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Investigating Medicinal Plants as Therapeutic Agents for Parkinson's Disease: Qualitative Analysis and target molecule identification

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I Manju, Roll Number: 2K21/MSCBIO/24 student of M.Sc. Biotechnology, hereby declare that the project dissertation titled - "**Investigating Medicinal Plants as Therapeutic Agents for Parkinson's Disease: Qualitative Analysis and target molecule identification"** which is submitted by me to the Department of Biotechnology**,** Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is original and not copied from any source with 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.

The details of the review article and conference paper are given below:

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Name of Authors: Manju, Devansh Sharma and Navneeta Bharadvaja*

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CERTIFICATE

I hereby certify that the Project Dissertation titled "**Investigating Medicinal Plants as Therapeutic Agents for Parkinson's Disease: Qualitative Analysis and target molecule identification"** which is submitted by Manju, Roll No.: 2K21/MSCBIO/24, Department of Biotechnology**,** Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is a record of the project work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to the University or elsewhere.

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ABSTRACT

The gradual loss of dopaminergic neurons in the substantia nigra area of the brain is a hallmark of Parkinson's disease (PD), a neurodegenerative condition. The investigation of alternative therapy alternatives is necessary since the therapeutic choices for PD that are now available have limits in terms of efficacy and side effects. Traditional medicine has used medicinal plants to treat a wide range of problems, and there is growing interest in using them as potential innovative therapeutic agents for PD. According to the study's methodology, a qualitative analysis of the selected medicinal plant varieties was carried out with an emphasis on their phytochemical composition and bioactive components. Additionally, the study employed in-silico techniques to identify potential target substances in extracts from plants that could be helpful as PD treatments. Computing tools have been utilised to predict the connection between the targeted phytochemicals and certain biochemical targets connected to the diagnosis of PD. The analysis of the binding affinities and interaction patterns between the phytochemicals and the target molecules has shown the importance of molecular docking. These interactions shed important light on the potential therapeutic applications of the recently discovered phytochemicals as well as their propensity to alter key PD-related pathways. The In silico research sheds light on the possible molecular targets and mechanisms of action of the discovered phytochemicals, while the qualitative examination offers a thorough profile of the phytochemical composition of the chosen plants. In order to validate the therapeutic efficacy of medicinal plants and their phytochemical elements in the cure of PD, the findings of this study could open opportunities for further experimental and eventually, clinical trials. This study will examine the possibility of a number of medicinal plants, including *Petunia hybrida, Plectranthus scutellatioides, Alcea rosea, Dahlia pinnata,* and *Chrysanthemum morifolium*, in an effort to discover novel therapies for PD.

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CHAPTER 1

1.1 INTRODUCTION

People have recently become increasingly conscious of the significance of employing plants for healing, and the world of plants has a great collection of possible medicines [1]. Plant-based medications are simple to get, reasonably priced, environmentally friendly, safe to use, and effective with little adverse effects. In order to identify novel medications that are effective against cancer, infections, and liver issues, scientists are currently examining some plants that have been utilised by humans for thousands of years to treat a wide range of diseases. The World Health Organisation (WHO) claims, the best source for many different types of medications is medicinal plants. About 80% of individuals in affluent nations take drugs derived from medicinal plants [2].

Around the world, natural remedies made from plants are still in high demand [3]. Bioactive substances found in medicinal plants are useful for treating a range of human ailments and promoting recovery. A medicinal plant is one that has components or precursors have potential for therapeutic application. Secondary metabolites and essential oils are widely distributed in these plants. However, it's important to keep in mind that phytochemicals, which are naturally occurring chemical compounds in plants, offer a range of protective properties despite not being necessary for nutrition, even though the primary focus of using medicinal plants is frequently on treating existing disorders rather than preventing them [4]. Primary and secondary metabolites are two kinds of phytochemicals that are present in plants in varying concentrations. They include flavonoids, tannins, saponins, alkaloids, and steroids and they help to give plants their flavour, colour, and fragrance. Additionally, they may be utilised to shield people against a diseases like cardiovascular disease, diabetes, arthritis, ageing, and cancer. Flavonoids, alkaloids, tannins, saponins, phenols, and other phytochemicals have anticancer, antiviral, antifungal, antidiabetic, and antibacterial effects that serve to prevent a variety of diseases [5].

The weakening of movement and motor abilities is a hallmark of PD [6]. It results from the degeneration of dopamine-producing neurons in the substantia nigra area of the

brain [7]. Examining the possible effects of plant metabolites on PD has recently received more interest.

The therapeutic herbs used in this investigation have been carefully chosen based on their potential for the discovery of novel medications as well as their historical and traditional uses in various medical systems. A wide variety of phytochemical diversity is ensured by the representation of several geographical locations and distinct plant groups. *Petunia hybrida, Plectranthus scutellarioides* (commonly known as Coleus), *Alcea rosea* (Hollyhock), *Dahlia pinnata*, and *Chrysanthemum morifolium* were the five medicinal plants selected for examining secondary metabolites. It was discovered that the plant extracts from these species included a wide variety of phytochemicals, such as saponins, flavanoids, tannins, terpenoids, alkaloids, and phenols. Through qualitative analysis and the identification of target molecules, this study intends to delve into the phytochemical composition and potential of medicinal plants as novel treatment agents for PD. This study uses qualitative analysis to determine the bioactive substances found in these plants and assess their potential medicinal uses. The main aim of this study is to deepen our understanding of these healing plants, lay a scientific foundation for their therapeutic benefits, and make a contribution to drug development, the recognition of conventional medicine, and possible industrial uses for their bioactive chemicals. The primary objective of this project is to use this information to help create natural chemicals derived from medicinal plants as viable and alternative therapies for PD.

1.2 PARKINSON'S DISEASE (PD)

PD, is the 2nd most prevalent neurological illness typically affects elderly people and has a big worldwide effect. Around 90% of PD cases are classified as "sporadic PD" (SPD), in which there is no obvious genetic relationship, and the remaining instances are known as "familial PD" (FPD), in which there are genetic ties to parents [8]. Resting tremors (shaking of the arms), sluggish movements, muscular stiffness, and balance issues are the four basic signs of PD [9]. Other signs include swallowing troubles, loss of smell, urine problems, sleep disorders, anxiety, sadness, and minor cognitive impairment are crucial for diagnosis. The illness is characterised by the degeneration of dopamine-producing

neurons in the substantia nigra pars compacta and the development of abnormal protein aggregates termed Lewy bodies [10]. Secondary metabolites, such as limonene, are important in PD because they may have therapeutic advantages and speed up the illness. For instance, the anti-inflammatory and antioxidant characteristics of limonene may help lessen the disease's neuroinflammation and oxidative stress.

1.3 PLANT METABOLITES

Metabolites, which include both intermediates and end products, are molecules produced during metabolism. In addition to functioning as a source of energy, metabolites also contribute to structure, operate as signals, control enzyme activity, have catalytic activity, perform protective functions, and engage in relationships with other species. The vast majority of the organic molecules that plants create are not directly related to growth and development. Plant metabolites fall into two categories: the primary and secondary. For basic metabolic functions including respiration and photosynthesis, primary metabolites are necessary [11]. During the growth period, the body produces primary metabolites, which are essential for preserving the body's physiological processes. Since they act as intermediate products in anabolic metabolism and provide the raw materials for crucial macromolecules, they are regarded as key metabolites. Amino acids, vitamins, and organic acids are a few examples of primary metabolites generated on a large scale [12].

Whereas, plant secondary metabolites have distinctive uses in medications, food additives, flavours, and different industrial materials [13]. The difficulties in producing these secondary metabolites have been overcome by the use of plant cell cultures [14]. A certain class of metabolites known as secondary metabolites are often created after the growth phase and do not directly contribute to growth, however they may help the organism survive. Nicotine and caffeine, which are recognised for their stimulant qualities, are examples of secondary metabolites [15]. Alkaloids are a type of secondary metabolites that have therapeutic uses for a wide range of diseases. The idiophase, or quiescent phase of growth, is when secondary metabolites are most commonly generated. In addition to steroids, additional examples of secondary metabolites include alkaloids,

antibiotics, phenolics, pigments, and essential oils [16]. **Fig 1.1**.Shows the information about plant metabolites along with examples.

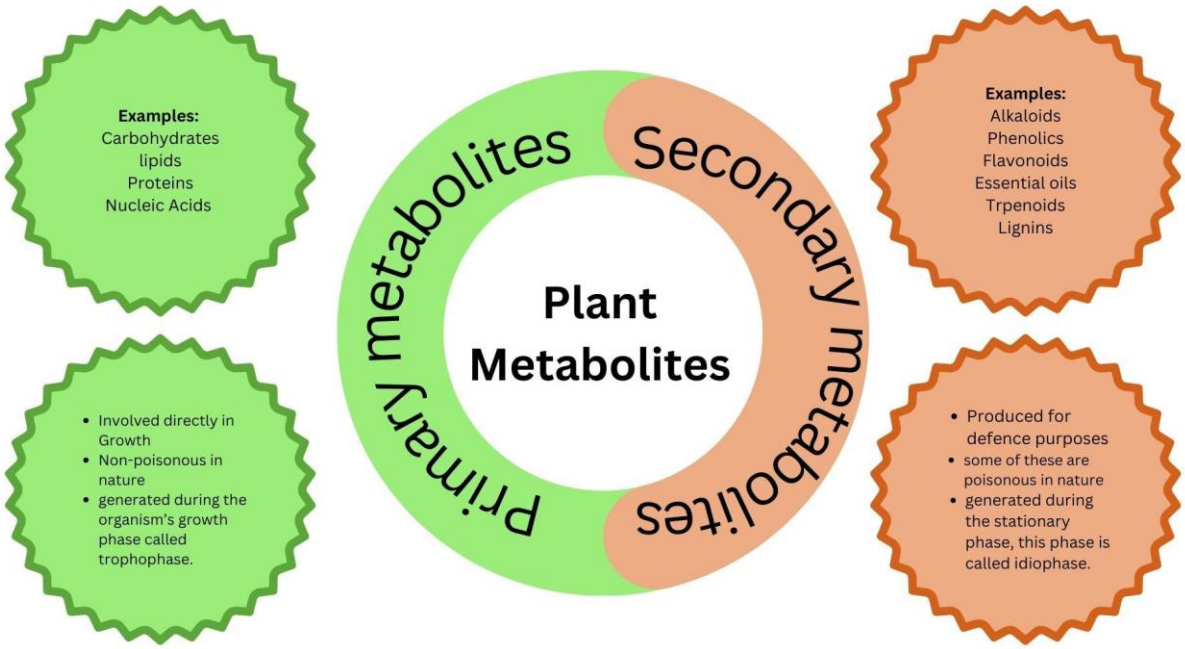


Fig 1.1. Illustration of major plant metabolite and its example.

