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Research Article

### IN-VITRO EVALUATION OF ANTI-PARKINSONISM ACTIVITY OF *Musa acuminata* 'Red Dacca'

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#### Abstract:

The present study aimed to evaluate the anti-Parkinson's potential of ethanolic extract of *Musa acuminata* 'Red Dacca' fruit using in vitro methods. *Musa acuminata* 'Red Dacca' fruit were collected, chopped, shade dried and then finally powdered.

The ethanolic extract of *Musa acuminata* 'Red Dacca' were prepared by using ethanol followed by maceration method. Preliminary phytochemical screening was performed by using standard operating procedures. The anti-Parkinson's activity of plant extract was assessed by using in vitro studies. In vitro studies are performed by 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide [MTT assay].

The preliminary phytochemical analysis indicated the presence of amino acids, phenolic compounds, carbohydrates, flavonoids. The extract inhibits cytotoxicity. In this study, the results demonstrated that the ethanolic extract of *Musa acuminata* 'Red Dacca' fruit possess anti-Parkinson's activity.

**Keywords:** Anti-Parkinson's activity, *Musa acuminata* 'Red Dacca', In vitro studies, Dopamine, Chemical Constituents, MTT assay.

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**INTRODUCTION:**

Medicinal flora represents natural resources utilized for their therapeutic attributes, frequently employed in the treatment or prevention of a range of health conditions. These flora, encompassing herbs, shrubs, and trees, have played a pivotal role in traditional medical practices throughout history, with numerous species now acknowledged by scientific communities for their medicinal advantages. They possess active constituents, such as alkaloids, flavonoids, and essential oils, which may exhibit antibacterial, anti-inflammatory, antioxidant, and other health-enhancing effects.<sup>[1]</sup>

Parkinsonism is essentially a clinical syndrome brought about by combining a number of motor symptoms that include tremors, muscle stiffness or rigidity, bradykinesia or slowness of movement, and postural instability. Such symptoms usually result from the dysfunction or degeneration of dopamine-producing neurons in a specific region of the brain called substantia nigra<sup>[2]</sup>. Parkinsonism is closely associated with Parkinson's disease as the primary cause of the disease but can also be caused by several other diseases like multiple system atrophy, progressive supranuclear palsy, or due to drug-induced parkinsonism. The symptoms of the disease are generally so progressive that they seriously impair a person's ability to lead an everyday life and bring down a person's quality of life in general.<sup>[3]</sup>

*Musa acuminata* 'Red Dacca', also known as Red banana or *Musa acuminata* colla (AAA) 'Red', is one of the known banana cultivars belonging to the family Musaceae. It is one of the many distinct cultivars that come with bright red to purple skin and sweet creamy flesh that has berry-like undertones<sup>[4]</sup>. Originating in Southeast Asia and closely related to India-where it is highly prized in both culinary and cultural senses the banana plant has spread so widely in tropical and subtropical regions around the world.

*Musa acuminata* 'Red Dacca' contains variety of chemical constituents that contribute to their nutritional and medicinal properties. It consists of natural sugar (glucose, fructose, sucrose); dietary fibre(pectin), vitamins(Vit C, Vit B, Vit A, Vit B9), minerals(potassium, magnesium, iron, calcium) It contains phytochemicals such as anthocyanin(a type of flavonoids) tannins (polyphenolic compounds), phenolic acids(ferulic acid).It consists of amino acids, the building block of proteins, while bananas are not a major protein source, they still contribute to overall protein intake. Amino acids such as tryptophan, tyrosine, serine, proline, alanine are found. It is also

imbued with potent anti-oxidants. It is also imbued with potent antioxidant like beta-carotene, lutein and zeaxanthin contains low levels of fats, with small proportion of both saturated and unsaturated fatty acids<sup>[5]</sup>.



