.

[22] K. A. Jellinger, "Neuropathological spectrum of synucleinopathies.," Mov. Disord.,50 vol. 18 Suppl 6, pp. S2-12, Sep. 2003, doi: 10.1002/mds.10557.

[23] V. N. Uversky, "The roles of intrinsic disorder-based liquid-liquid phase transitions in the 'Dr. Jekyll–Mr. Hyde' behavior of proteins involved in amyotrophic lateral sclerosis and frontotemporal lobar degeneration," Autophagy, vol. 13, no. 12, pp.2115–2162, 2017, doi: 10.1080/15548627.2017.1384889.

[24] L. C. Kwan and T. L. Whitehill, "Perception of speech by individuals with Parkinson's disease: A review," Parkinsons. Dis., vol. 2011, 2011, doi: 10.4061/2011/389767.

[25] A. Cardinale, R. Chiesa, and M. Sierks, "Protein misfolding and neurodegenerative diseases.," Int. J. Cell Biol., vol. 2014, p. 217371, 2014, doi: 10.1155/2014/217371.

[26] Al-Snafi, A. E. (2018) 'Chemical constituents, pharmacological effects and therapeutic importance of Hibiscus rosa-sinensis-A review', IOSR Journal Of Pharmacy, 8(7), pp. 101–119.

[27] Calabrò, S. (2015) 'Plant secondary metabolites', Rumen Microbiology: From Evolution to Revolution, (October), pp. 153–159. doi: 10.1007/978-81-322-2401-3_11.

[28] Cazzonelli, C. I. (2011) 'Carotenoids in nature: Insights from plants and beyond', Functional Plant Biology, 38(11), pp. 833–847. doi: 10.1071/FP11192.

[29] Chitra Jain; Shivani Khatana; Rekha Vijayvergia (2019) 'Bioactivity of secondary metabolites of various plants: A review', International Journal of Pharmaceutical Sciences and Research, 10(2), pp. 494–504. doi: 10.13040/IJPSR.0975-8232.10(2).494-04.

[30] Das, S., Krishi Viswavidyalaya, C. and Sharangi, A. B. (2017) 'Madagascar periwinkle (Catharanthus roseus L.): Diverse medicinal and therapeutic benefits to humankind', ~ 1695 ~ Journal of Pharmacognosy and Phytochemistry, 6(5), pp. 1695–1701.

[31] Domínguez, H. (2013) ‘Algae as a source of biologically active ingredients for the formulation of functional foods and nutraceuticals’, Functional Ingredients from Algae for Foods and Nutraceuticals, pp. 1–19. doi: 10.1533/9780857098689.1.

[32] Espín, J. C., González-Sarrías, A. and Tomás-Barberán, F. A. (2017) ‘The gut microbiota: A key factor in the therapeutic effects of (poly)phenols’, Biochemical Pharmacology, 139, pp. 82–93.doi: 10.1016/j.bcp.2017.04.033.

[33] Asare GA, Gyan B, Bugyei K, Adjei S, Mahama R, Addo P, Otu-Nyarko L, Wiredu E.K, Nyarko A. (2012). Toxicity potentials of the nutraceutical Moringa oleifera at supra-supplementation levels. Journal of Ethnopharmacology, 139: 265–272.

[34] Awodele O., Oreagbe I.A., Odoma S., da Silva J.A.T., Osunkalu V.O. (2012). Toxicological evaluationof the aqueous leaf extract of Moringa oleifera Lam.(Moringaceae). J Ethnopharmacol, 139: 300– 306.

[35] Bakre A.G., Aderibigbe A.O., Ademowo O.G. (2013). Studies on neuropharmacological profile of ethanol extract of Moringa oleifera leaves in mice. J Ethnopharmacol, 149: 783–789.

[36] Bamishaiye E. I., Olayemi F. F., Awagu E. F., and BamshaiyeO. M. (2011). Proximate and phytochemical composition of Moringa oleiferaleaves at three stages of maturation. Advance Journal of Food Science and Technology, 3(4): 233–237.