1.4 OBJECTIVES

The objectives of this project are

1. To determine the presence of secondary metabolites in the following medicinal plants.

- *Petunia hybrida*
- *Plectranthus scutellarioides*
- *Alcea rosea (Hollyhock)*
- *Dahlia pinnata*
- *Chrysanthemum morifolium*

2. To find out the potential target genes in Parkinson's disease using phytochemicals.

1.5 ORGANIZATION OF THESIS

The following thesis title as "**Investigating Medicinal Plants as Novel Therapeutic Agents for Parkinson's Disease: Qualitative Analysis and target molecule identification"** is a reviewed information gathered from various research and review article. The primary topic of the thesis is a qualitative examination of secondary metabolites from the plants *Petunia hybrida, Plectranthus scutellarioides, Alcea rosea, Dahlia pinnata,* and *Chrysanthemum morifolium*, including tannins, terpenoids, phenols, saponins, and alkaloids. Additionally, it uses phytochemicals to characterise the probable PD target genes.

Chapter 1 represents general introduction of medicinal plant, Parkinson's disease and Plant metabolites.

Chapter 2 is a study of literature that provides a thorough understanding of secondary metabolites alkaloids, terpenoids, phenols, flavonoids, tannins, and saponins. It also covers the information about five medicinal plants *Petunia hybrida, Plectranthus scutellarioides, Alcea rosea, Dahlia pinnata,* and *Chrysanthemum morifolium* and role of already known phytochemicals in PD.

Chapter 3 is an outline of the project's suggested approach, and it includes information on how to target certain genes to treat PD as well as the procedures for first testing for secondary metabolites.

Chapter 4 includes result, discussion and conclusion.

CHAPTER 2. REVIEW OF LITERATURE

2.1 MEDICINAL PLANTS

Herbs and plants with therapeutic qualities that are good for the human or animal body are referred to as medicinal plants. They make up a substantial fraction of plant species across the world and have long been important sources of substances with potential therapeutic applications. These plants include phytochemicals, chemical substances with a variety of medicinal advantages [17]. Cancer, diabetes, cardiovascular disease, neurological illness, gastrointestinal ailments, as well as viral and bacterial infections, have all been examined for their capacity to both prevent and treat using phytochemicals found in medicinal plants. There are several ways to use medicinal plants, including utilising the entire plant or particular portions such as the barks, flowers, stems, leaves, roots, fruits, etc. [18]. For the purpose of preventing and treating ailments, they can be made into extracts or other formulations. The primary and secondary metabolites that are created spontaneously by living things are referred to as "natural products." These naturally occurring substances include molecules that are derived from plants, animals, fungi, algae, prokaryotes, and other creatures, either in their purest form or combined with other molecules [19].

Traditional medicine practices has benefited greatly from the use of medicinal herbs in many different civilizations across the world. Alkaloids, flavonoids, terpenoids, and other bioactive substances are abundant in these plants. Numerous pharmacological characteristics, including anti-inflammatory, antibacterial, antioxidant, and anticancer effects, are displayed by these substances. Five unique medicinal plants were chosen for this study's qualitative analysis: *Petunia hybrida, Plectranthus scutellarioides, Alcea rosea, Dahlia pinnata,* and *Chrysanthemum morifolium* and the medicinal benefits of these plants have been mentioned in Table 2.1.

Table 2.1. Medicinal plant species' ethnobotanical data for analysing their phytochemical composition and potential health benefits

S.No.	PLANT SPECIES	COMMON NAME	PART USED	PRESENT PHYTOCHEMICALS	MEDICINAL BENEFITS
1.	*Petunia hybrida*	*Petunia*	Leaves	Volatile benzenoids/phenylpropanoids	Alleviate respiratory issues, diminish inflammation, and encourage improved breathing
2.	*Plectranthus scutellarioides*	Coleus	leaves	Dotricontane, Hentriacontane, Nonacosane, Tritriacontane, pentatriacontane, beta-sitosterol, stigmasterol	Treat diarrhoea, malarial fever, cough, chroni asthma
3.	*Alcea rosea*	Hollyhock	Leaves	4-Methylbenzaldehyde, Cholesterol, citral, Campestrol, alpha-phellandrene,	To cure wounds, burns and cuts. Used as a herbal tea or decoction to treat internal inflammation

				beta-sitosterol, stigmasterol, limonene	
4.	*Dahlia pinnata*	*Dahlia*	Leaves	Protein, tannins, flavonoids, phenols, and saponins	Treat infected grazes, rashes and cracks in skin. Prevents constipation, help in weight loss and good for diabetes
5.	*Chrysanthemum morifolium*	Guldavari	Leaves	13-cis- α-carotene, β-carotene, lutein, zeaxanthin, trans-β-carotene, 9-cis-β-carotene, and β-cryptoxanthin.	Anti-inflammaatory, antipyretic, sedative, antiarthritic, and antihypertensive

2.1.1 Botanical name: *Petunia hybrida*

Fig 2.1. *Petunia hybrida*

Classification-

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Solanales

Family: *Solanaceae*

Genus: *Petunia*

Species: *Petunia hybrida*

Petunia hybrida is a widely grown bedding plant that is well-liked all over the world [20]. The Solanaceae family of plants includes floral plants like P*etunia*. Commonly, they have smooth, oval leaves that range in colour from dark to light green and are basic, oval in shape. *Petunia* are most notable for their lovely blooms. These blooms can be used as beautiful garnishes or in salads due to their mild flavour [21]. Depending on the species, the *Petunia* fruit is a capsule that holds somewhere between 30 and 1000 seeds. The capsule has a conical form and a diameter of 7-8 mm. The base is the broadest, while the apex is the narrowest. *Petunia* seeds are tiny, with dimensions of 0.5–0.6 mm in diameter and 0.6–0.7 mm in length. Bees, butterflies, and moths are the principal visitors to *Petunia* plants, which are mostly dependent on insect pollinators for pollination [22]. There are

several horticultural *Petunia* cultivars that may be generally divided into two categories. The compact erect varieties are ideal for summer garden beds since they reach a height of 15–25 cm (6–10 inches). The spreading, long-stemmed balcony P*etunia*, on the other hand, grows to a height of around 46 cm (18 inches) and is frequently grown in hanging baskets and window boxes.

Petunias are a highly favoured decorative crop, not just for flower gardens but also for highway landscaping. It is a popular option due to the variety of colours available. Additionally, *Petunia* is commonly used as a model plant in studies of the flavonoid synthesis route, development of flowers, and male sterility [23]. *Petunia* has also been widely used in studies incorporating genetic engineering techniques for colour alteration because of its high transformation efficiency [24]. With the help of *Petunia* transformation, scientists' knowledge of plant biochemical pathways has significantly advanced. The study of genes involved in anthocyanin synthesis, which is what gives flowers their colour, has been one of the main areas of interest in *Petunia* transformation research. The phenylpropanoid pathway, which is strongly associated with the synthesis of anthocyanins, has several genes that have been well investigated [24]. Researchers have successfully created transgenic *Petunia* plants with economic value as aesthetic crops by using methods including gene overexpression, gene suppression, and gene silencing. These strategies have allowed researchers to acquire critical insights into this crucial system. Additionally, research has shown that the primary volatile substances released by P*etunia* flowers include phenylpropanoids and benzenoids. These substances are produced from phenylalanine, an aromatic amino acid. This finding enhances our comprehension of the biochemical makeup and aroma of *Petunia* blooms [25].

2.1.2 Botanical name: *Plectranthus scutellarioides*

Fig 2.2. *Plectranthus scutellarioides*

Classification-

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Lamiales

Family: *Lamiaceae*

Genus: *Plectranthus*

Species: *Plectranthus scutellarioides*

It is a perennial plant in the Lamiaceae family with the scientific name *Plectranthus* scutellarioides [L.]. It is frequently called painted nettle. The coleus's variegated leaves, which come in a range of hues and patterns, are one of its distinguishing characteristics. Because of its charming foliage, coleus is a popular decorative plant [26]. *Plectranthus* amboinicus, *Plectranthus laxiflorus, and Plectranthus barbatus* are three species that have a long history of ethnomedicinal usage. Numerous cultivars of one species, *P. scutellarioides,* are grown for their decorative value, particularly because of their eye-catching variegated leaves [26]. *P. scutellarioides* has also been utilised in traditional

medicine by several civilizations all over the world. Additionally, P. scutellarioides has been used for its therapeutic benefits by local inhabitants for a long time in many different places [27]. Many local populations have used *P.* scutellarioides for its medical benefits. Indian state of Arunachal Pradesh uses a hot water extract of *P. scutellarioides* combined with apple juice to treat scorpion bite symptoms. Similar to this, the Tlanchinol people of Mexico utilise this plant's infusions to cure digestive disorders. Notably, *P. scutellarioides* leaves are particularly abundant in trans-rosmarinic acid (RA), a phenolic molecule with anti-inflammatory and anti-microbial activities [28]. In experimental animals, coleus extracts can lower oxidative stress indicators due to their antioxidant capabilities. *P. scutellarioides* is a hallucinogen that is utilised in Oaxaca, Mexico [29]. Coleus includes rosmarinic acid, which inhibits signalling pathways and has anti-inflammatory effects by lowering the production of inflammatory cytokines. The generation or activity of inflammatory mediators including cytokines and prostaglandins has been found to be inhibited by coleus extract. Coleus has long been used to improve digestion and treat conditions including indigestion, bloating, and constipation. It is said to have digestive stimulating and carminative qualities. The bioactive elements in coleus may potentially have effects on the heart, including possible vasodilatation and hypotensive effects. Coleus is also employed in the treatment of cough, bronchitis, and other respiratory ailments.