**Figure No 1: *Musa acuminata* 'Red Dacca' fruit**

**MATERIALS AND METHODS:****MATERIALS**

- Ethanol
- Bromine Water
- Millon's Reagent
- Biuret Reagent
- Magnesium powder
- Hydrochloric acid
- Fehling's solution A
- Fehling's solution B
- Dragendorff's reagent
- Ferric chloride solution

**METHODS****Collection and authentication of Plant Materials**

The fruits were collected from certain areas of Kanyakumari district, Tamilnadu India in the month of November. The plant material was identified and authenticated from Department of Botany, Christian College, Kattakada. Then, the sample is thoroughly washed with fresh water and air dried at room temperature under shade for 15 days and pulverized into coarse powder by the aid of mechanical grinder. The powder material was stored in airtight container.



**Figure No 2: Dried powdered fruits of *Musa acuminata* 'Red Dacca'**

### Preparation of ethanolic fruit extract of *Musa acuminata* 'Red Dacca'<sup>[6]</sup>

- *Musa acuminata* 'Red Dacca' were washed under running tap water and dried under shade for 15 days and pulverized into coarse powder by the aid of mechanical grinder
- In which 500g of banana peel powder was macerated with 5 L of 95% ethanol.
- The mixture is continuously shaken at room temperature.
- After 1 week, the extract was strained with muslin cloth and the crude extract was concentrated to dryness under reduced pressure and controlled temperature and stored in airtight container for further use.
- The percentage yield of the extractions was calculated.



**Figure No 3: Ethanolic fruit extract of *Musa acuminata* 'Red Dacca'**

### Calculation of percentage yield

The percentage yield of extract was calculated using the formula.

$$\text{Percentage yield} = \frac{w_2}{w_1} \times 100$$

Where,

W1- Weight in grams of dried plant material.

W2- weight in grams of extracts obtained.

### Preliminary Phytochemical screening<sup>[7]</sup>

#### 1. Test for Amino acids

##### a. Biuret test

To 2ml of extract biuret reagent is added dropwise.

##### Test for tyrosine

##### b. Millon's Test

1ml of extract is taken in test tube. To it Millon's reagent is added and shown in flame.

#### 2. Test for phenolic compounds.

##### a. Bromine water test

Drops of bromine water is added to the test tube containing extract.

#### 3. Test for flavonoids

##### a. Shinoda test:

Add magnesium powder and few drops of concentrated hydrochloric acid or sulphuric acid to 2ml of extract.

#### 4. Test for tannins:

##### a. Ferric chloride test:

Mixed 2ml of the extract with few ml of 5% ferric chloride solution.

### *Invitro study of Musa acuminata* 'Red Dacca' *Invitro neuroprotective effect determination by MTT assay*<sup>[8]</sup>

**SHSY-5Y (Neuroblastoma cells)** cell line was purchased from NCCS Pune was maintained in Dulbecco's Modified Eagles media (HIMEDIA) from National Centre for Cell Sciences (NCCS), Pune, India.

The cell line was cultured in 25cm<sup>2</sup> tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO<sub>2</sub> incubator (NBS Eppendorf, Germany).

The viability of cells was evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.



**Figure No 7: CO<sub>2</sub> Incubator**

**Cells seeding in 96 well plate:**

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension ( $5 \times 10^3$  cells/well) was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator.

**Preparation of compound stock:**

1mg of the sample was weighed and completely dissolved in 1ml DMEM using a cyclomixer. The extract solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility. Rotenone(10µm) was added to induce toxicity.

**Cytotoxicity Evaluation**

After attaining sufficient growth, Rotenone(10µm) was added to induce toxicity and incubated for one hour. After 1 hours, freshly prepared each compound were added at concentrations of 25 µg/ml, 12.5 µg/ml, 6.25µg/ml, 3.1 µg/ml and 1.5 µg/ml of DMEM. Each concentration was added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator. Untreated control cells and Rotenone alone treated wells were also maintained.

**Cytotoxicity Assay by Direct Microscopic observation:**

Entire plate was observed after 24 hours of treatment in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images.

Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.



