INTRODUCTION

Dr. James Parkinson first described Parkinson's disease (PD) also known as the "Shaking palsy". It can be described as the chronic, progressive neurodegenerative disease which is characterized by the motor and nonmotor features. The shaking palsy is also known as the Paralysis Agitans [1]. The term parkinsonism is basically used to define the complex symptoms of the disease [2].

PD is among the most common disease of neurodegenerative disorders. Parkinson's disease foundation reports that almost 1 million people are affected with the disease currently [3, 4]. From the clinical point of view, the symptoms of PD are: bradykinesia, rigidity, resting tremor and postural instability. Levodopa is a neutral, large amino acid which is synthesized from L-thyroxine through hydroxylation, followed by decarboxylation to form the end product as the dopamine, which is a neurotransmitter that lacks the ability to pass the blood-brain barrier [5]. Thus, the levodopa is used as a drug which has the ability to pass the blood-brain barrier.

Levodopa is the precursor of dopamine. When levodopa or L-dopa is converted to dopamine and crosses the blood-brain barrier and helps in the proper functioning of the central nervous system via the synapsis. Levodopa converts to dopamine and crosses the blood-brain barrier and prevents the Parkinson's disease [6].

Levodopa is the symptomatic treatment of Parkinson's disease. It is considered as the most effective drug for the same and provides better symptomatic control, it is one of the potent drugs for the curing of the disease. This aromatic amino acid, L-3,4-dihydroxy phenylalanine has always been the most important drug from past half century. This L-dopa passes the blood-brain barrier and thereby increases the amount of dopamine near the brain cells, whereas the dopamine itself cannot do that and thus leads to the incurable disease. This dopamine and its precursor play an important role both in Parkinson's disease as well as the dopamine responsive dystonia [7].

Parkinson's disease is a neurological disease related to the central nervous system wherein the movement of the body is affected, which is sometimes accompanied by tremors. This disease is more common in the people belonging to the age group of 45-60 and above. This disease is not curable but the condition can only be improved by the proper medication. Parkinson's disease is a neurological disease related to the central nervous system wherein the movement of the body is affected, which is sometimes accompanied by tremors. This disease is more common in the people belonging to the age group of 45-60 and above. This disease is not curable but the condition can only be improved by the proper medication [8].

PubChem being the online database for the chemical structures, the phytochemicals which were downloaded using IMPPAT, the phytochemical structure and canonical smiles were downloaded as PubChem gives proper data for each input these ids and smiles were then again verified using Classifyfire which is a database, which basically aims at classifying the compound's family and subgroups. This classification helps in understanding the classification of each phytochemical and its similarities with other phytochemicals of other plants. Whereas the PubChem is an online database which is designated to solely give information related to the chemical information, having its resource at the U. S. National Centre for Biotechnology Information (NCBI). This was launched in the year 2004. PubChem organizes the data into three inter-linked databases, which includes Substance, Compound and Bioassay [9]. PubChem is the public repository for information on chemicals [10]. The desire to classify almost all the chemical compounds up to the subfamily and subclass was made possible through a comprehensive, flexible and completely computable as well as purely structure-based chemical taxonomy along with a computer program was made into existence. ClassiFire having data for almost ~4800 different categories of chemical compounds, and up to 11 different levels of categories of classification whereas each category is defined as per the nomenclature and in English. The database is freely accessible [11].

Mucuna pruriens (Linn.)

L-dopa is naturally present in the Mucuna pruriens (Linn.) this is commonly known as The Magic Velvet Beans or Kauchbee in Hindi. The plant belongs to the Leguminosae family; subfamily Papilionaceae plant plays a major role in the treatment of Parkinsonism [12].

REFERENCES

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The amount of levodopa present in the seeds are quantified using HPLC densitometry and the concentration ranges from 0.58-6.42(%, dr. wt.) [13, 14].

**Cassia fistula (Linn.)**

The tree is commonly known as the golden shower tree, and is usually known for its medicinal purposes, also showing the mild laxative property which was found effective in children and pregnant ladies. In the traditional medicines this plant is known in Ayurveda to Unani and is used as infusion, decoction or powder [15]. The seeds of cassia fistula contain nearly about 24% of protein which is higher than most of the plants having protein and is also considered as the important source of lectins [16]. According to the Pharmacological studies, the plant is used for Central Nervous System Activities apart from other medicinal uses [17].