2.1.3 Botanical name: *Alcea rosea*

Fig 2.3. *Alcea rosea*

Classification-

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Malvales

Family: Malvaceae

Genus: *Alcea*

Species: *Alcea rosea*

The Malvaceae family includes the flowering plant *Alcea rosea*, sometimes known as hollyhock, which is mostly grown for decorative purposes [30]. It often doesn't need staking. Throughout the investigation of traditional medicine, the flowers, leaves, stem, and roots of the hollyhock plant have all been utilised for their medicinal properties [31]. Hollyhock is used both medicinally and gastronomically. The petals are especially appreciated for its dark red pigment, which is used to colour a variety of food items, including confectionery, fruit jellies and jams, sausages, non-alcoholic drinks, wine, and other dishes. The stem, leaves, roots, and flowers are also edible. The flower's red anthocyanin pigment is also used as a litmus test indicator, and the petals can be utilised

to make a brown dye [32]. Numerous advantageous qualities of *Alcea rosea* have been discovered, including antiurolithiatic, immunomodulatory, antiulcerogenic, antibacterial, anticancer, analgesic, anti-inflammatory, and febrifuge actions. Due to these qualities, it is utilized as an astringent, febrifuge, demulcent, emollient, diuretic, and anti-inflammatory agent in traditional medicine [33]. There are several conventional medical applications for *Alcea rosea*. It is used to treat bedwetting problems, reduce inflammation, and stop bleeding gums in the mouth. The flowers' demulcent, diuretic, and emollient qualities are well known. They have been applied to the management of chest issues. Additionally, a decoction produced from the blossoms is used to improve blood flow and treat ailments including haemorrhages, dysmenorrhea, and constipation [34].

2.1.4 Botanical name: *Dahlia pinnata*

Fig 2.4. *Dahlia pinnata*

Classification-

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Asterales

Family: Asteraceae

Genus: *Dahlia*

Species: *Dahlia pinnata*

A common decorative plant in the Asteraceae family is *Dahlia pinnata*, sometimes referred to as garden *Dahlia*. It is cultivated all over the world for its broad variety of flower sizes, colours, and forms [35]. This perennial herbaceous plant grows from 70 to 120 centimetres tall and has rhizomes and tuberous roots. It produces big, single, double, and multicoloured blooms. Only the inflorescence has branches; the stem is upright [36]. Sandalwood or loamy soils with a pH range of 7.0 to 8.0 and moderate fertility are preferred for *Dahlia pinnata*. The presence of anthocyanins is what gives *Dahlia* blooms their striking shades of red, purple, and black. Dahlias are quite simple to grow, and because of their protracted flowering season, they are very common in gardens.

Despite being largely grown as a decorative, *Dahlia pinnata* has been proven to have antioxidant qualities [37]. Studies have revealed that *Dahlia pinnata* contains a variety of antioxidant substances, including flavonoids, phenolic acids, and anthocyanins. These substances have the ability to suppress oxidative processes and have free radical scavenging action. Given that oxidative stress is a risk factor for chronic illnesses and the ageing process, *Dahlia pinnata* antioxidant capability have health advantages [36]. Although the traditional medical usage of *Dahlia pinnata* is not widely known, its anti-inflammatory and antioxidant characteristics may promote brain function and be beneficial for neurodegenerative diseases. Although certain members of the, The *Dahlia* genus has historically been used in traditional medicine to treat skin disorders and digestive problems, but further investigation is needed to determine *Dahlia pinnata's* particular therapeutic advantages.

2.1.5 Botanical name: *Chrysanthemum morifolium*

Fig 2.5. *Chrysanthemum morifolium*

Classification-

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Asterales

Family: Asteraceae

Genus: *Chrysanthemum*

Species: *Chrysanthemum morifolium*

Long used in traditional Chinese medicine, *Chrysanthemum morifolium* is also known as "Ju Hua" in China. Its origins are in China, from where it spread to other Asian nations which comprises Japan, Nepal, Korea, India, and Thailand. Due to the tremendous value of its flowers in both traditional and commercial settings, it is the second most costly blooming crop in the world, behind roses. *Chrysanthemum* flowers are renowned for their many health advantages, including their calming, anti-arthritic, anti-inflammatory, and

antipyretic qualities. Additionally, several *Chrysanthemum* species are widely used in agriculture, the pharmaceutical sector, and food production [38]. Significant phyto-compounds such lignans, phenolic acids, and flavonoids are present in C. morifolium [39]. In a research, goat meat patties cooked at various temperatures contained flower extract from *Chrysanthemum morifolium*. Heterocyclic amine levels in deep-fried patties were successfully decreased by the floral extracts [40]. *Chrysanthemum* tea, derived from the dried flowers of florist's *chrysanthemum*, or *Dendranthema indicum* (formerly *C. indicum*), has an extensive history of usage in traditional Chinese medicine. It is commonly employed as a natural remedy for hypertension and is reputed to be effective in treating fevers, headaches, and inflammation. [41]. *Chrysanthemum* leaves have long been employed in ethnomedicine for a number of ailments, including the treatment of fever, skin infections, eye irritation, migraines, and excessive body heat. The plant exhibits a number of biological processes, including as antidiabetic, cardioprotective, neuroprotective, anti-obesity, anti-inflammatory, anti-cancer properties. These advantageous effects are hypothesised to be caused by bioactive compounds such as steroids, volatile oils, terpenoids, flavonoids and their glycosides, polysaccharides, volatile oils, and polyphenols [42]. *Chrysanthemum* extracts have long been used to treat inflammation because they prevent the release of inflammatory mediators, which reduces pain and swelling. Additionally, because to its expectorant characteristics that aid in relieving congestion and promoting respiratory comfort, *Chrysanthemum* has been applied to treat respiratory issues in conventional medicine like coughs, colds, and bronchitis. The plant may also help detoxification processes and guard against liver damage due to its hepatoprotective characteristics, which might improve liver health.

2.2 SECONDARY METABOLITES OF PLANTS

2.2.1 Alkaloid - Alkaloids were formerly thought to be alkaline substances that were isolated from plants and had biological action [43]. Alkaloids are important drugs like emetine to cure oral intoxication and vincristine and vinblastine to treat tumours. They also contain neuroactive substances like nicotine and coffee. Due to their poisonous

qualities, alkaloids can be used by plants as defensive substances to successfully ward off diseases and predators [44]. Alkaloids, a wide class of chemical substances found in plants, are distinguished by the presence of a nitrogen atom in a heterocyclic ring. Around 12,000 different alkaloids are produced by them, and according to their carbon skeleton structures, they may be divided into a number of families. Alkaloids in plants are thought to have a variety of roles in plant physiology, including being metabolic waste products, nitrogen storage reservoirs, growth regulators, defense mechanisms against predators, and even replacements for minerals like calcium and potassium [45]. A well-known alkaloid called colchicine is known for its capacity to prevent plant cell division. Classification of alkaloids along with significance has been shown in **Fig 2.6.**

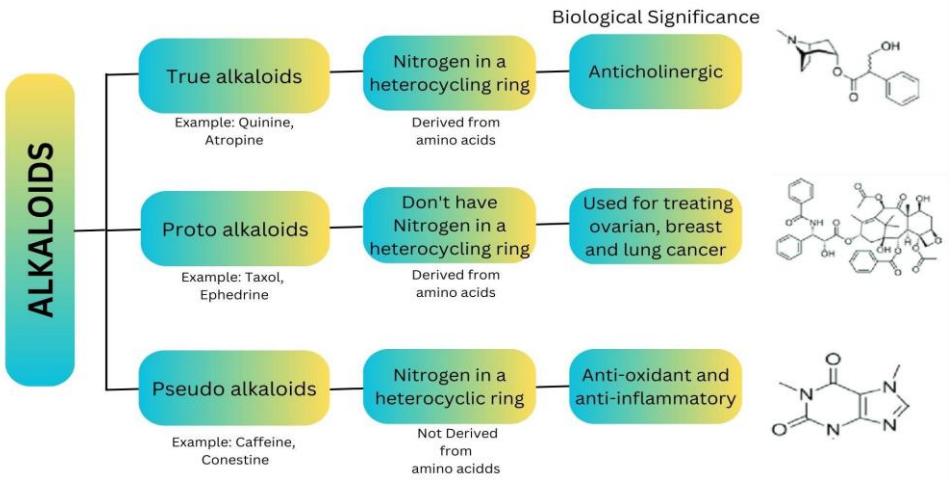


Fig 2.6. Classification of alkaloids along with biological significance.

2.2.2 Flavonoids - Vegetables, fruits, cereals, tea, and other naturally occurring polyphenolic substances contain large amounts of flavonoids. These substances play a vital role in several biological processes and plants' reactions to environmental conditions. They are known as plant secondary metabolites [46]. Plant flavonoids are crucial parts of various biological processes. They support the development, growth and ripening process of the plant. Additionally, flavonoids work as powerful UV filters and protect against a variety of biotic and abiotic stressors. These substances also have the power to protect plants from herbivores, bacteria, fungus, and viruses. Additionally, flavonoids function as chemical messengers, frequently working with bacteria and mycorrhiza [47]. Flavonoids offer a broad range of beneficial biochemical and anti-inflammatory effects

that have been connected to several illness, including as cancer, Alzheimer's disease (AD), atherosclerosis, and others. They are necesaary ingredients in pharmacological, medicinal, cosmetic, and nutraceutical uses and are known to provide a variety of health-promoting advantages. This is mostly due to its capacities to govern vital enzyme activities within cells and its anti-carcinogenic, anti-inflammatory, antioxidative, anti-mutagenic, and other properties [48]. It is well known that flavonoids strongly inhibit a wide range of enzymes, including phosphoinositide 3-kinase, lipoxygenase, cyclooxygenase (COX), and xanthine oxidase (XO). **Fig 2.7.** Shows the classes and subclasses of flavonoids.

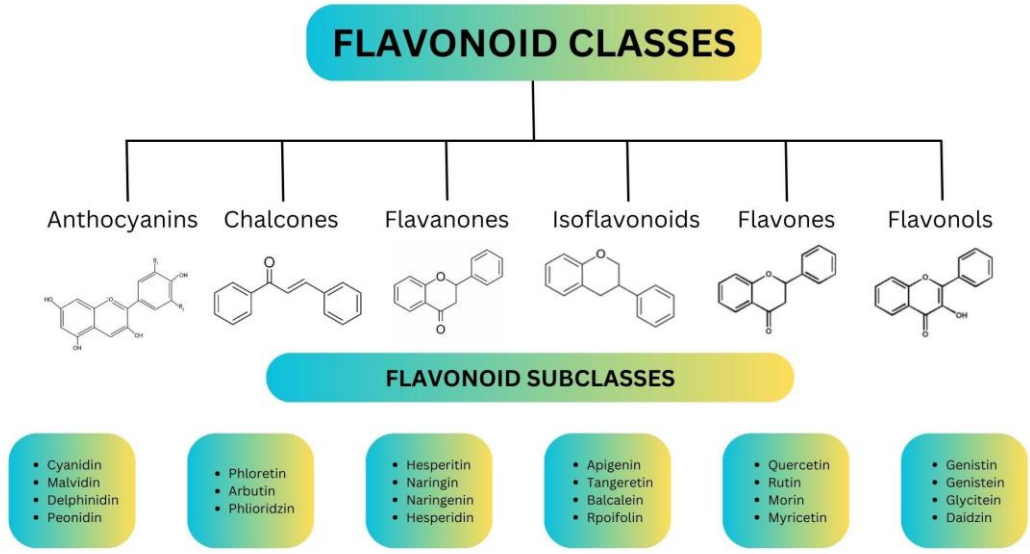


Fig 2.7. Flavonoid classes and subclasses.

