Original Article

Phytochemical screening and evaluation of antitumour activity of Mucuna pruriens (L.) DC

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ABSTRACT

This paper represents the phytochemicals present in the shade dried leaf extract of Mucuna pruriens (L.) DC. In this research work qualitative assessment of the presence of different phytochemicals was performed. After that the extract was assessed for antitumour assay following brine shrimp cytotoxicity experiment. The sample/extract was found to be positive in antitumour assay. Hence this extract can be of ecofriendly use to control the tumorous growth of the biological systems.

Introduction

Mucuna pruriens (L.) DC. belonging to family leguminoseae is indigenous to tropical countries. They are found in Asia, America, Africa and the pacific Island, cultivated in some parts for the sake of its golden-brown velvety legumes. All parts of Mucuna pruriens (L.) DC. possess valuable medicinal properties [1-3] and there is a heavy demand of Mucuna in India drug market. After the discovery that Mucuna seeds contains L-dopa, an anti-Parkinson disease drug, its demand in international market has increased many fold [4] and demand has motivated Indian farmers to start commercial cultivation.

Present study involves the phytochemical screening of the leaf extract of Mucuna pruriens (L.) DC. and evaluation of the antitumour activity of the leaf extract of above said plant. This study also incorporates some additional evaluation of this extract as an ecofriendly antitumour agent that can be applied in living biological systems.

Materials and methods

Collection of plant

Whole plant of Mucuna pruriens (L.) DC. was collected in the month of July 2009 from experimental garden of Department of Botany, University of Kalyani, and was identified in the Department of Botany, University of Kalyani, Nadia.

Preparation of the extract

5 kg shade dried leaves of Mucuna pruriens (L.) DC. was grounded to a fine powder and then extracted for three times in 3 liter of 90% aqueous ethanol at room temperature for 7 days in dark [5]. The extract was filtered and concentrated under reduced pressure and dark greenish residue was obtained (up to volume 100 ml). The residual mass obtained was then subjected to phytochemical screening followed by antitumour assay.

Preparation of sample solution

The test solution was prepared by dissolving the dark greenish residue in few drops of propylene glycol and then diluting with sterile water [6] in the concentration of 125 mg ml-1 to 1 mg ml-1. Few drops of propylene glycol diluted with sterile water were used as control. All the dilutions were sterilized by filtration using membrane filter (0.02 μ pore size).

Phytochemical screening of the leaf extract

Screening for Aldehydes and ketones

With 1 ml of the sample, saturated solution of 2, 7-diamino fluorescein is added. To make the solution acetic by capillary 2 to 3 drops of glacial acetic acid is added [7].

Screening for Alkaloids

Boil 2.6 g bismuth carbonate and 7 g sodium iodide with 25 ml glacial acetic acid for a few minutes. After 12 hours filter off the precipitated sodium acetate. Then mix 20 ml of the red-brown filtrate with 80 ml ethyl acetate and add 0.5 ml water. This is the stock solution and it is stored in a dark bottle.

Mix 1 ml stock solution with 100 ml glacial acetic acid, 2.4 ml ethyl acetate and 1 ml of the sample [7].

Screening for Alkaloids and amines

With 1 ml of the sample, add 5 drops 65% nitric acid to 10 ml ethanol. After prolonged heating of the mixture at 120°C inspect it in UV light [7].
Screening for Corticosteroids

2 ml of 50% sulfuric acid and 2 ml of 2% methanolic solution of 4-hydroxybenzaldehyde was mixed freshly before use. Then 1 ml of the sample was added and heated for 3-4 min at 105°C [7].

Screening for Ergot alkaloids

Dissolve 0.125 g of 4-dimethyl amino benzaldehyde in a cooled mixture of 65 ml 97% sulfuric acid and 5 ml water and add 0.05 ml 5% aqueous iron (III) chloride solution. With this mixture add 1 ml of the sample solution [7].

Screening for Essential oils

Dissolve 0.06 g of diphenyl picryl hydrazyl in 10 ml chloroform. With this mixture add 1 ml of the sample. The experimental set was heated for 5-10 min at 110°C [7].