Figure No 8: Phase contrast microscope

**Cytotoxicity Assay by MTT Method:**

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator for 4 hours. After incubation period, the supernatant was removed and 100µl of MTT solubilization solution (DMSO) was added to solubilize the formazan crystals.

The absorbance value was measured by using microplate reader at wavelength of 540nm<sup>[9]</sup>.



Figure No 9: Microplate reader

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ viability} = \frac{\text{Mean OD Samples} \times 100}{\text{Mean OD of control group}}$$

**RESULTS:****Preparation of fruit extract of *Musa acuminata* 'Red Dacca' and the calculation of percentage yield.**

The fruits were collected from certain areas of Kanyakumari district, Tamil Nadu, India. The powdered plant material was extracted with ethanol for 72 hours by using maceration. Then it is stored in air tight container until further use. The percentage yield of the fruit extract is shown in Table No. 1

**Preliminary phytochemical screening**

The fruit extract of *Musa acuminata* 'Red Dacca' were carried out chemical test for identification of active constituents. The presence of amino acids, flavonoids, alkaloids, tannins, carbohydrates was determined by

conducting chemical test such as Millon's test, Shinoda test, Dragendorff's test, ferric chloride test, Fehling's test. Summary of preliminary phytochemical screening of ethanolic fruit extract of *Musa acuminata* 'Red Dacca' is shown in the table No.2

#### A. Test for flavonoids:

##### Shinoda test:

In Shinoda test, appearance of Yellow to orange colourization indicates the presence of flavonoids.



Figure No 10: Result of Shinoda test

#### C. Test for tannins:

##### Ferric chloride test:

In ferric chloride test, appearance of dark bluish green colourisation indicates the sample contains tannins.

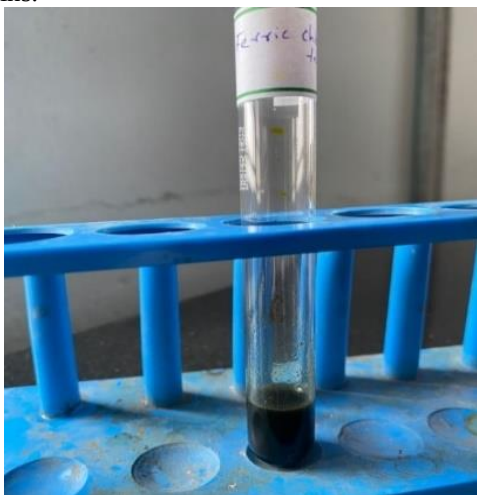


Figure No 11: Result of Ferric chloride test

#### D. Test for Amino acids

##### Biuret test:

In biuret test, appearance of violet colourisation indicates the presence of amino acids.

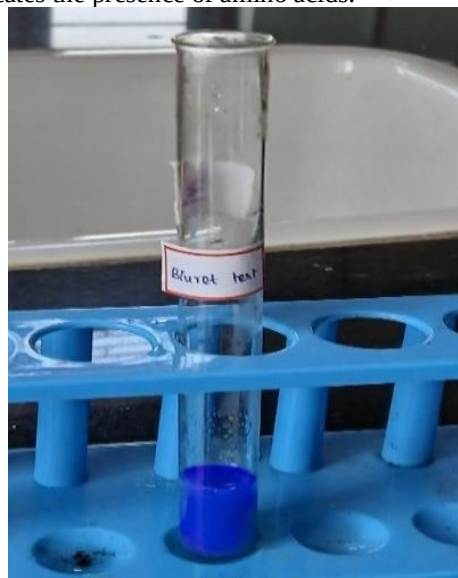


Figure No 12: Result of Biuret test

#### E. Test for tyrosine

##### Millon's Test:

In Millon's test, appearance of reddish-brown precipitate indicates the presence of tyrosine.



Figure No 13: Result of Millon's test

**F. Test for phenolic compounds.**Bromine water test:

In bromine water test, appearance of white cloudy precipitate indicates the presence of phenols.