[37] Parron C., Poucet B. and Save E. (2006). Cooperation between the hippocampus and the entorhinal cortex in spatial memory: A disconnection study. Behavioural Brain Research, 170, 99–109.

[38] Potterat O. (1997). Antioxidant and free radical scavengers of natural origin. Current Organic Chemistry, 1: 415–440.

[39] Singh B.N., Singh B.R., Singh R.L., Prakash D., Dhakarey, R., Upadhyay G., Singh H.B.(2009). Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of Moringa oleifera. Food Chem Toxicol 47:109–116.

[40] Singh R., RajasreeP.H. andSankar C. (2012). Suppression of neurofilament degradation by protease inhibitors extracted from Moringa Oleifa leaves in experimental spinal cord injury Journal of Pharmacy Research,5(10): 4987-4990.

[41] Spencer J.P.E.,Whiteman, M., Jenner, P., Halliwell, B. (2002). 5-S-cysteinyl-conjugates of catecholamines induce cell damage, extensive DNA base modification and increases incaspase-3 activity in neurons. J. Neurochem.,81: 122–129.

[42] Sreelatha S., Padma P.R. (2009). Antioxidant activity and total phenolic content of Moringa oleifera leaves at two stages of maturity. Plant Foods Hum Nutr., 64: 303–311.

[43] Stohs S.J. and Hartman M.J. (2015). Review of the Safety and Efficacy of Moringa oleifera. Phytotherapy Research, 29: 796–804.

[44] Sutalangka C., Wattanathorn J., Muchimapura S., and Thukham-mee W. (2013). Moringa oleifera Mitigates Memory Impairment and Neurodegeneration in Animal Model of Age-Related Dementia. Oxidative Medicine and Cellular Longevity, Volume 2013, Article ID 695936, 9 pages

[45] Tsao R., Akhtar M.H. (2005). Nutraceuticals and functional foods. I. Currenttrend in phytochemical antioxidant research. Journal of Food, Agricultureand Environment, 3: 10–17.

[46] Vauzour D., VafeiadouK., SpencerJ.P.E. (2007). Inhibition of the formation of the neurotoxin5-Scysteinyl-dopamine by polyphenols. Biochem. Biophys. Res. Commun., 362: 340–346.

[47] Smita Parekh, Anjali Soni, Nyctanthes arbor-tristis: Comprehensive review on its pharmacological, antioxidant and anticancer activities, Journal of Applied Biology and Biotechnology,2020:8(01):95-104.

[48] Anowar Hussain, Anand Ramteke, Flower extract of Nyctanthes arbor-tristis modulates glutathione level in hydrogen peroxide treated lymphocytes, Pharmacognosy Res,2012:4(4):230-233. DOI10.4103/0974-8490.102272.

[49] Milind M Meshram, Swatee B Rangari, Shashank B Kshirsagar, Suraj Gajbhiye, Madhurim R Trivedi et al. Nyctanthes arbor-triris a herbal panacea, International Journal of Pharmaceutical Science and Research,2012, Page No: 2432-2440, DOIhttp://dx.doi.org/10.13040/IJPSR.0975-8323.3(8).2432- 40.

[50] Laxmi Verma, Vaibhav Tamrakar, Narul Haque, Anil Kumar. Antifungal activity of different parts of Nyctanthes arbor-tristis Linn. (Parijat) against clinical pathogens, Shodh Darpan (Special Issue),2016:1(4). ISSN No. 2454-1516.

[51] Vidyavati Hiremath, Hiremath BS, Mohapatra S, Arun Kumar Das. Literary review of Parijata (Nyctanthus arbor-tristis Linn.) An herbal medicament with special reference to Ayurveda and Botanical literature, Biomed Pharmacol J, 2016, 9(3), DOIhttps://dx.doi.org/10.13005/bpj/1043.