2.2.3 Terpenoids - Terpenoids are a class of chemical substances that are naturally present in diverse organisms. They are also known as isoprenoids. They comprise compounds like terpenes and diterpenes and are formed from the isoprene 5-carbon molecule. A total of 224 terpenoids have been found in the pollen of male Cannabis plants. These substances may play a key role in attracting pollinator-dependent creatures or deterring pathogenic organisms [49]. Terpenoids are divided into groups according to the amount of carbons they contain and how they are structurally arranged, which is done by first arranging isoprene units in a linear pattern, then cyclizing and rearranging the carbon skeleton. The isoprene rule, an empirical principle, governs this structural

organisation. Terpenoids can be produced either the plastidial 2-C-methyl-D-erythriol 4-phosphate (MEP) route or the cytosolic mevalonate (MVA) system [50]. The main chemical elements of essential oils are called monoterpenoids, and well known for their aromatic properties. Terpenoids are frequently created by hydrolyzing carbocationic intermediates generated from geranyl pyrophosphate, especially those with an alcohol functional group. Sesquiterpenoids are produced by hydrolyzing intermediates generated from farnesyl pyrophosphate, whereas diterpenoids are produced by hydrolyzing intermediates derived from geranylgeranyl pyrophosphate.

2.2.4 Phenol - Phenols, often referred to as benzenols, carbolic acids, or phenolic acids, are chemical substances that are distinguished by the presence of a OH group that is directly linked to an aromatic carbocyclic nucleus. They often have the appearance of white crystalline crystals and are volatile. It is essential to remember that phenols are separate from alcohols and have unique characteristics. This difference results from their increased acidity, which is explained by both the comparatively weak link between the oxygen and hydrogen atoms in the hydroxyl group and the strong resonance coupling between the aromatic ring and the oxygen atom [51]. The shikimic acid and the acetic acid pathway are the two primary processes for the production of phenolic chemicals. The total phenolic content of dark-colored vegetables like black beans, kidney beans, and black gramme is shown to be greater when evaluated quantitatively. A particular class of phenolic compounds known as phenolic acids have benzene rings, one or more hydroxyl groups, and carboxylic groups [52]. According to their carbon structure, phenols may be split into two main groups: Cinnamic acid and Benzoic acid derivatives. Common phenols called hydroxybenzenes are not commonly present in higher plants. However, two additional well-known phenols are quinol and catechol, with quinol being more extensively distributed in the plant kingdom than catechol [53]. A diet rich in polyphenols can aid in the prevention of major illnesses including Parkinson's, Alzheimer's, and cardiovascular issues. Additionally, polyphenols have anti-inflammatory, hypotensive, and anti-diabetic characteristics. Plants mostly include phenolic acids, which are made up of hydroxycinnamic acids, as their main class of polyphenols. Sinapic acid, Ferulic acid, Coumaric acid, Chlorogenic acid, and Caffeic acid are a few of the several subtypes of

hydroxycinnamic acids found in plants. The most prevalent of these hydroxycinnamic acids is caffeic acid. While hydroxybenzoic acids are mostly found as glucosides in plants, and there are simple esters of hydroxycinnamic acids with either glucose or hydroxyl carboxylic acids. Strong antioxidant properties in phenolic acids help the body's own defences against free radicals as well as scavenge them.

2.2.5 Tannins - Tannins, sometimes referred to as tannic acid, are phenolic compounds that are water soluble and have molecular weights more than 500. They are able to remove proteins from aqueous solutions by precipitating them. Tannins, which are generated by plants as secondary metabolites, are widely distributed throughout many different plant species. Due to their astringent qualities, which render plant tissues unpleasant to eat, they play a function in controlling plant development and serving as a defence against predators. Condensed tannins, also known as proanthocyanidins, and hydrolyzable tannins, are the two largest divisions among the four types of tannins [54]. Tannins enhance the flavour, texture, and structure of wine while also acting as powerful antioxidants. Red wines and other wines that are fermented on the skins usually have enough tannins from natural sources. However, for some wines like floral or fruit wines, the addition of tannins may be helpful. Proanthocyanidins, commonly referred to as condensed tannins, are compounds created by the oligomerization of flavan-3-ol molecules like epigallocatechin, epicatechin, and catechin [55]. The major portion of tannins, which are made up of oligomers of flavan-3-ols and flavan-3,4-diols, are found in the bran of legumes. It's important to keep in thoughts that green tea, grapes, and chocolate are well-known for being excellent sources of tannins as well [56]. Due to their interactions with proteins, carbohydrates, and digestive enzymes, tannins are frequently viewed as nutritionally unfavourable since they reduce the nutritional content of meals. Complexes are created as a result of these interactions, which may negatively impact nutrient absorption and digestion. Through the activity of polyphenol oxidase, tannins can also cause browning reactions in meals, darkening the dish and perhaps affecting its appeal. It's crucial to remember that tannins also have advantageous qualities. In some situations, they can serve as antibacterial and anticarcinogenic agents, potentially promoting health.

2.2.6 Saponins - quinoa seeds have saponin, a substance with a bitter taste, in its outermost layers. A varied collection of substances known as saponins is extensively distributed across the plant world. Their chemical constitution, which consists of a triterpene or steroid aglycone connected to one or more sugar chains, can be used to differentiate them [57]. Saponins are able to operate as surfactants because of their amphipathic nature. They can interact with phospholipids and cholesterol, among other elements of cell membranes, because of this characteristic. More than 100 plant groups have been reported to contain saponins, which are also present in some marine sources including starfish and sea cucumbers [58]. Monocotyledonous plants, such as those from the Agavaceae, Dioscoreaceae, and Liliaceae families, are the main sources of steroidal saponins. On the other hand, dicotyledonous plants, such as those in the Leguminosae, Araliaceae, and Caryophyllaceae families, mostly contain triterpene saponins [59]. Saponins have the capacity to create foam and can also cause red blood cell hemolysis [60]. Saponins, which are plant glycosides, are used to suppress the development of tumour cells by processes including cell cycle arrest and death; their efficiency is shown by their IC50 values. Saponins have also demonstrated potential anti-tumorigenic capabilities. After removing lipids using petroleum ether or chloroform, the procedure of extracting saponins from plant material often requires the use of a polar solvent [61]. The use of several purifying methods follows. A number of chromatographic methods have been used to separate distinct saponins. However, the isolation, separation, and quantification steps of the saponin analysis are a difficult and potentially error-prone procedure that involves careful attention and exact techniques.

2.3 Role of phytochemicals in PD- Due to their wide variety of phytochemical components, plants found in nature or herbal treatments have shown their capacity to offer protection and healing of a number of illness, including neurological conditions [62]. The tendency to use a variety of plant-based natural compounds as possible medications has significantly increased recently. Diallyl trisulfide, quercetin, Curcumin, epigallocatechin-3-galate (EGCG), and flavonoids are a few common phytochemicals and rest of the phytochemicals are mentioned in **Table 2.2.** These phytochemicals control hormone metabolism, strengthen the immune system, and lessen platelet aggregation [63].

Table 2.2. Mode of action of various phytochemicals in Parkinson disease.

S.No.	Phytochemicals	Source	Secondary Metabolites	Mode of action	References
1.	Celastrolis	*Tripterygium wilfordii*	Terpenes	- blocks the depletion of DOPAC and DA levels - reduces the loss of dopaminergic neurons	[64]
2.	Baicalein	*Scutellariabaicalensis*	Flavonoids	- Inhibiting apoptosis - Increase PC12 cells viability.	[65]
3.	Acacetin	*Chrysanthemum morifolium*	Flavonoids	- Reducing inflammatory mediators - Decreasing loss of dopaminergic neurons	[66]
4.	Fustin	*Rhus verniciflua*	Flavonoids	- suppression of cell apoptosis - Activation of p38 phosphorylation	[67]
5.	Silymarin	*Silybum marianum*	Flavonoids	- increasing antioxidant agents - antiapoptotic effect	[68]
6.	Salvianic acid	*Salviamiltiorrhiza*	Phenolic acid	- Antiapoptotic - Reducing ROS formation	[69]
7.	Rosmarinic acid	*Rosmarinus officinalis*	Phenolic acid	- block intracellular ROS production - Inactivation of	[70]

				caspase-3	
8.	Ginkgolide B	*Ginkgobiloba extracts*	Terpenes	- protect dopaminergic neurons	[71]
9.	Zingerone	*Zingiber officinale*	Alkaloids	- enhances the antioxidative defense	[72]
10	Acetylcoryn oline	*Corydalis bungeana*	Alkaloids	- Controlling the death of dopaminergic neurons, the decrease in DA levels, and the aggregation of α-syn protein	[73]

Even though there is no approved treatment or cure for PD, researchers are constantly researching these options. Studies have looked at the possible advantages of phytochemicals produced from plants as well as other natural sources to alleviate the symptoms of PD. The antioxidant and anti-inflammatory properties of phytochemicals, such as 4-methyl benzaldehyde, have been demonstrated. PD development is hypothesised to be influenced by oxidative stress and inflammation. 4-Methylbenzaldehyde may help safeguard neurons from harm by lowering oxidative stress and inflammation. Citrus fruit peels contain limonene, a naturally occurring substance. It has proven to have anti-inflammatory and antioxidant effects. Additionally, limonene may have neuroprotective properties that prevent the deterioration of dopamine-producing neurons. Lemon grass and lemons are two plants that contain citral as part of their essential oils. It possesses anti-inflammatory and antioxidant properties. Several plants, including eucalyptus and cinnamon, produce essential oils that include the naturally occurring compound alpha phellendrene. The oxidative stress and inflammation linked to PD may be mitigated by these qualities. To completely comprehend their potential therapeutic benefits and establish the most efficient dosages and formulations,

more study is required. In addition, PD treatment is complicated and frequently combines many strategies, such as medication, physical therapy, and lifestyle changes. Future research on phytochemicals like the ones described may focus on adjunctive or complementary therapies, but it is crucial to seek advice and suggestions from medical experts first.

2.4 In silico analysis -

Computational techniques have been successfully used to create several novel medicinal molecules [74]. The phrase "in silico" refers to computer-based simulations or tests that are carried out using computer models or algorithms. In silico analysis is used to develop hypotheses, make predictions, and gather insights in a timely and cost-effective manner [74]. PubMed, a comprehensive library of biological literature, bioinformatics journals, and specialised bioinformatics databases like BioGRID, STRING, and KEGG are a few examples of well-liked databases and tools for in silico analytical literature.

