In-Vitro Evaluation of Anti-mitotic Activity of Different Extracts of Mucuna pruriens Linn. Seed

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ABSTRACT

Mucuna pruriens is a medicinal plant with therapeutic potential, belongs to the family Fabaceae, also known as monkey tamarind or velvet bean, and is known for its anti-mitotic activity. Recently, many biological activities of Mauna Pruriens L. seeds have been reported, including antioxidant, anti-cholesterol, anti-Parkinson, anti-diabetic, sexually enhancing, anti-inflammatory, antimicrobial, anti-cancer, and antivenom activities. In this study, several seed extracts from Mauna pruriens L. have been tested for Antimitotic activity. The assay results concluded that the ethanolic seed coat extract and ether seed coat extract of Mauna pruriens showed inhibition of green gram seed growth of 7.2±4.6 mm and ether seed coat extract showed inhibition of 0.01±1.5mm with a concentration of 20mg/ml after 24hrs, 48hrs and 72hrs, compared with percentage inhibition of standard Methotrexate of 0.001±1.1mm with a concentration of 0.1. Among all the seed extracts, ether extract showed more anti-mitotic activity compared with ethanolic extract. In the point of view of seed germination the control, i.e., distilled water, has shown higher seed germination compared to standard and other ethanolic and ether seed coat extracts.

Keywords: In-vitro Anti-mitotic activity, Mauna Pruriens, L. seed. Methotrexate, Green Gram.

INTRODUCTION

Mucuna prurience Plants have been used as an excellent source of medicine from the outset, which established a foundation of traditional medicine. Such traditional medicinal plants play a vital role in addressing the global health needs of today and their use will increase in the future. Belongs to the family fabaceae, also known as tamarind monkey or velvet bean, and is known for its anti-mitotic activity. In this study, a number of Mauna pruriens L seed extracts were tested for Antimitotic activity. A green gram was used to assess seed sprouting. This entire drug showed Antimitotic activity Methotrexate, vincristine vinblastine, HST-K, apart from which Methotrexate was taken due to good effective inhibited cell growth. I was picked Mauna pruriens having Antimitotic activity, based on the plant three different solvents; water, ether, and ethanol were collected to extract samples. Mauna pruniens seed contains many chemical components that are responsible for the achievement of various physiological and therapeutic responses. The extracts of ethanolic and ether extract are then subjected to the various qualitative tests for the detection of Mauna pruriens seed constituents like alkaloids, glycosides, tannins, carbohydrates, coumarins, saponins, flavonoids, proteins etc. Owing to the side effect of chemical drugs, the use of medicinal plant extracts for the treatment of human diseases has greatly increased in the past few decades. The phytochemical in plants act as a medicine; therefore, plants have been used as a source of medicine for thousands of years. I have been taken standard drug Methotrexate, control water, ethanolic extract, ether extract used for sampling, according to experimental procedures. All these procedure tested on green gram for the purpose of seed germination process.

MATERIALS AND METHODS

Materials

Monkey tamarind seeds were procured from local market. Ether was procured from S.D. Fine chemicals, Mumbai. Methotrexate drug was obtained as a gift sample from Aurobindo pharma Ltd., Hyderabad. All the other chemicals were procured of analytical grade.

Methods

Monkey tamarind seeds of good quality were purchased from local market and they were cleaned properly and sun dried. 500gm of the seeds were taken and converted into a coarse powder, which was later used in preparing extraction processes.

Extraction of seeds

Ether extract

10 g of seeds were weighed and transferred to soxhlet apparatus and the seeds were extracted with ethanolic at 35°C for 3-4 cycles. The extract was collected and the alcohol was evaporated after extraction by using rotary...
evaporator connected to a vacuum pump. The final extract in semi-solid form was dried by placing in desiccators. A rotary evaporator, yielding the extracted compound and their percentage yield is calculated respectively. The extracted crude drug was used for further photochemical evaluation studies.

**Ethanolic extract**

10 g of seeds were weighed and transferred to soxhlet apparatus and the seeds were extracted with ethanolic at 35°C for 3-4 cycles. The extract was collected and the alcohol was evaporated after extraction by using rotary evaporator connected to a vacuum pump. The final extract in semi-solid form was dried by placing in desiccators. A rotary evaporator, yielding the extracted compound and their percentage yield is calculated respectively and used for further the extracted crude drug phytochemical evaluation studies.

\[
\text{Weight of extract} \times 100 \\
\text{Percentage yield (\%w/w)} = \frac{\text{Weight of drug taken}}{\text{Weight of extract}}
\]

**Extractive value**

The extractive values were recorded in different solvents with a view to study the distribution of various constituents Monkey tamarind of seed. Accurately weighed 4.0 g of coarsely powdered air-dried material was placed in a glass stoppered conical flask and macerated with 100 ml of the solvent for 6 hrs, shaking frequently, and then allowed to stand for 16 hrs. The mixture was filtered rapidly taking care not to lose any solvent. 24 ml of the filtrate was transferred to a tarred thin porcelain dish and evaporated to dryness on water bath.

The residue was dried at 103°C for 5 h, cooled in a Desiccators for 30 min, and weighed without delay and Calculated the percentage w/w of extractive with Reference to air-dried drug.

\[
\text{Extractive value (\%)} = \frac{W2-W1}{W2-\text{W1}} \times 100
\]

Where \( W_{1} \) = weight of empty dish
\( W_{2} \) = weight of dish +residue

**Phytochemical Screening**

The process of detection of various constituents in a Monkey tamarind seed extract is known as phytochemical screening. The Monkey tamarind seed contain numerous chemical constituents that are responsible for eliciting various physiological and therapeutic responses. The extracts of ethanolic and ether extract are then subjected to the various qualitative tests for the detection of Monkey tamarind seed constituents like alkaloids, glycosides, tannins, carbohydrates, coumarins, saponins, flavonoids, proteins etc.