[52] Reema Srivastava, Deepali Trivedi, Gauri Shukla, Pankaj Srivastava, Nyctanthus arbor-tristis: A wonder Indian herbal drug needs healthcare attention, Biomedical Journal of Scientific and technical research, published on June 11, 2018, ISSN: 2574-1241, DOI:10.26717/BJStr.2018.05.001199.

[53] Champa Rani, Sunaina Chawla, Manisha Mangal, AK Mangal, Subhash Kajla, AK Dhawan et al, Nyctanthes abor-tristis Linn. (Night Jasmine): A sacred ornamental plant with immense medicinal potential, Indian Journal of Traditional Knowledge,2011:11(3):427-435.

[54] Jadhav VS, Ghawate VB. Evaluation of combined wound healing activity of ethanolic extracts of leaves of Murraya koenigii and Nyctanthes arbortristis on rats, Drug Invention Today,2017:9:2. ISSN: 0975-7619.

[55] David M Arnoff, Eric G Neilson. Antipyretics: Mechanisms of action and clinical use in fever suppression, The American Journal of Medicine,2001:111(4):304-15. DOI: 10.1016/s0002- 9343(01)00834-8

[56] Mrunal K Shirsat, Gupta SK, Bele D, Vaya R, Dwivedi, Goyal S et al. Antipyretic effect of whole plant extract of Nyctanthes arbortristis Linn., Research and Review: A Journal of Pharmaceutical Science,2011:2(1):7-10.

[57] Shalini Tripathi PK, Tripathi M, Vijay kumar, Ch V Rao, Singh PN. Anxiolytic activity of leaf extract of Nyctanthes arbor-tristis in experimental rats, Pharmacologyonline,2010:2:457-463.

[58] Abou-Sleiman P. M., Healy D. G., and Wood N. W. (2004) Causes of Parkinson's disease: genetics of DJ-1. *Cell Tissue Res.* **318**, 185–188.

[59] Abou-Sleiman P. M., Healy D. G., Quinn N., Lees A. J., and Wood N. W. (2003) The role of pathogenic DJ-1 mutations in Parkinson's disease. *Ann. Neurol.* **54**, 283–286.

[60] Annesi G., Savettieri G., Pugliese P., D'Amelio M., Tarantino P., Ragonese P., et al. (2005) DJ-1 mutations and parkinsonism-dementia-amyo trophic lateral sclerosis complex. *Ann Neurol.* **58**, 803–807.

[61] Bader V., Ran Zhu X., Lubbert H., and Stichel C. C. (2005) Expression of DJ-1 in the adult mouse CNS. *Brain Res.* **1041**, 102–111.

[62] Bandyopadhyay S. and Cookson M. R. (2004) Evolutionary and functional relationships within the DJ-1 super-family. *BMC Evol. Biol.* **4**, 6.

[63] Chen L., Cagniard B., Mathews T., Jones S., Koh H. C., Ding Y., et al. (2005) Age-dependent motor deficits and dopaminergic dysfunction in DJ-1 null mice. *J. Biol. Chem.* **280**, 21,418–21,426.

[64] Clark L. N., Afridi S., Mejia-Santana H., Harris J., Louis E. D., Cote L. J., et al. (2004) Analysis of an early-onset Parkinson's disease cohort for DJ-1 mutations. *Mov. Disord.* **19**, 796–800.

[65] Dekker M. C., Eshuis S. A., Maguire R. P., Veenma-van der Duijn L., Pruim J., Snijders P. J., et al. (2004) PET neuroimaging and mutations in the DJ-1 gene. *J. Neural Transm.* **111**, 1575–1581.

[66] Dawson T. M. and Dawson V. L. (2003) Molecular pathways of neurodegeneration in Parkinson's disease. *Science* **302**, 819–822.

[67] Gasser T., Müller-Myhsok B., Wszolek Z. K., et al. (1998) A susceptibility locus for Parkinson's disease maps to chromosome 2p13. *Nat. Genet.* **18**, 262–265.