In Silico analysis has the following benefits over conventional experimental methods: 1) Cost- and time-effective. 2) Makes it possible to analyse huge datasets and complicated biological systems. 3) Aids in the creation of hypotheses and the forecasting of experimental results.

limitations on In silico analysis: 1) Reliance on information already known and available, which may be inaccurate or biased. 2) Potential differences between predicted outcomes from in silico and actual experimental outcomes. 3) The precision of computing models and methods is limited

CHAPTER 3: EXPERIMENTAL

3.1 CHEMICAL USED -

Conc. Sulphuric acid, Dilute ammonium hydroxide, Alcoholic Potassium hydroxide, Wagner reagent, Chloroform, Hydrochloric acid, Lead acetate, Ferric chloride, distilled water.

3.2 EQUIPMENTS -

Test tubes, conical flask, beakers, Whatman filter paper No 42, pipette, measuring cylinder, hot plate, weighing balance, Heating mantle, mortar and pestle, cultural tubes, test tube stand, centrifuge,Tissue paper, glass rod, falcon tubes, china dish, aluminum foil, etc.

3.3 PLANT MATERIALS -

Leaves of *Petunia hybrida, Plectranthus scutellarioides, Alcea rosea, Dahlia pinnata, Chrysanthemum morifolium.*

3.4 METHODOLOGY

3.4.1 Collection of plant material

At Delhi Technological University, leaves were taken from a range of plants, including *Petunia hybrida*, *Plectranthus* scutellarioides, *Alcea rosea*, *Dahlia pinnata*, and *Chrysanthemum morifolium*. Throughout the month of January, the collection was conducted. To harvest leaves, only healthy plants were used. The leaves were cautiously gathered to assure cleanliness, and any latex or dirt was then rinsed off.

3.4.2 Drying

- **Shade Dried -** After being collected from the five plants (*Petunia hybrida*, *Plectranthus* scutellarioides, *Alcea rosea*, *Dahlia pinnata*, and *Chrysanthemum morifolium*), to get rid of any potential dirt, the leaves had been washed twice: once with normal water and other

with double distilled water. In the plant biotechnology lab at Delhi Technological University, the leaves were then uniformly distributed on newspaper sheets and let to dry for 15 days at room temperature. The leaves were crushed and carefully grind using a pestle and mortar, forming a fine powder. In order to simplify further analysis, this powder was then put onto a china dish and covered with aluminium foil.

- **Sun Dried -** After being collected from the five plants (*Petunia hybrida*, *Plectranthus* scutellarioides, *Alcea rosea*, *Dahlia pinnata*, and *Chrysanthemum morifolium*), to get rid of any potential dirt, the leaves had been washed twice: once with normal water and other with double distilled water. The leaves were then equally scattered on sheets of newspaper and exposed to sunlight for a duration of 15 days at Delhi Technological University. The leaves were crushed and carefully grind using a pestle and mortar, forming a fine powder. In order to simplify further analysis, this powder was then put onto a china dish and covered with aluminium foil.

3.4.3 Extract Preparation

- **Fresh Sample** - The petioles were cut off the leaves of *Petunia hybrida*, *Plectranthus* scutellarioides, *Alcea rosea*, *Dahlia pinnata*, and *Chrysanthemum morifolium*. To remove any dust, the leaves were washed under running water and then twice sterilised in milli-Q water. Using mortar and pestle crushed the leaves into a fine paste.. The plant extract was made in a 200 ml flask using 1 gram of leaf powder and 100 ml of milli-Q water. After boiling for five minutes, the flask was heated for ten minutes at 50 °C on a heating mantle. Once the container had cooled to room temperature, the material was poured into a 50 ml falcon tube. The centrifugation of the extract at 3000 rpm for 5 minutes, followed by the use of Whatman filter paper to separate the supernatant from the leaf pellets, produced the aqueous extract of the leaves. Then, this extract was kept for further phytochemical investigation.

- **Shade dried –** To make the leaf's aqueous extract, In a 200 ml flask, one gram of leaf powder was combined with 100 ml of milli-Q water. The liquid was then heated on a

heating mantle for a further five minutes, bringing it to a boil at a temperature of 50 C. After boiling, the flask was allowed to cool at room temperature. The flask's contents were then transferred to a 50 ml falcon tube and chilled. The extract-containing falcon tube was thereafter spun at 3000 rpm for 5 minutes. Whatman filter paper was used to separate the liquid supernatant from the leaf pellets during centrifugation. The ensuing aqueous extract made from the leaf is represented by the collected supernatant.

- **Sun dried -** 1 gram of the leaf powder was combined with 100 ml of milli-Q water in a 200 ml flask to create the aqueous extract from the leaf. Using a heating mantle, the liquid was then brought to a boil for 5 minutes at a temperature of 50 C. After the flask had boiled, the liquid was poured into a 50 ml falcon tube and allowed to cool at room temperature. The resultant solution was then centrifuged for five minutes at 3000 rpm in a falcon tube. Whatman filter paper was used to separate the liquid supernatant from the remaining leaf material after centrifugation. The ensuing aqueous extract made from the leaf is what the collected supernatant represents.

3.4.4 Qualitative estimation of secondary metabolites

- **Akaloids Test**

  Wagner's Test is the qualitative assay for alkaloids. 2.25 grams of iodine and 1.25 grams of potassium iodide were precisely weighed using a weighing scale to make Wagner's reagent. The components were then mixed with 250 ml of milli-Q water. At last 1 ml of the ready Wagner's reagent was carefully pipetted into 1 ml of the produced plant extract.

- **Flavonoids Test**

  Each variety of leaf's extract was made, put in a different test tube, and given the appropriate label. 1 ml of each leaf extract was measured and divided among the test tubes in order to conduct the experiment. To each test tube containing the leaf extract, 10% lead acetate solution was then added.

- **Terpenoids Test**

  3 ml of the extract were placed in a test tube, and 1.5 ml of pure H2SO4 and 1 ml of chloroform were carefully poured down the side.

- **Phenol Test**

  Phenol was assessed qualitatively using the Lead acetate test. Add 3 ml of a 10% lead acetate solution into the 5 ml of extract. The mixture was then thoroughly stirred or mixed to ensure adequate mixing.

- **Tannins Test**

  Five distinct leaves' extracts were collected and labelled as fresh, shade-dried, and sun-dried. Five ml of each extract were put to different test tubes. The neutral 5% ferric chloride solution was then diluted and a few drops were added to each test tube containing the leaf extract.

- **Saponins Test**

  Five distinct leaves' extracts were gathered and labelled as fresh, shade-dried, and sun-dried. 0.5 mg of each extract was put to different test tubes. The leaf extract was then added to each test tube along with a few ml of distilled water. After giving the combination a good shake, observe the results.

Table 3.1. Protocol for Qualitative test

S.no.	Preliminary Test	Procedure	Expected Results
1.	Alkaloids	Extract + Wagner's Reagent	Reddish brown
2.	Flavonoids	Extract + 10% lead acetate	Yellow Precipitate
3.	Terpenoids	3 ml Extract + 1 ml Chloroform + 1.5 ml of concentrated H2SO4	Reddish Brown color
4.	Phenol	3ml of 10% lead acetate solution + 5 ml extract	White precipitate

5.	Tannins	5% ferric chloride solution + 5ml extract	Dark green color
6.	Saponins	0.5 mg of extract + distilled water	Formation of frothing

3.5 TOOLS USED

The term "in silico tools" refers to computer techniques and algorithms used to simulate and analyse biological processes and data. In bioinformatics and genomics research, these techniques are frequently used to forecast and examine different characteristics of genes, including the discovery of targeted genes. In silico methods may be used to analyse certain genes that are linked to a particular characteristic, illness, or biological function in order to predict targeted genes. Gene4PD (http://genemed.tech/gene4pd), an integrative genomic database for PD, SwissTargetPrediction (SwissTargetPrediction), VENNY 2.1, Pubchem (for obtaining the SMILES structure of targeted phytochemicals), and SwissDock (for determining the targeted genes forPD) are examples of in silico tools.

3.5.1 Gene4PD - All of the targeted genes from this approach have the ability to stop PD from progressing. 15223 genes in all were found to be associated with PD.

3.5.2 SwissTargetPrediction - For swiss target prediction, the website (SwissTargetPrediction) needs to be accessed. Then, the PubChem database may be used to derive the canonical SMILES of the phytochemicals. Insert the SMILES into the Swiss target prediction box and choose "Predict targets" to make your predictions. Based on the supplied canonical SMILES, the programme will present you with a list of prospective targets after the prediction procedure is finished.

3.5.3 Venny 2.1.0 - Only 6 genes were found to be shared targets among the 15223 genes that were examined in order to identify the common targets.

3.5.4 Pubchem- to extract the 3D structure files and canonical SMILES of phytochemicals.

3.5.5 Protein data bank (PDB) - Using PDB, one may obtain the target proteins' three-dimensional structure.

3.5.6 SwissDock - Prior to docking the target protein with the chosen phytochemicals, prepare a target and ligand from the BIOVIA discovery studio. After removing hetero atoms (hetatms) and crystalline H2O molecules, polar hydrogen atoms were added. After being created, the target protein's structure was saved in PDB format. In order to save them, ligand (compound) SDF structures were converted to MOL2 format using the visualisation tool BIOVIA Discovery Studio. Swissdock is an online tool or website that predicts the molecular interactions between target proteins and small molecules like medicines, inhibitors, ligands, etc. by using results from local or blind docking in the form of CHARMM energies (http://www.swissdock.ch/). The SwissDock system allows users to email the results after receiving files providing the structures of proteins and ligands.

CHAPTER 4: RESULTS

4.1 Qualitative estimation of Alkaloid in *Petunia hybrida, Plectranthus scutellarioides, Alcea rosea, Dahlia pinnata, Chrysanthemum morifolium* for fresh, sun and shade dried leaves

A chemical test called the Wagner test is used to find alkaloids in a sample. Prepare a little quantity of the test ingredient (such as a plant extract). The test sample should be mixed with a few drops of Wagner's reagent, which is a potassium iodide and iodine solution. Watch the colour shift that takes place. A reddish-brown precipitate or colour is a sign of an alkaloid test that is positive.