**Lathyrus odoratus (Linn.)**

The plant is represented by more than 200 species worldwide, which includes 75 taxa, of which 18 species are endemic. The plant shows various therapeutic properties due to its pharmacological activities like anti-oxidant, anti-cancer, anti-inflammatory etc. The plant also has nutraceutical properties as well [18].

**Glycine max (L. Merr.)**

Soybean or (Glycine max L. Merr.) is considered as the fourth most important crop in the world when it comes to harvesting and production. It is also considered as one of the cheapest sources of oil as well as the protein source [19]. The plants show various therapeutic properties due to its pharmacological activities like anti-oxidant, anti-cancer, anti-inflammatory etc. The plant also has nutraceutical properties as well [20, 21].

**Mimosa pudica (L.)**

Mimosa belonging to the family Mimosae, commonly known as the Lajvanti or chumui in local Hindi language and touch me not plant in English [22]. The plant is identified to have antiasthmatic, aphrodisiac, analgesic, and anti-depressant properties. The phytochemical studies reveal the presence of alkaloids and flavonoids, including some non-protein amino acids like Mimosine. The plant shows the active presence of terpenoids, flavonoids, Glycosides, alkaloids etc. [23].

**Tecoma stans (L.) Juss Ex. Kunth**

Tecoma belonging to the family Bignoniaceae is also commonly known as the yellow elder tree, which exactly comes under erect shrub or a small tree [24].

The plant has numerous monoterpene alkaloids, triterpenoids and phenolics, like chlorogenic, caffeic, vanillic etc. The bioactive compounds that are present are exhibiting various pharmacological activities for e.g. Antioxidant, antimicrobial and antifungal activities [25].

**Phytochemical profiling**

**Collection of plants**

The selected plant seeds were purchased online through Amazon.com, Inc. (https://www.amazon.in/) [26], through a verified seller of horticulture grade.

**Sample preparation**

**Antibacterial activity**

The plant seeds were made into fine powder using the home grinder. The fine powder obtained was stored in the airtight containers. This powder was used for testing the antibacterial activity.

**Estimation of Levodopa**

The seed powder was used for the estimation of the compound. The mobile solvent was prepared with acetonitrile: water in the ratio 50:50, the extraction solvent was also kept the same.

**RESULTS**

**Estimation of levodopa in plant samples**

The retention time for \( \text{M. pruriens} \) was noted down to be 6.132 with a tail factor of 1.82 whereas the concentration was observed in mAU of 22366.000; therefore the amount of levodopa was quantified from the fig. 1 as well as from fig. 7 and was found out to be 705.5985µg/20 µl.

The retention time for \( \text{C. fistula} \) was noted down to be 6.682 with a tail factor of 1.59, whereas the concentration was observed in mAU of 5695.000, therefore the amount of levodopa quantified from the fig. 2 as well as from fig. 7 and was found out to be 257.8075µg/20 µl.

The retention time for \( \text{L. odoratus} \) was noted down to be 6.832 with a tail factor of 1.56, whereas the concentration was observed in mAU of 100,000, therefore the amount of levodopa quantified from the fig. 3 as well as from fig. 7 and was found out to be 100.000µg/20 µl.

The retention time for \( \text{G. max} \) was noted down to be 6.948 with a tail factor of 1.46, whereas the concentration was observed in mAU of 100,000; therefore the amount of levodopa was quantified from the fig. 4 as well as from fig. 7 and was found out to be 100.000µg/20 µl.

The retention time for \( \text{M. pudica} \) was noted down to be 6.422 with a tail factor of 1.32, whereas the concentration was observed in mAU of 5062.000, therefore the amount of levodopa was quantified from the fig. 5 as well as from fig. 7 and was found out to be 192.0065 µg/20 µl.
Fig. 1: Quantification of L-dopa present in the seeds of M. pruriens

Fig. 2: Quantification of L-dopa present in the seeds of C. fistula

Fig. 3: Quantification of L-dopa present in the seeds L. odoratus
The retention time *T. stans* was noted down to be 6.788 with a tail factor of 1.55 whereas the concentration was observed in mAU of 100,000, therefore the amount of levodopa was quantified from the fig. 6 as well as from fig. 7 and was found out to be 100 µg/20 µl.
The concentration of the compound was calculated using the standard graph of L-dopa, which was prepared using the pure L-dopa of HPLC grade.