[68] Good P. F., Hsu A., Werner P., Perl D. P., and Olanow C. W. (1998) Protein nitration in Parkinson's disease. *J. Neuropathol. Exp. Neurol.* **57**, 338–342.

[69] Hague S., Rogaeva E., Hernandez D., Gulick C., Singleton A., Hanson M., et al. (2003) Early-onset Parkinson's disease caused by a compound heterozygous DJ-1 mutation. *Ann. Neurol.* **54**, 271–274.

[70] Hicks A. A., Petursson H., Jonsson T., Stefansson H., Johannsdottir H. S., Sainz J., et al. (2002) A susceptibility gene for late-onset idiopathic Parkinson's disease. *Ann. Neurol.* **52**, 549–555.

[71] Jin J., Meredith G. E., Chen L., Zhou Y., Xu J., Shie F. S., et al. (2005) Quantitative proteomic analysis of mitochondrial proteins: relevance to Lewy body formation and Parkinson's disease. *Brain Res. Mol. Brain Res.* **134**, 119–138.

[72] Kim R. H., Peters M., Jang Y., Shi W., Pintilie M., Fletcher G. C. et al. (2005a) DJ-1, a novel regulator of the tumor suppressor PTEN. *Cancer Cell* **7**, 263–273.

[73] Le W. D., Xu P., Jankovic J., Jiang H., Appel S. H., Smith R. G., and Vassilatis D. K. (2003). Mutations in NR4A2 associated with familial Parkinson disease. *Nat. Genet* **33**, 85–89.

[74] Lee S. J., Kim S. J., Kim I. K., Ko J., Jeong C. S., Kim G. H., et al. (2003) Crystal structures of human DJ-1 and Escherichia coli Hsp31, which share an evolutionarily conserved domain. *J. Biol. Chem.* **278**, 44,552–44,559.

[75] Shinbo Y., Taira T., Niki T., Iguchi-Ariga S. M., and Ariga H. (2005) DJ-1 restores p53 transcription activity inhibited by Topors/p53BP3. *Int. J. Oncol.* **26**, 641–648.

[77] Singleton A. B., Farrer M., Johnson J., Singleton A. B., Farrer M., Johnson J., et al. (2003) α-Synuclein locus triplication causes Parkinson's disease. *Science* **302**, 841–844.

[78] Taira T., Takahashi K., Kitagawa R., Iguchi-Ariga S. M., and Ariga H. (2001) Molecular cloning of human and mouse DJ-1 genes and identification of Sp1-dependent activation of the human DJ-1 promoter. *Gene* **263**, 285–292.

[79] Van Duijin C. M., Dekker M. C., Bonifati V., Galjaard R. J., Houwing-Duistermaat J. J., Snijders P. J., et al. (2001) Park7, a novel locus for autosomal recessive early-onset parkinsonism, on chromosome 1p36. *Am. J. Hum. Genet.* **69**, 629–634.

[80] Zimprich A., Biskup S., Leitner P., Lichtner P., Farrer M., Lincoln S., et al. (2004) Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron* **44**, 601–607.

turnitin

Similarity Report ID: oid:27535:36231896

PAPER NAME
shruti mtech thesis final - Copy.docx

AUTHOR
shruti

WORD COUNT
10825 Words

CHARACTER COUNT
61481 Characters

PAGE COUNT
66 Pages

FILE SIZE
3.4MB

SUBMISSION DATE
May 26, 2023 12:30 PM GMT+5:30

REPORT DATE
May 26, 2023 12:31 PM GMT+5:30

8% Overall Similarity

The combined total of all matches, including overlapping sources, for each database

- 4% Internet database
- 3% Publications database
- Crossref database
- Crossref Posted Content database
- 4% Submitted Works database

Excluded from Similarity Report

- Bibliographic material
- Quoted material
- Small Matches (Less then 8 words)

Summary

75

turnitin

Similarity Report ID: oid:27535:36231896

8% Overall Similarity

Top sources found in the following databases:

- 4% Internet database
- Crossref database
- 4% Submitted Works database
- 3% Publications database
- Crossref Posted Content database

TOP SOURCES

The sources with the highest number of matches within the submission. Overlapping sources will not be displayed.