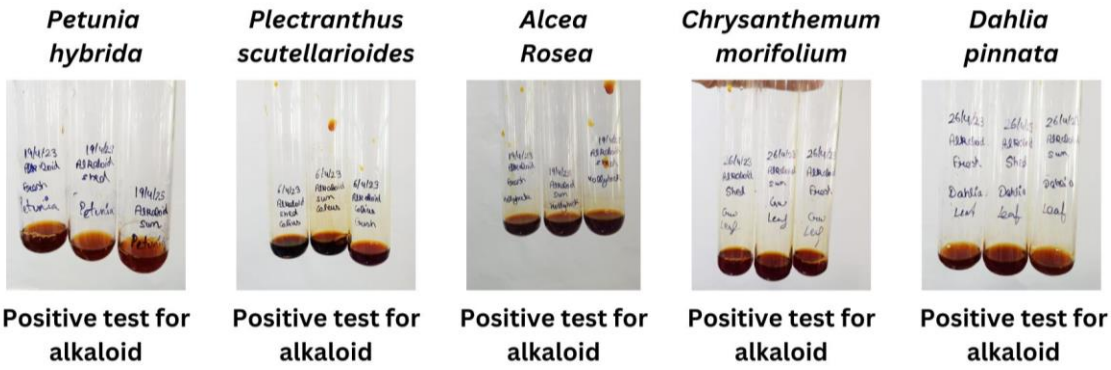


Fig 4.1 Qualitative test of alkaloid for sun, shade and fresh leaves of all the plants.

Table 4.1. Result of alkaloid test of all medicinal plant species.

S.no.	Plant	Fresh	Sun-dried	Shade dried
1.	***Petunia hybrida***	+	+	+
2.	***Plectranthus scutellarioides***	+	+	+
3.	***Alcea rosea***	+	+	+
4.	***Chrysanthemum morifolium***	+	+	+
5.	***Dahlia pinnata***	+	+	+

4.2 Qualitative estimation of Flavonoids in *Petunia hybrida, Plectranthus scutellarioides, Alcea rosea, Dahlia pinnata, Chrysanthemum morifolium* for fresh, sun and shade dried leaves

A typical chemical test to find flavonoids in a material is the qualitative test for flavonoids using lead acetate. Prepare a little quantity of the test ingredient (such as a plant extract). Drops of lead acetate solution should be added to the test sample. Because it creates a combination with flavonoids and causes a colour shift, lead acetate is frequently utilised. Watch the colour shift that takes place. The development of a yellow or yellowish-brown precipitate is indicative of a successful test for flavonoids.

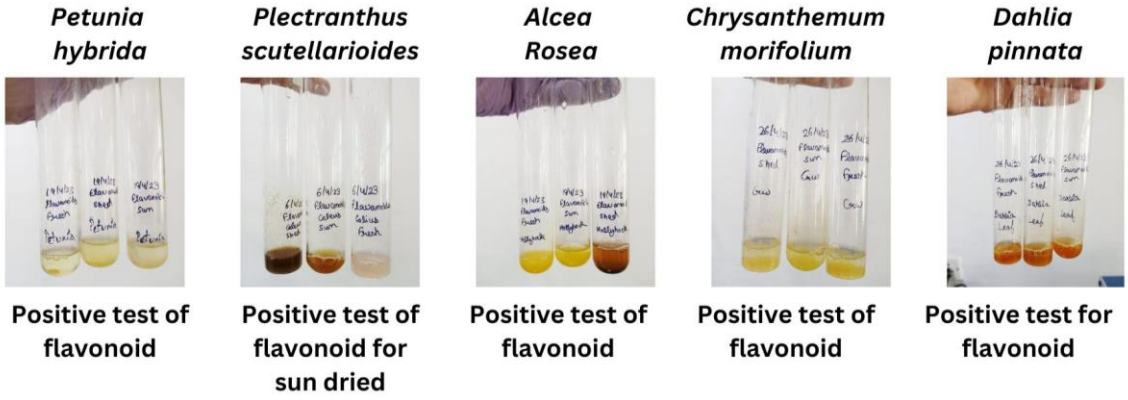


Fig 4.2. Qualitative test of Flavonoid for sun, shade and fresh leaves of all the plants.

Table 4.2. Result of Flavonoid test of all medicinal plant species.

S.no.	**Plant**	**Fresh**	**Sun-dried**	**Shade dried**
1.	***Petunia hybrida***	+	+	+
2.	***Plectranthus scutellarioides***	-	+	-
3.	***Alcea rosea***	+	+	+
4.	***Chrysanthemum morifolium***	+	+	+
5.	***Dahlia pinnata***	+	+	+

4.3 Qualitative estimation of Terpenoids in *Petunia hybrida, Plectranthus scutellarioides, Alcea rosea, Dahlia pinnata, Chrysanthemum morifolium* for fresh, sun and shade dried leaves

Salkowski test is a qualitative terpenoid assay. In a test tube, place a little quantity of the sample (plant extract or chemical). Chloroform should be added in little amounts to the test tube containing the sample. Concentrated sulfuric acid needs to be progressively added down the sides of the test tube, enabling it to split into a layer at the bottom. To combine the ingredients, gently shake the test tube. Observe how the mixture's colour changes. The development of a reddish brown colour near the interface is a sign that terpenoids are present.

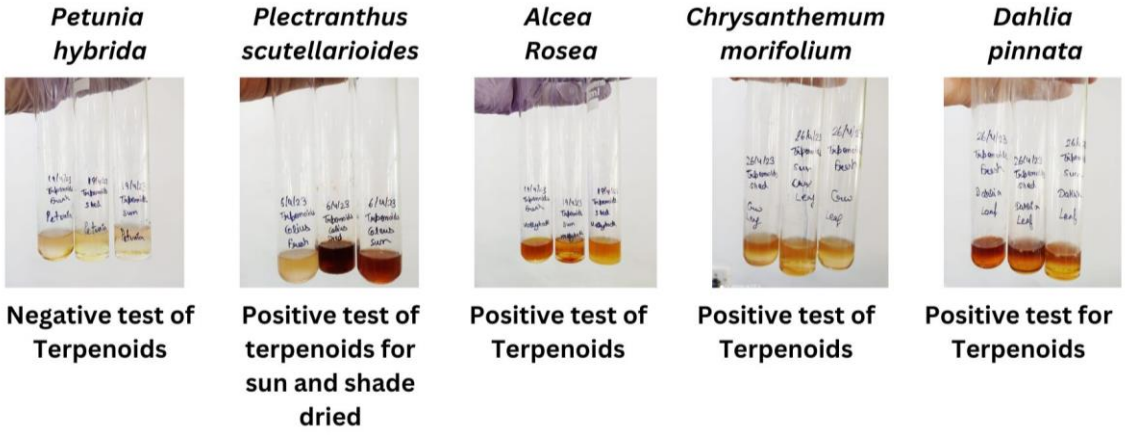


Fig 4.3. Qualitative test of Terpenoids for sun, shade and fresh leaves of all the plants.

Table 4.3. Result of Terpenoid test of all medicinal plant species.

S.no.	**Plant**	**Fresh**	**Sun-dried**	**Shade dried**
1.	***Petunia hybrida***	-	-	-
2.	***Plectranthus scutellarioides***	-	+	+
3.	***Alcea rosea***	+	+	+
4.	***Chrysanthemum morifolium***	+	+	+
5.	***Dahlia pinnata***	+	+	+

4.4 Qualitative estimation of Phenol

The qualitative test for phenol is lead acetate. A little part of the samples should be placed in a test tube. Several drops of lead acetate solution should be added to the test tube containing the sample. A white or yellowish precipitate is an indication of the presence of phenolic compounds.

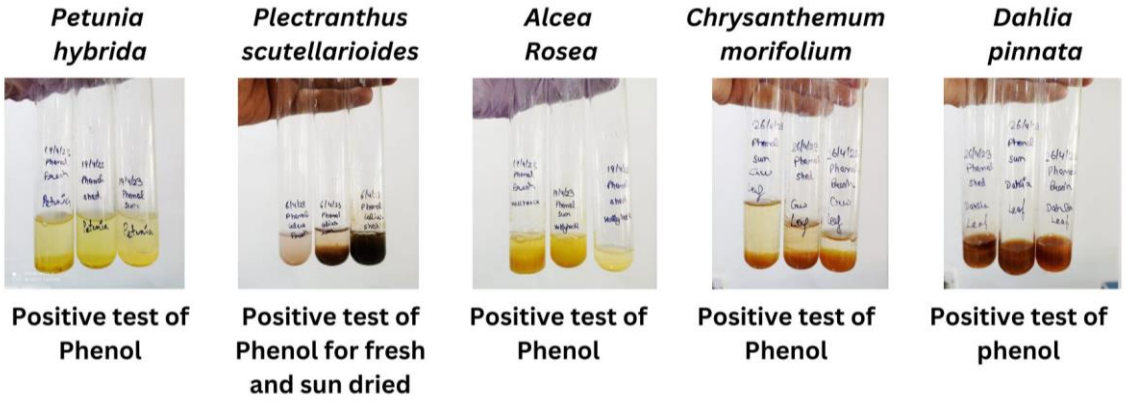


Fig 4.4. Qualitative test of Phenol for sun, shade and fresh leaves of all the plants.

Table 4.4. Result of Phenol test of all medicinal plant species.

S.no.	Plant	Fresh	Sun-dried	Shade dried
1.	***Petunia hybrida***	+	+	+
2.	***Plectranthus scutellarioides***	+	+	-
3.	***Alcea rosea***	+	+	+
4.	***Chrysanthemum morifolium***	+	+	+
5.	***Dahlia pinnata***	+	+	+

4.5 Qualitative estimation of Tannins

The ferric chloride test is a qualitative method for detecting tannins. By combining a tiny quantity of ferric chloride with water, you may make a ferric chloride solution. Put a little bit of the sample in a test tube. The produced ferric chloride solution should be diluted and a few drops added to the test tube containing the sample. The development of a dark green, blue-black, or greenish-black colour or a greenish-black precipitate is a sign that tannins are present.