The quantification of levodopa was done through reverse phase HPLC, using Acetonitrile: water in the ratio 50:50 as the mobile phase. After the run time of 15 min and temperature set to 35 °C, the tail factor as well as the retention time of the compounds were analyzed with the standard graph of Levodopa. A graph was also plotted for retention time vs tail factor to understand the difference between the plant species in this perspective.

HPLC was conducted on all the six plants, whereas *T. stans* belonging to entirely different family and sub-class i.e. Bignoniaceae and gamopetalae whereas rest of the five plants belongs to the family of Leguminosae and sub-class polypetalae.

The concentration of the compound was calculated with the standard, where Mucuna pruriens showed the presence of the compound in the quantifiable quantity, whereas the other plant samples neither matched the retention time as well as the tail factor with the standard as well as the Mucuna pruriens. The concentration of the compound was also not in the quantifiable quantity.

The concentration in *M. pruresis* was found to be 705.5985 µg/20 µl [table 1]

For the further understanding of the absence of levodopa in other plant samples, *in silico* methods were adopted for better understanding. The result showed no presence of similarity in the phytochemicals present in them. Showing no similarity even among the plant samples belonging to the family Leguminosae.

Drug family was identified using Classyfire to verify with ChemMine and Cytoscape.

### Statistical analysis of the data

For further analysis statistical analysis was conducted on the data [table 2], the main aim to do the analysis was to understand the significance of plants in relation to the compound obtained.

### Anti-microbial activity towards selected bacteria

Following colonies were seen [table 3] for *E. coli* and *Bacillus*, for the following concentration of the seed powder of *M. pruresis*
Fig. 9: Graphical representation of concentration of Levodopa

Table 2: Tests of between-subjects effects ANOVA-one way dependent variable: plant species

| Source          | Type III sum of squares | df | Mean square | F     | Sig. |
|-----------------|-------------------------|----|-------------|-------|------|
| Corrected Model | 12.833                  | 3  | 4.278       | 1.833 | .372 |
| Intercept       | 45.633                  | 1  | 45.633      | 19.557| .048 |
| L-Dopa Conc.    | 12.833                  | 3  | 4.278       | 1.833 | .372 |
| Error           | 4.667                   | 2  | 2.333       |       |      |
| Total           | 91.000                  | 6  |             |       |      |
| Corrected Total | 17.500                  | 5  |             |       |      |

R Squared = .733 (Adjusted R Squared = .333)

a = Corrected model with the significance value of 0.372 which is greater than 0.05 thus, P>0.05 i.e. there is no significance, b = Intercept with the significance value of 0.048 which is lesser than 0.05 thus P<0.05 i.e. there is significance.

Table 3 Colonies for M. pruriens

| Time in min | 0.1g Plant sample | 0.5g Plant sample | 1.0g Plant sample | 0.1g Plant sample | 0.5g Plant sample | 1.0g Plant sample | Control |
|-------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|---------|
| 30          | 73                | 0.0               | 0.0               | 84.0              | 5.0               | 247.0             | 378.0   |
| 60          | 80                | 0.0               | 0.0               | 129.0             | 9.0               | >500              | 528.0   |
| 120         | 119               | 0.0               | 0.0               |                   |                   |                   |         |

Following colonies were seen [table 4] for E. coli and Bacillus, for the following concentration of the seed powder of C. fistula

Table 4: Colonies of C. fistula

| Time in min | 0.1g Plant sample | 0.5g Plant sample | 1.0g Plant sample | 0.1g Plant sample | 0.5g Plant sample | 1.0g Plant sample | Control |
|-------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|---------|
| 30          | 584.0             | >500              | 4.0               | >500              | >500              | >500              | 4.0     |
| 60          | >500              | >500              | 32.0              | >500              | >500              | >500              |         |
| 120         | >500              | >500              | 138.0             | >500              | >500              | >500              | 1459    |

Following colonies were seen [table 5] for E. coli and Bacillus, for the following concentration of the seed powder of L. odoratus.