1	A. Saravanan, R. Jayasree, P. Senthil Kumar, Sunita Varjani, R.V. Hema... Crossref	1%
2	bnrc.springeropen.com Internet	<1%
3	Esra Bilen, Ümmühan Özdemir Özmen, Servet Çete, Saliha Alyar, Ahme... Crossref	<1%
4	dost.hochiminhcity.gov.vn Internet	<1%
5	Monash University on 2017-09-20 Submitted works	<1%
6	researchsquare.com Internet	<1%
7	University of the Pacific on 2023-05-22 Submitted works	<1%
8	tkmcas.ac.in Internet	<1%

Sources overview

9	researchgate.net Internet	<1%
10	University of Sheffield on 2010-08-26 Submitted works	<1%
11	vdocuments.mx Internet	<1%
12	HCUC on 2023-05-17 Submitted works	<1%
13	academic.oup.com Internet	<1%
14	ao.um5s.ac.ma Internet	<1%
15	tandfonline.com Internet	<1%
16	Anindita Mitra, Ria Biswas, Angshuman Bagchi, Rita Ghosh. "Insight int... Crossref	<1%
17	University of Stellenbosch, South Africa on 2020-02-07 Submitted works	<1%
18	fenix.tecnico.ulisboa.pt Internet	<1%
19	docecity.com Internet	<1%
20	mdpi.com Internet	<1%

Sources overview

21	Higher Education Commission Pakistan on 2015-06-16 Submitted works	<1%
22	Higher Education Commission Pakistan on 2022-10-16 Submitted works	<1%
23	Oluwagbenga Adeogun, Adedotun Adekunle, Anofi Ashafa. "Chemical c... Crossref	<1%
24	scholars.hkbu.edu.hk Internet	<1%
25	Higher Education Commission Pakistan on 2022-11-19 Submitted works	<1%
26	Saginaw Valley State University on 2012-04-13 Submitted works	<1%
27	University of Greenwich on 2022-04-25 Submitted works	<1%
28	innovareacademics.in Internet	<1%
29	Dinesh, Murugesan, Selvaraj Mohana Roopan, and Chinnadurai Imman... Crossref	<1%
30	KLE Academy of Higher Education and Research on 2023-05-12 Submitted works	<1%
31	Midlands State University on 2019-10-29 Submitted works	<1%
32	National Institute of Technology, Rourkela on 2014-05-27 Submitted works	<1%

Sources overview

33	Universiti Teknologi MARA on 2019-12-07 Submitted works	<1%
34	jamanetwork.com Internet	<1%
35	jocpr.com Internet	<1%
36	Higher Education Commission Pakistan on 2013-08-03 Submitted works	<1%
37	Mansoura University on 2019-09-09 Submitted works	<1%
38	Recent Trends in Biotechnology and Therapeutic Applications of Medi... Crossref	<1%
39	Ritesh Agrawal, Pratima Jain, Subodh Narayan Dikshit, Radhe Shyam B... Crossref	<1%
40	The Scientific & Technological Research Council of Turkey (TUBITAK) ... Submitted works	<1%
41	Universiti Malaysia Pahang on 2021-01-28 Submitted works	<1%
42	University College London on 2021-01-13 Submitted works	<1%
43	University of California, Los Angeles on 2004-11-11 Submitted works	<1%
44	University of Witwatersrand on 2022-11-16 Submitted works	<1%

Sources overview

45	flex.flinders.edu.au Internet	<1%
46	journaljpri.com Internet	<1%
47	nopr.niscpr.res.in Internet	<1%

PUBLICATION

Date: 26 May 2023
To: "Navneeta Bharadvaja" navneetab@dce.ac.in
cc: manojkdhar@rediffmail.com, "Shruti ." shruti00011199@gmail.com
From: "Vegetos (VTOS)" Prasanth.Dhandapani@springer.com
Subject: Your Submission VTOS-D-22-00650R3

Dear Dr. Bharadvaja,

We are pleased to inform you that your manuscript, "Biotechnology based strategies for secondary metabolites enhancement: a review", has been accepted for publication in Vegetos.