Screening for Flavonoids

With 1 ml of the sample add 25% aqueous solution of basic lead acetate. After heating for 2 min at 650°C it is inspected in the long wavelength of UV light [7].

Screening for Glycolipids

A mixture of 2 ml 10% ethanolic diphenylamine solution, 10 ml 37% hydrochloric acid and 8 ml glacial acetic acid was prepared. In that mixture 1 ml of the sample was added. After that it was heated for 5-10 min at 100°C [7].

Screening for Glycosides

With 1 ml of the sample, 5 ml of 0.5% methanolic magnesium acetate solution was added. The mixture was heated for 5 min at 90°C [7].

Screening for Ketoses

Dissolve 0.3 g of anthrone in 5 ml acetic acid and add to the solution 5 ml 96% ethanol, 3 ml 85% phosphoric acid and 1 ml water. In that reaction mixture add 1 ml of the sample and it was heated for 5-6 min at 110°C[7].

Screening for Lipids

With 1 ml of the sample solution 0.01% ethanolic solution of fluorescein was mixed. Then the mixture was incubated for 10 min. After the incubation the mixture was dried with warm [7].

Screening for Organic phosphate esters

1% anhydrous cobalt (II) chloride solution in acetone. Then the mixture was added to 1 ml of the sample solution. The reaction mixture was incubated at 40-50°C for 5 min [7].

Screening for Peroxides

Dissolve 1.5 g of N, N-dimethyl-1, 4-diphenylene di ammonium dichloride in a mixture of 128 ml methanol, 25 ml water and 1 ml glacial acetic acid. In that mixture 1 ml of the sample solution was added [7].

Screening for Persulfates

Dissolve 0.05 g of benzidine in 100 ml 1 N acetic acid. In that mixture 1 ml of the sample solution was added [7].

Screening for Phenols

Dissolve 0.1 g of vanillin in 10 ml 97% sulfuric acid. In that reaction mixture 1 ml of the sample was added and heated at 120°C until the intensity of the colour changes [7].

Screening for Phosphoric acid for keto sugars

Dissolve 0.3 g of 5,5-dimethyl cyclohexane-1,3-dione (dimedone) in 5 ml of ethanol and add 2 ml 85% ortho phosphoric acid. In this solution 1 ml of the sample was added incubated for 15-20 min at 110°C [7].

Screening for Polyalcohol

Mixture I: 0.5% aqueous sodium meta- periodate solution; Mixture II: Add 50 ml water; 20 ml acetone and 10 ml 0.2 N hydrochloric acid to a solution of 1.8 g benzidine in 50 ml ethanol. Now in 1 ml of the sample add 1 ml of Mixture I. This was incubated for 5 min. at room temperature. Then 1 ml of Mixture II was added [7].

Screening for Polyphenyls

Dissolve 0.3 g cerium (IV) sulfate in 100 ml 65% nitric acid. In that reaction mixture 1 ml of the sample was added and heated for 15-20 min at 120°C [7].

Screening for Polysaccharides

Dissolve 0.3 g of anthrone in 5 ml acetic acid and add to the solution 5 ml 96% ethanol, 3 ml 85% phosphoric acid and 1 ml water. In that reaction mixture 1 ml of the sample was added and heated for 5-6 min at 110°C [7].

Screening for Sugar alcohols

Add with stirring 1 ml saturated aqueous silver nitrate solution to 2 ml of acetone, and then add water drop wise until the silver nitrate just dissolves. In that reaction mixture 1 ml of the sample was added and heated for 1 hr into a chamber saturated with ammonia vapours (protected against light) at 80°C until the sample has turned light brown, and remove the excess silver nitrate with 10% sodium thiosulfate solution [7].

Screening for Sugar phosphates

Dissolve 0.5 g of ammonium molybdate in 2 ml water, add 0.5 ml 25% hydrochloric acid and 0.5 ml 70% perchloric acid. After cooling to room temperature fill up to 5 ml with acetone. Allow the solution to stand for at least one day prior to use. In that mixture 1 ml of the sample solution was added. After adding of the sample the set was irradiated for 2 min with an IR lamp from a distance of 30 cm and heated for 5-10 min at 110°C [7].