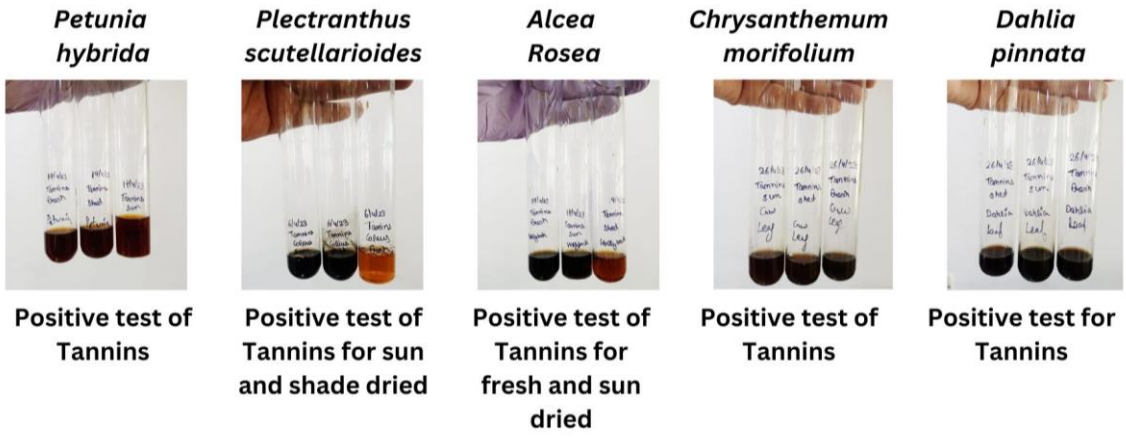


Fig 4.5. Qualitative test of Tannins for sun, shade and fresh leaves of all the plants.

Table 4.5. Result of Tannins test of all medicinal plant species.

S.no.	Plant	Fresh	Sun-dried	Shade dried
1.	***Petunia hybrida***	+	+	+
2.	***Plectranthus scutellarioides***	-	+	+
3.	***Alcea rosea***	+	+	-
4.	***Chrysanthemum morifolium***	+	+	+
5.	***Dahlia pinnata***	+	+	+

4.6 Qualitative estimation of Saponins

Put a little bit of the sample in a test tube. The test tube containing the sample should be filled with distilled water. Mix well. Observe any foaming or frothing that develops while the mixture is stirred or shaken. A steady, enduring froth or foam that lasts for a considerable amount of time is an indication that saponins are present.

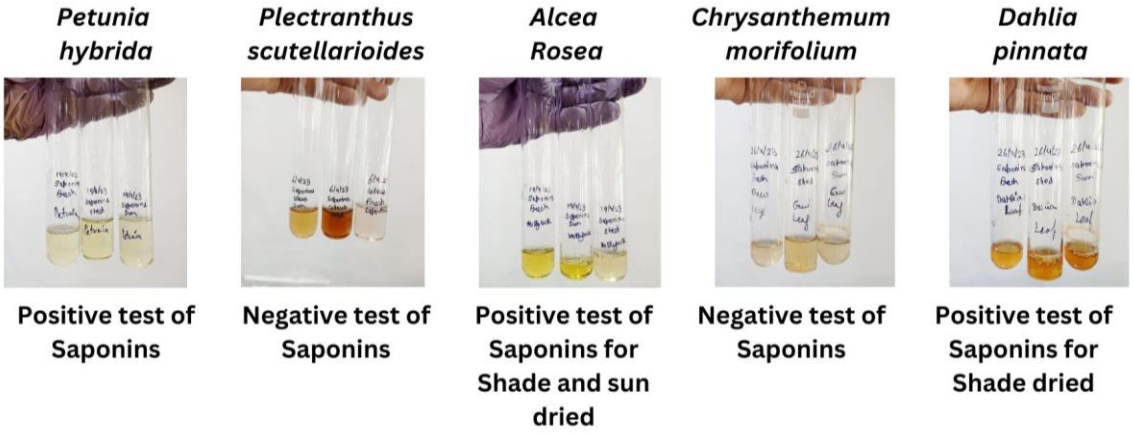


Fig 4.6. Qualitative test of Saponins for sun, shade and fresh leaves of all the plants.

Table 4.6. Result of Saponin test of all medicinal plant species.

S.no.	Plant	Fresh	Sun-dried	Shade dried
1.	***Petunia hybrida***	+	+	+
2.	***Plectranthus scutellarioides***	-	-	-
3.	***Alcea rosea***	-	+	+
4.	***Chrysanthemum morifolium***	-	-	-
5.	***Dahlia pinnata***	-	-	+

4.7 Target identification interaction analysis

TARGET	4-methyl benzaldehyde	Citral	Limonene	alpha-phellenderene	CONTROL
PSEN1	-5.85	-6.46	-6.21	-6.25	-5.71
ADORA1	-6.08	-6.97	-6.52	-6.6	-6.01
NR1H3	-6.08	-6.64	-5.95	-6.06	-5.9
DAO	-6.07	-6.72	-6.18	-6.1	-5.9
ALDH1A1	-6.22	-6.95	-6.51	-6.47	-5.38
ADORA3	-6.34	-7.19	-6.25	-6.44	-5.09

Fig 4.7. Heat map of binding energies (Kcal/mol)

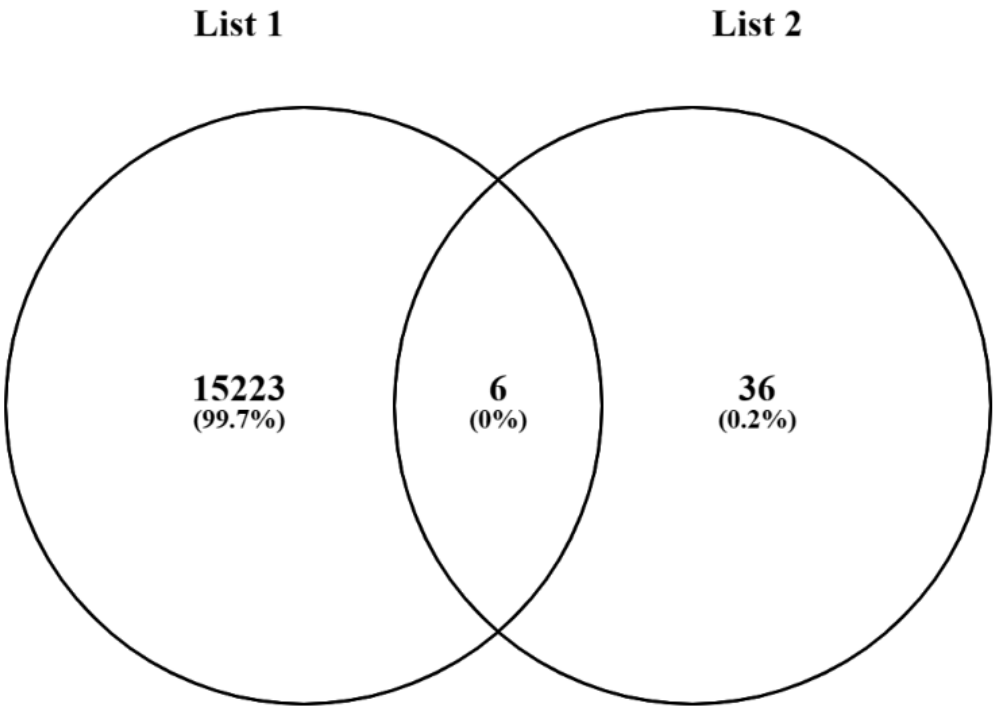


Fig 4.8. Venn diagram for the identification of common targeted genes from Venny 2.1

DISCUSSION

PD is a neurological situation that usually shows up in later life and causes bradykinesia and at least one symptom, such as stiffness or resting tremors. Through a mix of qualitative analysis and target molecule identification, this study objective was to investigate the potential of therapeutic herbs as innovative treatment agents for PD. Finding bioactive substances that may have neuroprotective benefits requires a qualitative investigation of secondary metabolites in medicinal plants. Five therapeutic plants were chosen for investigation in this study: *Petunia hybrida*, *Plectranthus scutellatioides, Alcea rosea, Dahlia pinnata,* and *Chrysanthemum morifolium*. These plants were chosen based on their traditional uses and the presence of secondary metabolites with established pharmacological functions.

The qualitative analysis revealed the presence of secondary metabolites in the researched plants, including saponins, tannins, flavonoids, trpenoids, alkaloids, and phenols. Alkaloids were present in every one of the medicinal plants examined, indicating the presence of potentially therapeutic bioactive compounds. It is generally recognised that secondary metabolites with anti-inflammatory and antioxidant properties include flavonoids, phenols, and tannins. The pathophysiology of PD includes essential factors including inflammation and oxidative stress. The majority of the medicinal herbs analysed had high levels of phenols, tannins, and flavonoids, which suggests that they may have neuroprotective advantages in PD by reducing inflammation and oxidative stress. Terpenoids, a separate class of secondary metabolites, were present in several of the investigated plants. Terpenoids were only detected in a few samples in this study, but considering their potential neuroprotective advantages, additional investigation is required to determine how they may be utilised to treat PD.

In addition to the qualitative investigation, in silico analysis was carried out to identify the target molecules for PD treatment. Several computational techniques, including Gene4PD, Swiss Target Prediction, Venny 2.1.0, PubChem, PDB, and Swissdock, were used to identify potential target molecules. The results showed that citral had a greater binding energy to the target molecule ADORA1 than the control. The PSEN1-targeting ligand also possessed higher binding energy. These results suggest that

citral may be a viable therapeutic ligand for PD due to its good binding characteristics with the identified target molecules.

Overall, this study offers insightful information about the potential of herbal remedies as innovative PD treatment agents. The studied medicinal plants contained bioactive substances such phenols, terpenoids, saponins, phenols, alkaloids, and flavonoids, according to the qualitative analysis. These substances have pharmacological characteristics that could support their neuroprotective actions. Citral was also recognised by the in silico research as a possible therapeutic ligand due to its better affinity for target molecules.

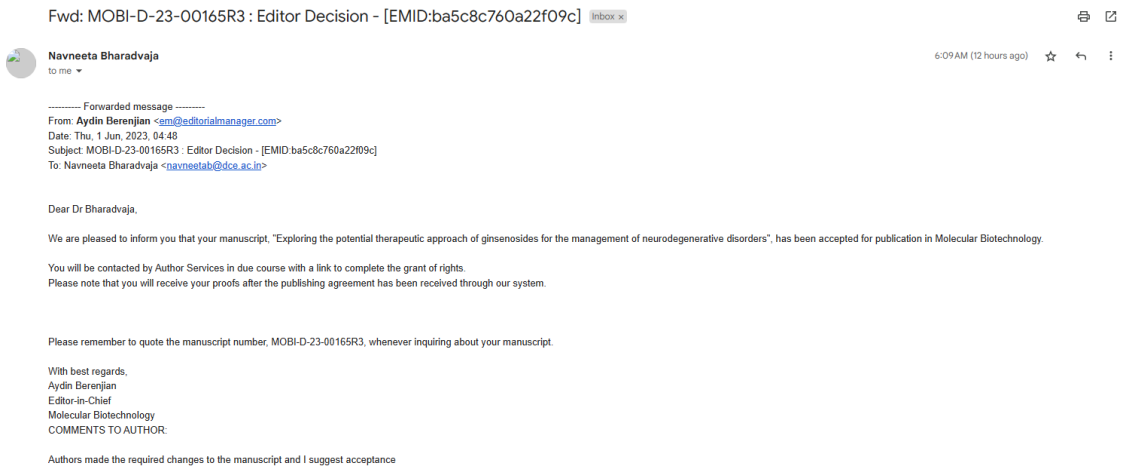
More study, particularly in vitro and in vivo investigations, are necessary to verify the stated effects and understand the underlying mechanisms of action. A multi-target strategy may also be crucial to achieving the greatest treatment outcomes for PD.