**Antimitotic activity**

The anti-mitotic activity of Mucuna prurience

**Seed germination assay**

Seed germination assay was evaluated by using green gram seeds.

**Experimental design**

Green gram seeds were collected from the local market and each seed weighed individually. 10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml, concentrations of seed coat extracts were prepared. Methotrexate was used as standard drug. Distilled water was used as a control. Equal weights of seeds were added in the sample in Petri plates containing different concentration. The Petri plates were left at room temperature for 24hrs for imbibitions of water. After 24hrs and 72 hrs drug treatment dried on dry tissue paper and weighted. The time of sprouting was extended to 72hrs and photographs were taken.

Percentage of inhibition = 
\[
\frac{(\text{weight of dish +residue})}{(\text{wt D-wt E})/((\text{wt D-wt M}) \times 100)}
\]

Where, Wt D = seed weight in distilled water
Wt E = seed weight in extract sample
Wt M = seed weight in Methotrexate

**Table 1:** Drug treatment of green gram seeds in seed germination.

<table>
<thead>
<tr>
<th>Group number</th>
<th>Drug treatment</th>
<th>Concentration (mg/ml)</th>
<th>Treatment schedule (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (distilled water)</td>
<td>0</td>
<td>24 to 72</td>
</tr>
<tr>
<td>2</td>
<td>Standard (Methotrexate)</td>
<td>0.1</td>
<td>24 to 72</td>
</tr>
<tr>
<td>3</td>
<td>Ether extract</td>
<td>10</td>
<td>24 to 72</td>
</tr>
<tr>
<td>4</td>
<td>Ether extract</td>
<td>20</td>
<td>24 to 72</td>
</tr>
<tr>
<td>5</td>
<td>Ethanolic extract</td>
<td>30</td>
<td>24 to 72</td>
</tr>
<tr>
<td>6</td>
<td>Ethanolic extract</td>
<td>40</td>
<td>24 to 72</td>
</tr>
</tbody>
</table>

**RESULTS**

Seed Germination Assay results

The assay results showed that the ethanolic seed coat extract of green gram has significantly increased the percentage inhibition after 24hrs, 48hrs and 72hrs treatment which was comparable with percentage inhibition of Methotrexate.

The ethanolic extract of 40mg/ml concentration showed seed growth of 7.2±4.6mm i.e., percentage inhibition of seed germination of the standard drug-Methotrexate of 0.1mg/ml showed seed growth of 0.001 ±1.1mm.
The assay results showed that the ether seed coat extract of green gram has significantly decreased the percentage of inhibition after 24hrs, 48hrs, and 72hrs, treatment which was comparable with percentage of inhibition of Methotrexate.

The ether extract of concentration 20mg/ml showed seed growth of 0.01±1.5 mm

In the point of view of seed germination, the control i.e., distilled water has shown the higher seed germination compared to standard and other ethanolic and ether seed coat extracts. As the concentration of ethanolic seed coat extracts was increased, the seed growth was found to be decreased. As the concentration of ether seed coat extracts was increased, the seed germination was found to be decreased. Thus, the % inhibition was increased as the concentration of ethanolic and ether seed coat extracts was increased and among the two extracts, ether extract showed more % of inhibition of seed growth, which indicated that the ether extract of Mucuna pruriens showed more anti-mitotic activity and it is compared with the standard drug Methotrexate.

### Table 2: The average seed lengths in control, standard and in extracts after 72 h.

<table>
<thead>
<tr>
<th>Group number</th>
<th>Drug treatment</th>
<th>Concentration (mg/ml)</th>
<th>Treatment schedule (hrs)</th>
<th>Average Seed growth (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control (distilled water)</td>
<td>0</td>
<td>24 to 72</td>
<td>28.5±5</td>
</tr>
<tr>
<td>2.</td>
<td>standard (Methotrexate)</td>
<td>0.1</td>
<td>24 to 72</td>
<td>0.001±1.1</td>
</tr>
<tr>
<td>3.</td>
<td>Ether extract</td>
<td>10</td>
<td>24 to 72</td>
<td>1.4±4.1</td>
</tr>
<tr>
<td>4.</td>
<td>Ether extract</td>
<td>20</td>
<td>24 to 72</td>
<td>0.01±1.5</td>
</tr>
<tr>
<td>5.</td>
<td>Ethanolic extract</td>
<td>30</td>
<td>24 to 72</td>
<td>14.5±10</td>
</tr>
<tr>
<td>6.</td>
<td>Ethanolic extract</td>
<td>40</td>
<td>24 to 72</td>
<td>7.2±4.6</td>
</tr>
</tbody>
</table>

**Figure 1:** Green gram experiment: treatment of Green gram seeds with different solvents a) Green Gram Seeds b) with water (control), c) Ether 20mg/ml, d) Ethanolic 30mg/ml and e) Methotrexate (standard).

**CONCLUSIONS**

This study of the Antimitotic activity of M. pruriens (monkey tamarind) suggests that the plant has a potential Antimitotic activity. This activity showed that the presence of many major chemical compounds that is responsible for obtaining various physiological and therapeutic responses. This plant could therefore be used as a potential source of medicines for the treatment of cancer. It can be concluded that the ethanolic and ether seed coat extract of green gram has significantly increased the percentage inhibition after 24hrs, 48hrs and 72hrs treatment which was
compared with percentage inhibition of Methotrexate. The ether extract of concentration 20mg/ml has shown the Antimitotic activity of 0.01±1.5mm i.e., percentage inhibition of seed germination as significant as the standard drug Methotrexate 0.001mg/ml±1.1mm.

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