Table 5: Colonies of L. odoratus

| Time in minutes | 0.1g Plant sample | 0.5g Plant sample | 1.0g Plant sample | 0.1g Plant sample | 0.5g Plant sample | 1.0g Plant sample | Control |
|-----------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|---------|
| 30              | 584.0             | >500              | 32                | >500              | >500              | >500              | 4.0     |
| 60              | >500              | >500              | 138               | >500              | >500              | >500              |         |
| 120             | >500              | >500              | 152               | >500              | >500              | >500              | 1496.0  |

Following colonies were seen [table 6] for E. coli and Bacillus, for the following concentration of the seed powder of G. max.

Table 6: Colonies of G. max

| Time in min | 0.1g Plant sample | 0.5g Plant sample | 1.0g Plant sample | 0.1g Plant sample | 0.5g Plant sample | 1.0g Plant sample | Control |
|-------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|---------|
| 30          | 40.0              | 96.0              | 48.0              | 72.0              | 80.0              | 80.0              | 59.0    |
| 60          | 80.0              | 72.0              | 144.0             | 56.0              | 64.0              | 144.0             |         |
| 120         | 80.0              | 80.0              | 124.0             | 112.0             | 104.0             | 120.0             | 80.0    |

Following colonies were seen [table 7] for E. coli and Bacillus, for the following concentration of the seed powder of M. pudica.
Table 7: Colonies of M. pudica

|          | E. coli | Bacillus |
|----------|---------|----------|
|          | Time in min | Plant sample | Plant sample | Plant sample | Plant sample | Plant sample | Control |
| 30       | 0.1g     | 80.0       | 88.0         | 136.0        | >500         | >500         | >500    | 50.0    |
| 60       | 0.5g     | 64.0       | 88.0         | 120.0        | >500         | >500         | >500    | -       |
| 120      | 1.0g     | 56.0       | 80.0         | 96.0         | >500         | >500         | >500    | 80.0    |

Following colonies were seen [table 8] for E. coli and Bacillus, for the following concentration of the seed powder of T. stans.

Table 8: Colonies of T. stans

|          | E. coli | Bacillus |
|----------|---------|----------|
|          | Time in min | Plant sample | Plant sample | Plant sample | Plant sample | Plant sample | Control |
| 30       | 0.1g     | 104.0      | 168.0        | 176.0        | 200.0        | 192.0        | 144.0   | 200.0   |
| 60       | 0.5g     | 120.0      | 160.0        | 224.0        | 224.0        | 192.0        | 272.0   | -       |
| 120      | 1.0g     | 112.0      | 128.0        | 184.0        | 208.0        | 156.0        | 224.0   | 400.0   |

Following colony forming units were seen [table 9] for E. coli and Bacillus, for the following concentration of the seed powder of M. prureins.

Table 9: Colony forming units for M. prureins

|          | E. coli | Bacillus |
|----------|---------|----------|
|          | Time in min | Plant sample | Plant sample | Plant sample | Plant sample | Plant sample | Control |
| 30       | 0.1g     | 13.1644    | -            | -            | -            | -            | 11.9031 |
| 60       | 0.5g     | 13.2041    | -            | -            | -            | -            | -       |
| 120      | 1.0g     | 13.3766    | 12.5051      | 13.4116      | 12.2553      | -            | 14.0237 |

Following colony forming units were seen [table 10] for E. coli and Bacillus, for the following concentration of the seed powder of C. fistula.

Table 10: Colony forming units for C. fistula

|          | E. coli | Bacillus |
|----------|---------|----------|
|          | Time in min | Plant sample | Plant sample | Plant sample | Plant sample | Plant sample | Control |
| 30       | 0.1g     | 14.2294    | -            | -            | -            | -            | 11.9031 |
| 60       | 0.5g     | 14.0674    | -            | -            | -            | -            | -       |
| 120      | 1.0g     | -          | -            | -            | -            | -            | 14.4760 |

Following colony forming units were seen [table 11] for E. coli and Bacillus, for the following concentration of the seed powder of L. odoratus.

Table 11: Colony forming units for L. odoratus

|          | E. coli | Bacillus |
|----------|---------|----------|
|          | Time in min | Plant sample | Plant sample | Plant sample | Plant sample | Plant sample | Control |
| 30       | 0.1g     | 12.9031    | 13.2833      | 12.9823      | 13.51584     | 13.1072      | 13.3802  |
| 60       | 0.5g     | 13.2041    | 13.1584      | 13.4594      | 13.0492      | 13.3181      | 13.3802  |
| 120      | 1.0g     | -          | 13.3181      | 13.4082      | 13.4082      | 13.6021      | 13.2041  |

Following colony forming units were seen [table 12] for E. coli and Bacillus, for the following concentration of the seed powder of G. max.