CONCLUSION

With several different compounds being utilised to treat both acute and chronic human illness, secondary metabolites have grown to be a significant source of herbal medicines. The objective of this review study was to investigate the potential of herbal medicines to serve as innovative therapies for PD. By performing a qualitative analysis and target molecule identification, the study highlights the fascinating function that naturally occurring compounds derived from plants can have in the treatment and management of this neurodegenerative disorder. The results of the review show that medicinal plants are a rich source of bioactive compounds with neuroprotective, anti-apoptotic, antioxidant, and anti-inflammatory effects. These qualities are crucial for tackling the underlying causes of PD, such as oxidative stress, inflammation, and neuronal cell death. The review emphasises various modes of action by which medicinal plants exercise their therapeutic benefits by identifying particular target compounds, such as phytochemicals and their derivatives. Numerous cellular functions, including the regulation of dopamine levels, avoidance of neuroinflammation, enhancement of antioxidant defence mechanisms, and promotion of neurogenesis, may be affected by these substances. A number of therapeutic plants were also discovered through this study's qualitative analysis, and their results in preclinical and clinical testing have been favourable. Examples include, among others, *Chrysanthemum morifolium, Alcea rosea*, *Plectranthus scutellatioides*, *Dahlia pinnata,* and *Petunia hybrida*. In PD models, these plants' bioactive substances have shown neuroprotective and symptomatic relief abilities.

In order to completely understand the safety, effectiveness, and ideal dose of medicinal plant-derived chemicals for PD treatment, it is vital to remember that further study is required. To ensure compatibility and prevent negative effects, it is also important to extensively study any possible interactions between these substances and traditional PD treatments.

APPENDICES – CONFERENCE DETAILS



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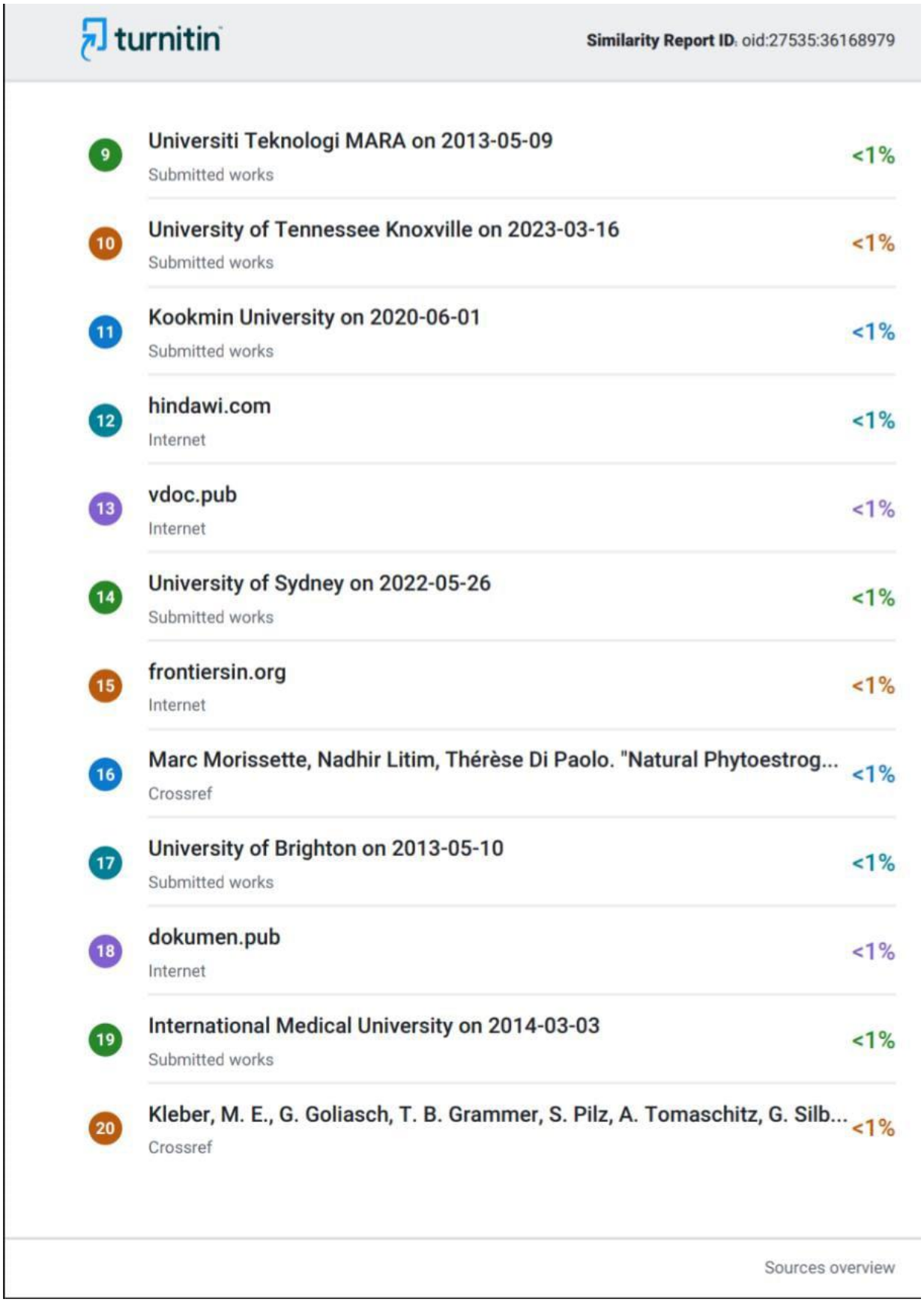

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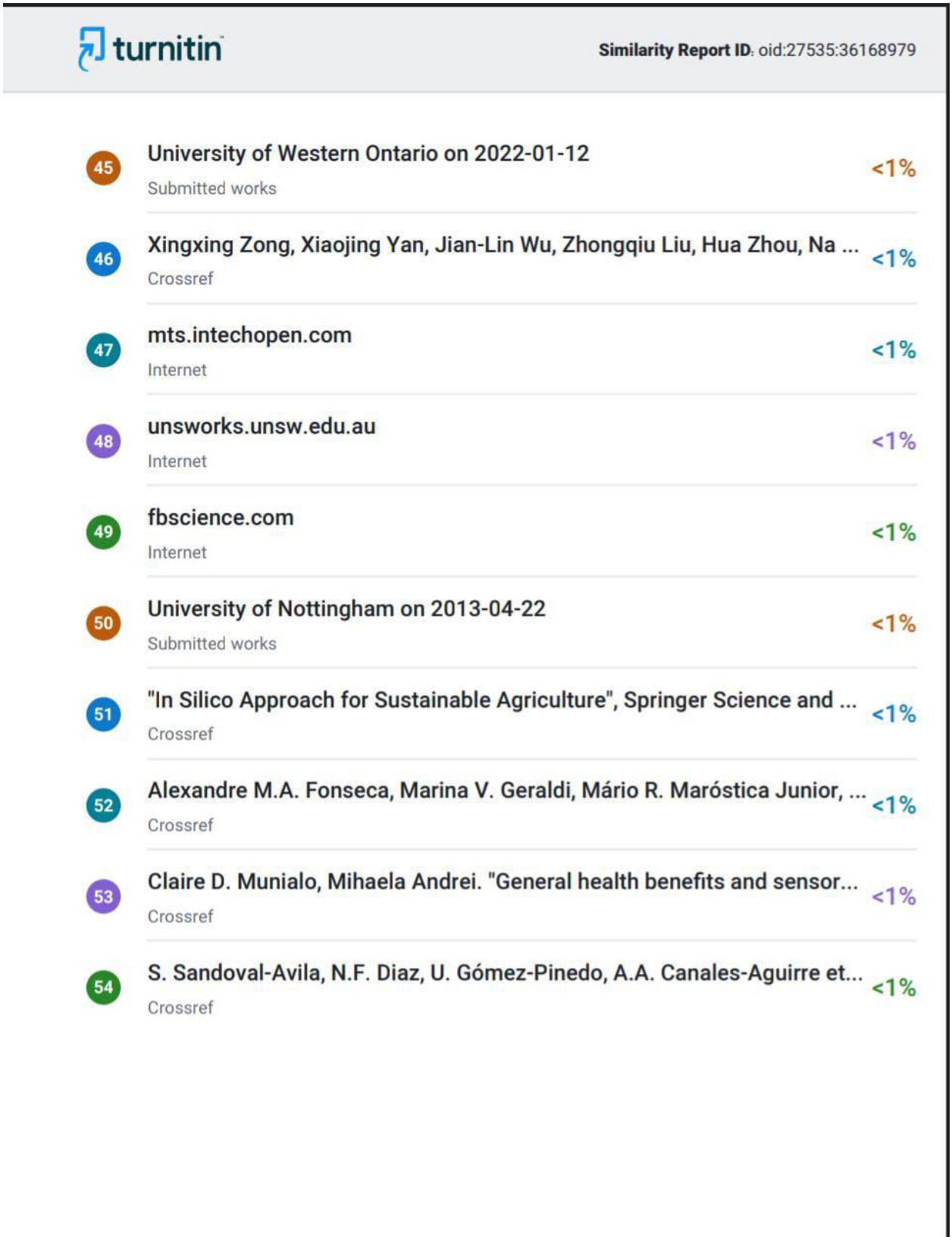

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Name of Conference: IEEE Bangalore Humanitarian Technology Conference (IEEE B-HTC 2023)

Organizers Details: JSS Academy of Higher Education and Research, JSS Hospital, Mysuru, INDIA

Status: Accepted and Presented

Dates of conference: 24th and 25th March 2023

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Name of Authors: Manju and Navneeta Bharadvaja*

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CERTIFICATE

I hereby certify that the Project Dissertation titled "**Investigating Medicinal Plants as Therapeutic Agents for Parkinson's Disease: Qualitative Analysis and target molecule identification**" which is submitted by Manju, Roll No.: 2K21/MSCBIO/24, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is a record of the project work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to the University or elsewhere.

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