Screening for Reducing sugars

Dissolve 4 g of diphenylamine, 4 ml of aniline and 5 ml of 85% phosphoric acid in 10 ml acetone. The 1 ml of the sample solution was added and heated for 10 min at 85°C [7].

Screening for Steroid sapogenins

Dissolve 30 g zinc chloride in 100 ml methanol and filter off from the insoluble matter. Now add 1 ml of the sample in 1 ml of the above reaction mixture. The total set was heated for 1 hour at 105°C and cover the layer immediately with a glass plate for protection against the influence of moisture [7].
Screening for Steroids and triterpene glycoside

Mix carefully and with cooling freshly before use 1 ml of acetic anhydride, 1 ml of 97% sulfuric acids and 1 ml of the sample solution. The mixture was cooled with addition of 2 ml of ethanol. The total set was incubated for 10 min at 110°C [7].

Screening for Sterols

Mix carefully and with cooling freshly before use 1 ml acetic anhydride with 1 ml 97% sulfuric acid, 1 ml of the sample solution and add the mixture with cooling to 3 ml of ethanol. After treatment: The total set was incubated for 10 min at 110°C [7].

Screening for Steroids

In 1 ml of the sample solution, 1 ml of 20% ethanolic solution of tungstophosphoric acid was added. The mixture was heated at 120°C [7].

Screening for Straight chain lipids

1 ml of the sample solution was added in 2 ml of 30% ethanolic solution of cyclodextrin. The total set was incubated for 10 min. at room temperature and placed into a chamber containing iodine vapour [7].

Evaluation of antitumour activity

Brine Shrimp Cytotoxicity assay was performed with the condensed leaf extract of Mucuna pruriens (L.) DC. [8-9]. Brine shrimp eggs were hatched in a shallow rectangular dish (22×32×12 cm), one third of which was filled with saline water. An aluminium divider with several 2 mm holes was clamped in the dish to make to unequal compartments. The eggs (50 mg) were sprinkled into the larger compartment which was darkened while the smaller compartment was illuminated. The set was maintained at 300C-320C and after 48 hours the phototropic nauplii was collected by pipette from the lighter side, having been separated by the divider from their shells.

The shrimps were transferred to each sample(s) vial using a 23 cm disposable pipette and saline water was added to adjust the volume to 5 ml. The nauplii could be counted in the stem of the pipette against a lighted background. A drop of dry yeast suspension (3 mg in 5 ml of saline water) was added as food to each vial. The vials were maintained under illumination at room temperature. Surviving shrimps was counted after every 3 hours up to 24 hours and the percentage of death at each dose and control was determined.

Death (%) = test-control×100/control

Three replicates was prepared for each dose level and after 24 hours LC50 values was determined.

Statistical analysis

The observed values were expressed as mean ± standard deviation. All the values reflected in the table are statistically analyzed by paired t test with control and found significantly different at 5% level (P<0.05)

Results

Phytochemical screening

From table 1 it can be concluded that the leaf extract contains aldehydes and ketones, alkaloids, alkoloids and amines, corticosteroids, essential oils, flavonoids, glycolipids, ketoses, organic phosphate esters, phenols, polysaccharides, sugars, reducing sugars, steroids and triterpene glycoside.

Antitumour activity screening of the crude extract

The LC50 value of this sample was 50 mg/ml (Table 2). Death of shrimps increases with the increase concentration of crude extract.

Table 1: Phytochemical screening of the crude leaf extract of Mucuna pruriens (L.) DC.