You will receive an e-mail in due course regarding the production process.

Please remember to quote the manuscript number, VTOS-D-22-00650R3, whenever inquiring about your manuscript.

With kind regards,
Subodh Bhatnagar, Ph.D.
Editor in Chief
Vegetos

Comments to the author (if any):

****

Please note that this journal is a Transformative Journal (TJ). Authors may publish their research with us through the traditional subscription access route or make their paper immediately open access through payment of an article-processing charge (APC). Authors will not be required to make a final decision about access to their article until it has been accepted.

Authors may need to take specific actions to achieve compliance with funder and institutional open access mandates. If your research is supported by a funder that requires immediate open access (e.g. according to Plan S principles) then you should select the gold OA route, and we will direct you to the compliant route where possible. For authors selecting the subscription publication route our standard licensing terms will need to be accepted, including our self-archiving policies. Those standard licensing terms will supersede any other terms that the author or any third party may assert apply to any version of the manuscript.

Find out more about compliance: https://www.springernature.com/gp/open-research/funding/policy-compliance-faqs

This letter contains confidential information, is for your own use, and should not be forwarded to third parties.

Electronic ISSN
2229-4473

Abstracted and indexed in

Baidu	Google Scholar	ProQuest-ExLibris Summon
CAB Abstracts	IFIS Publishing	SCImago
CLOCKSS	Japanese Science and Technology Agency (JST)	SCOPUS
CNKI	Meta	TD Net Discovery Service
CNPIEC	Naver	UGC-CARE List (India)
Dimensions	OCLC WorldCat Discovery Service	Wanfang
EBSCO Discovery Service	Portico	
EMBiology	ProQuest-ExLibris Primo	

Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Abstracting & Indexing

Letters in Applied NanoBioScience is covered by the following databases and archives:

- SCOPUS

Scopus Title Evaluation Team <titlesuggestion@scopus.com>
Către:

Title: Letters in Applied NanoBioScience
ISSN / E-ISSN: / 2284-6808
Publisher: AMG Transcend Association

Dear Dr. Alexandru Mihai Grumezescu,

Congratulations, your Letters in Applied NanoBioScience has been accepted for Scopus.

The Scopus Content Selection & Advisory Board (CSAB) has reviewed your application a

- Crossref
- Scilit (MDPI)

Manuscript ID	**lianbs-2944**
Manuscript Status	Paper accepted
Title	In silico approach to identify key active plant derived natural compounds in Parkinson's disease
Journal	Letters in Applied NanoBioScience
Type	Article
License	n/a

Abstract Parkinson's disease is the condition which is characterized by loss of memory, dopamine level, oxidative stress and several other comprehensible impairments. DJ-1 protein plays a vital role in the treatment of this disease due to its chaperon activity. Plumbago zeylanica have been reported in the past studies to protect neurons and motor activity in neurodegenerative disease. The present study deals with different selected phytochemicals of the plant since they have vast medicinal and therapeutic activities. Computational studies of total six compounds were carried out with the targeted protein having PDB ID: 4ZGG via molecular docking. Potential inhibitory effect was noticed with selected six compounds but plumbagin has shown the best binding energy using docking. The results were further validated using online server patchDock. Ligands were passed through different filters and ADMET analysis was done. Target was also validated via ramachandran plot. Additionally, the best docked compound having best binding energy was also compared with the conventional drug of this disease i.e. Levodopa and the phytocompound was found to be more effective than it. This study thus provides purposeful insights that Plumbagin could be the promising candidate to combat this disease.

Authors	Shruti . , Navneeta Bharadvaja *
Author Emails	shruti00011199@gmail.com, navneetab@dce.ac.in
Submission Date	15 April 2023