**Figure No 15: Result of Bromine water test**

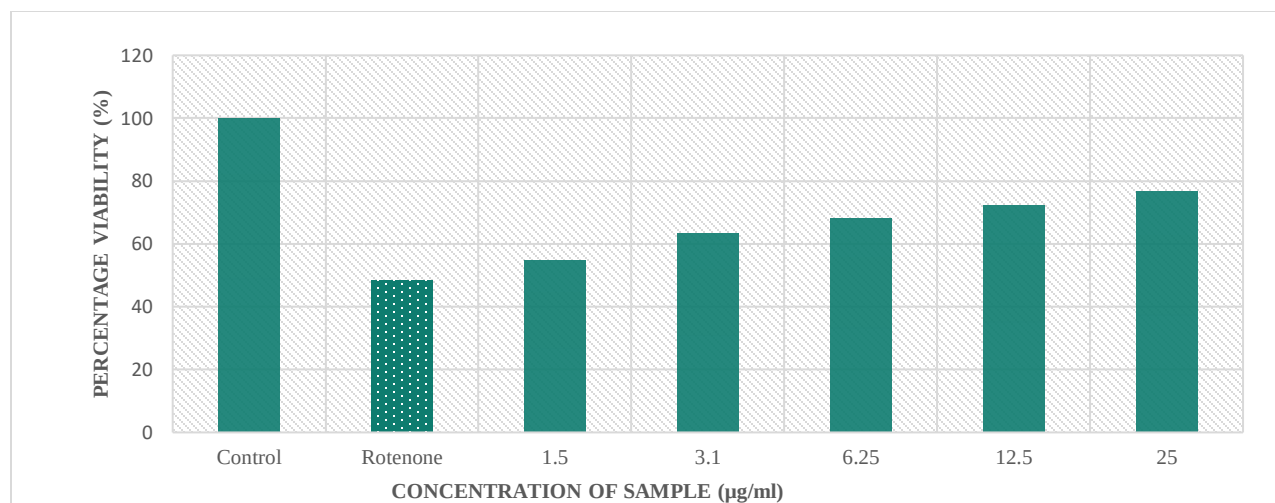
**Invitro analysis of *Musa acuminata* 'Red Dacca'****Invito neuroprotective effect determination by MTT assay**

At increasing concentrations, ethanolic extract of fruit of *Musa acuminata* 'Red Dacca' effectively provides neuroprotective effects, as there is increase in percentage cell viability is seen. Thus, the sample studied exhibited neuroprotective effects i.e. the sample has anti-Parkinsonism property. This is an indicator that the test sample has the potential to be developed as anti- Parkinsonism drugs.

EEFMART - Ethanolic Extract of fruit  
*Musa acuminata* 'Red Dacca'

**Graphical representation****Statistical analysis:**

| Cell line - SHSY5Y |        |        |        |                        |                        |                        |         |                    |           |
|--------------------|--------|--------|--------|------------------------|------------------------|------------------------|---------|--------------------|-----------|
| EEFMART            |        |        |        |                        |                        |                        |         |                    |           |
|                    | OD1    | OD2    | OD3    | Percentage viability 1 | Percentage viability 2 | Percentage viability 3 | Average | Standard Deviation | Std error |
| Control            | 0.3984 | 0.4015 | 0.401  | 100                    | 100                    | 100                    | 100     | 0                  | 0         |
| Rotenone           | 0.1845 | 0.1995 | 0.1984 | 46.3102                | 49.6887                | 49.4763                | 48.4917 | 1.89221            | 1.09247   |
| 1.5                | 0.2252 | 0.2135 | 0.2174 | 56.5261                | 53.1756                | 54.2145                | 54.6387 | 1.71507            | 0.9902    |
| 3.1                | 0.2563 | 0.2474 | 0.2549 | 64.3323                | 61.6189                | 63.5661                | 63.1724 | 1.39887            | 0.80764   |
| 6.25               | 0.2618 | 0.2765 | 0.2796 | 65.7129                | 68.8667                | 69.7257                | 68.1018 | 2.11296            | 1.21992   |
| 12.5               | 0.2956 | 0.2845 | 0.2867 | 74.1968                | 70.8593                | 71.4963                | 72.1841 | 1.77189            | 1.023     |
| 25                 | 0.3054 | 0.3025 | 0.3121 | 76.6566                | 75.3425                | 77.8304                | 76.6098 | 1.24464            | 0.71859   |