<table>
<thead>
<tr>
<th>Screening for</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroids and triterpene glycoids</td>
<td>Appearance of corticosteroids indicates the presence of steroids group. Appearance of orange color indicates the presence of flavonoids.</td>
</tr>
<tr>
<td>Alkaloids and amines</td>
<td>Appearance of yellow color indicates the presence of alkaloids. Appearance of greenish color indicates the presence of flavonoids.</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Appearance of yellow color indicates the presence of terpenoids.</td>
</tr>
<tr>
<td>Sugar alcohols</td>
<td>No changes in color indicating the absence of Sugar alcohols.</td>
</tr>
<tr>
<td>Sugar phosphates</td>
<td>Appearance of greenish color indicates the presence of sugar phosphates.</td>
</tr>
<tr>
<td>Inhibiting agents</td>
<td>Changes in color indicating the presence of inhibiting agents.</td>
</tr>
<tr>
<td>Steroid compounds</td>
<td>No changes in color indicating the absence of steroid compounds.</td>
</tr>
<tr>
<td>Steroid glycosides</td>
<td>Changes in color indicating the presence of steroid glycosides.</td>
</tr>
<tr>
<td>Triterpene glycosides</td>
<td>Changes in color indicating the presence of triterpene glycosides.</td>
</tr>
<tr>
<td>Sugars</td>
<td>Appearance of Blue color indicates the presence of Sugars in the sample.</td>
</tr>
<tr>
<td>Starch</td>
<td>No changes in the color of the sample during incubation under long wave of UV light.</td>
</tr>
<tr>
<td>Lignin</td>
<td>No changes in the color of the sample during incubation under long wave of UV light.</td>
</tr>
<tr>
<td>Organic phosphate esters</td>
<td>Appearance of Blue color indicates the presence of Organic phosphate esters.</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>No changes in the color of the sample during incubation under long wave of UV light.</td>
</tr>
<tr>
<td>Polypeptides</td>
<td>Changes in the color of the sample during incubation under long wave of UV light.</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>Appearance of blue color indicates the presence of Polysaccharides.</td>
</tr>
<tr>
<td>Phenol</td>
<td>No changes in the color of the sample during incubation under long wave of UV light.</td>
</tr>
</tbody>
</table>

Table 2: Determination of antitumour activity of the crude extract of Mucuna pruriens (L.) DC.

<table>
<thead>
<tr>
<th>Conc. (mg/ml)</th>
<th>No. of survivors after 24 h</th>
<th>LC50 (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>12.0±0.0</td>
<td>11.4±0.3</td>
</tr>
<tr>
<td>100</td>
<td>12.0±0.0</td>
<td>11.3±0.2</td>
</tr>
<tr>
<td>75</td>
<td>12.0±0.0</td>
<td>11.3±0.4</td>
</tr>
<tr>
<td>50</td>
<td>12.0±0.0</td>
<td>11.3±0.6</td>
</tr>
<tr>
<td>25</td>
<td>12.0±0.0</td>
<td>11.3±0.8</td>
</tr>
<tr>
<td>10</td>
<td>12.0±0.0</td>
<td>11.3±0.0</td>
</tr>
<tr>
<td>1</td>
<td>12.0±0.0</td>
<td>11.3±0.0</td>
</tr>
<tr>
<td>Control</td>
<td>12.0±0.0</td>
<td>11.3±0.0</td>
</tr>
</tbody>
</table>

The observed values were expressed as mean ± standard deviation.

Calculation was done with the help of spread sheet software Microsoft Excel 2010.

*Indicates significance at (P<0.05)
Conclusion

From previous reports we can say that steroids, triterpene and triterpene glycoside have some antitumour potentialities. This type of metabolites are found frequently in the plants of Zingeberaceae. Among the popular triterpene, there are 2, 7, (14), 10 Bisabolatriene- 1,9,12 triol found in the rhizomatic extract of Curcuma longa L. [10]. This triterpene have a potent cytotoxic effect for which the extract of turmeric can be used widely as an antitumour agent.

From the above results it can be conclude that, the crude extract of the experimental plant contains steroids and triterpene glycoside which may possess some antitumour activity. Hence, the extract can be applied as a better ecofriendly bioformulation of cytotoxic compounds. Further studies can be performed to locate that antitumour activity of that particular metabolite present in the extract.

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References