Table 12: Colony forming units for G. max

|          | E. coli | Bacillus |
|----------|---------|----------|
|          | Time in min | Plant sample | Plant sample | Plant sample | Plant sample | Plant sample | Control |
| 30       | 0.1g     | 13.2041    | 13.2455      | 13.4346      | -            | -            | 13.0000  |
| 60       | 0.5g     | 13.1072    | 13.2455      | 13.3802      | -            | -            | -       |
| 120      | 1.0g     | 13.0492    | 13.2041      | 13.2833      | -            | -            | 13.2041  |

Following colony forming units were seen [table 13] for E. coli and Bacillus, for the following concentration of the seed powder of M. pudica.

Table 13: Colony forming units for M. pudica

|          | E. coli | Bacillus |
|----------|---------|----------|
|          | Time in min | Plant sample | Plant sample | Plant sample | Plant sample | Plant sample | Control |
| 30       | 0.1g     | 13.2041    | 13.2455      | 13.4346      | -            | -            | -       |
| 60       | 0.5g     | 13.1072    | 13.2455      | 13.3802      | -            | -            | 13.0000  |
| 120      | 1.0g     | 13.0492    | 13.2041      | 13.2833      | -            | -            | 13.2041  |
DISCUSSION

The estimation of L-dopa was carried out in *M. pruriens* using HPLC, and found that it contains 2% of Levodopa by weight, Levodopa is commercially used for the treatment of Parkinson’s disease [32].

Estimation of Levodopa through HPLC method is considered to be the most convenient, simple, and accurate method [33].

It was observed that the other samples did not contain Levodopa, and the results were statistically analyzed using the one-way ANOVA test, no significance difference was observed in the present study between the plant species and the concentration of the Levodopa.

The result was further clarified by Cytoscope and ChemMine where, the phytochemicals of all the plant species were checked for similarity and showed no similarity in the phytochemical class, though they belong to the same family Leguminosae.

Thus, further studies can be conducted to understand the formation of L-dopa in *M. pruriens*. It takes place through the shikimic pathway, converting the simple carbohydrate precursors, which are derived from the glycolysis and pentose phosphate pathway to aromatic amino acids tyrosine, phenylalanine, tryptophan. Whereas the plants belonging to the same family does not show any similarity in the presence of phytochemicals as well as the compound [34]. The compound L-dopa belongs to the family of catecholamines, which are the aromatic compounds with a phenyl ring and 3,4-hydroxy groups. This catecholamines are active compounds in the plant, but these when enter the human body, behave as neurotransmitters [35].

The other plant samples though from the same family, shows absence of the active compound mainly because of the absence of carboxylic derivatives, in them. This L-dopa not only is useful to humans in terms of a neurotransmitter but also the synthetically synthesized levodopa has adverse effects on human health, whereas the Levodopa extracted from plants is twice more effective and has probably no side-effects [36]. The compound is found effective for the treatment of the disease, as per the study conducted on 170 patients between November 1969-December 1972, the drug showed improvement in 126 patients, thus making its potentiality marked as the drug which can improve the conditions of PD patients [37].

The concentration of levodopa was higher in *M. pruriens* than in any other plant; the cotyledon powder is the significant source of the compound levodopa. The other plant samples though from the same family Leguminosae, belonging to the same family does not show any similarity and showed no similarity in the phytochemical class, though they belong to the same family Leguminosae.

The plants showed positive results in the microbial test, no significance difference was observed in the present study between the plant species and the concentration of the Levodopa.

As the present studies state, that the active compound Levodopa is only seen present in *M. pruriens*, the formation of this compound is through the shikimate pathway. Since 5 plants were studied from the same family and 1 plant from another family as a comparative standard, showed no presence of the active compound, further studies can be conducted in understanding the formation of the compound whereas the Genes responsible for it.

The anti-microbial activity of the plants towards the selected bacteria proved to be positive as it is mainly due to the presence of flavonoids and other compounds which inhibit the bacteriostatic property. Further studies can be conducted in isolating the compounds responsible for the bacteriostatic property.

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CONFLICT OF INTERESTS

Declare none

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