**Effect of ethanolic fruit extract of *Musa acuminata* 'Red Dacca' in MTT assay**

### DISCUSSION:

Medicinal plants have been used for centuries for their therapeutic properties, and many have gained scientific recognition for their health benefits. *Musa acuminata* 'Red Dacca', a red banana cultivar, is known for its rich phytochemical composition, making it a subject of interest in modern medicine. This study explores the potential of its ethanolic fruit extract in combating Parkinsonism, a neurological disorder primarily caused by dopamine neuron degeneration. The research involved the extraction of bioactive compounds from *Musa acuminata* 'Red Dacca' using ethanol and subsequent phytochemical screening to identify key constituents. The extract was found to contain antioxidants, flavonoids, tannins, phenolic acids, and neuroprotective amino acids like tyrosine, which may contribute to its medicinal properties. To test its potential neuroprotective effects, the study used SH-SY5Y neuroblastoma cells, a widely accepted in vitro model for Parkinson's disease research. Rotenone, a neurotoxin known to induce Parkinson-like damage, was used to create a toxic environment, and the cells were treated with different concentrations of red banana extract to assess its protective effects.

### CYTOTOXICITY ASSAY BY MTT METHOD

The MTT assay was used to evaluate cell viability, revealing that the extract had a dose-dependent protective effect against rotenone-induced toxicity. At higher concentrations, the extract significantly increased cell survival rates, indicating antioxidant and cytoprotective properties. These findings suggest that *Musa acuminata* 'Red Dacca' may help in reducing oxidative stress, preventing dopamine neuron

damage, and enhancing cell survival in Parkinson's disease models. However, while the in vitro results are promising, further in vivo and clinical studies are needed to confirm its efficacy in human subjects.

In conclusion, this study highlights the potential of *Musa acuminata* 'Red Dacca' as a natural neuroprotective agent, offering hope for new treatment strategies against Parkinsonism. The presence of antioxidants, bioactive phytochemicals, and essential nutrients supports its role in brain health and neurodegeneration prevention. While traditional medicine has long utilized red bananas for their health benefits, modern research now provides scientific validation for their therapeutic properties. Future studies should explore mechanisms of action, bioavailability, and long-term effects, paving the way for natural, plant-based interventions in neurodegenerative diseases.

### CONCLUSION:

The findings of this study highlight the potential neuroprotective properties of *Musa acuminata* 'Red Dacca' particularly in combating oxidative stress and neuronal degeneration associated with Parkinsonism. The presence of bioactive compounds such as flavonoids, phenolic acids, and tyrosine suggests that this red banana cultivar may support dopamine regulation and enhance neuronal survival. The in vitro results, demonstrating increased cell viability in rotenone-exposed neuroblastoma cells, provide a strong foundation for further exploration of its therapeutic benefits. While the study establishes a

promising link between *Musa acuminata* ‘Red Dacca’ extract and neuroprotection, its full clinical relevance remains to be determined through in vivo studies and human trials.

Moving forward, research should focus on understanding the precise mechanisms through which *Musa acuminata* ‘Red Dacca’ exerts its neuroprotective effects, its bioavailability in the human body, optimal dosage, and long-term safety profile. If future studies validate its efficacy, *Musa acuminata* ‘Red Dacca’-based formulations could emerge as natural adjuncts to existing Parkinson’s treatments, potentially offering a safer, plant-derived alternative for neuroprotection. This research bridges the gap between traditional medicinal knowledge and modern scientific validation, reinforcing the importance of natural compounds in addressing neurodegenerative diseases.

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