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Study on pharmacological activity screening of *Derris robusta*

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Abstract

Derris robusta plant extract used to assess its different biological activity. Extract was made by soaking the dried plant powder in methanol. After comparing with the standard we found that Methanol extract of the sample gave the activity against all the experimented microbes of ZI (zone of inhibition) against *E. coli* and *B. subtilis*. After performing the antioxidant, thrombolytic, antidiarrheal, hypoglycemic and cytotoxic activity assay of methanol extract of sample plant we saw that it has a good biological activity that can be used as a potential traditional medicine.

Keywords: *Derris robusta*, antioxidant, antimicrobial, antidiarrheal, hypoglycemic, thrombolytic activity

Introduction

Derris Robusta also known as *Dalbergia robusta* is a [Error! Reference source not found.] deciduous, branches slightly sparsely pubescent, glabrescent tree species in the genus *Derris* & family Fabaceae found in India which grows up to 25 metres tall, with bole that is normally up to 30 cm in diameter but with some specimens up to 65cm. This plant mainly found in India but also in Bangladesh, Myanmar, Thailand, Laos, Vietnam, Indonesia. According to [Error! Reference source not found.] the plant contains Rotenone which is effective against a range of horticultural pests, such as aphids, caterpillars, and also against external body parasites like ticks, lice, fleas and flies.

The early use of derris root [Error! Reference source not found.] as a fish poison and as an ingredient of arrow poison lead to its development as an insecticide. Some examples from this genus like a poultice of roots or bark of *Derris microphylla* [Error! Reference source not found.] is used to treat itch in Malaysia. Another species named *Derris elliptica* is used as [Error! Reference source not found.] the medicine of cutaneous, subcutaneous parasitic infections. And also it's root shows phytochemistry of fish-poisons, insecticides, arachnicides and arrow-poisons. According to [Error! Reference source not found.] various parts of *Derris elliptica*, *Derris indica* and *Derris trifoliata* shows a varied level of broad spectrum antibacterial activity.

In ancient and recent time, this genus shows a great importance in pharmacological activities. So, several tests for the identification of Antioxidant, Anti-microbial, cytotoxicity, thrombolytic activities and other phytochemical screening tests were done to identify whether the plant have any pharmacological active ingredients in it or not.

Methods and Materials

Collection of plant materials

The leaf part of *Derris Robusta* plant was collected in May 2017 from Chittagong hill tract. After collection, the National Herbarium Bangladesh (NHB), Mirpur, and Dhaka authenticated the plant material and provided a plant identification number, which was 47698.

Preparation of the extract

At first, the leaves part was washed with fresh water to remove the unwanted dust particles and plant scrap. After that, the cleaned leaves were dried under the sun for a day. Then the leaves were again dried for 1 hour at 30-40 °C in hot air oven. By using a high capacity-grinding machine, the dry and crusty leaves were ground. After that, at a normal ambient temperature (22-25 °C) around 900 g of ground powder was soaked in 2.5 L of methanol for a period of 2 days with occasional stirring. With the help of cotton filter (pore size: 110mm) filtration was done and rotary evaporator was used at 100 rpm at 30 °C to evaporate the maximum amount of solvent. For vaporizing the solvent completely from the extract, the leaf extract was kept under laminar airflow cabinet. Moreover, it was used to avoid any possibility of microbial growth in the extract while drying. Finally, 22.4 g of plant leaf extract was obtained and kept in dry and cool place and proper labeling was done. After that, this extract was used to conduct

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antioxidant, brine shrimp lethality assay, thrombolytic, antidiabetic, antimicrobial and hypoglycemic studies.

Chemicals

The chemicals were gallic acid [Sigma-Aldrich, USA], sodium chloride [Sigma-Aldrich, USA], Folin-Ciocalteu reagent [Sigma-Aldrich, USA], vincristine sulphate [Sigma-Aldrich, USA], 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) [Sigma-Aldrich, USA], sodium carbonate [Merck, India] and ascorbic acid (ASA) [Merck, India], dimethyl sulfoxide (DMSO) [Fisher Scientific, UK], Castor oil (WELL's Health Care, Spain), 0.9% sodium chloride solution (normal saline) (Orion Infusions Ltd., Bangladesh), charcoal meal (10% activated charcoal in 5% gum acacia), and loperamide (Square Pharmaceuticals Ltd., Bangladesh) were used for antidiarrheal activity test, and dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA) and sodium chloride (Sigma) were used for cytotoxic activity test. All the chemicals used in this study were of analytical grade.

Anti-oxidant activity

Total phenolic content (TPC)

The phenols were oxidized by Folin-Ciocalteu in ionic phenolic solution. When the solution became yellow to dark blue, it is understood that the oxidation has been completed. After that, this color changed mixture measured in 760 nm in UV spectrophotometer. Finally, the value of the absorbance plotted in gallic acid calibration curve and data was evaluated as gallic acid equivalents (GAE).

Total Flavonoid content

Aluminum chloride was used to determine the total amount of flavonoids. Firstly, 0.5 ml of plant extract was made up to 1 ml of final volume with reaction medium (MeOH/H₂O/CH₃COOH=14:5:1) which was then mixed with Aluminum chloride reagent (4 ml, 133 mg of AlCl₃ × 6 H₂O and 400 mg of CH₃COONa dissolved in 100 ml H₂O). After 5 minutes, the absorbance was measured at 430 nm. Based on the calibration curve, total flavonoid content was calculated and it was expressed as gram equivalents.

DPPH free radical scavenging assay

The antioxidant activity of *Derris robusta* was determined by performing DPPH free radical scavenging assay. To run this assay, different concentrations of plant extracts were mixed with 2, 2-diphenyl-1-picrylhydrazyl (DPHH) solution. In methanol or aqueous solution, free radicals were generated due to delocalization of the free electrons and a deep purple colored solution is produced. Then absorbance of different concentration solutions was measured at 517 nm in UV spectrophotometer. The decreasing value of DPHH at 517 nm is directly proportional to the radical scavenging activity.

Percentage of inhibition of DPHH free radical (1%) was calculated by using the following equation:

$$(1\%) = (\text{Absorbance of blank} - \text{Absorbance of sample}) / \text{Absorbance of blank} \times 100$$

50% of inhibition of the extract concentration was calculated from the graph and the percentage of inhibition was plotted against extract concentration.

Cytotoxic activity

Brine shrimp lethality assay

In this assay, *Artemia salina* shrimp was used. Its offspring was hatched in replicated seawater to cultivate nauplii. Here, calculated amount of dimethyl-sulfoxide (DMSO) was added with sample and desired concentration of sample was

prepared by dilution. The counted nauplii were placed in vials that contained approximately 5 mL simulated seawater with visual inspection. With the help of micropipette, various concentrations of samples were added to tubes. Here, vincristine sulfate was used as standard. The sample containing tubes were then placed in a dry place for 24 hours at room temperature. At the last, after 24 hours, the survived nauplii were counted. Percentage (%) of mortality was calculated by using the following equation:

$$\text{Percentage of mortality} = (\text{Number of nauplii taken} - \text{Number of nauplii alive}) / \text{Number of nauplii taken} \times 100$$

50% of lethal concentration of extract concentration was calculated from the graph-plotted percentage of mortality against concentration.

Thrombolytic activity

The normal blood flow to the cells and tissues can be hampered due to thrombus as it blocks the blood vessel, which can lead to lack of blood and oxygen. There are some thrombolytic medications like utokinase, clopifogrel, and streptokinase remove this thrombus and cells and tissues are remained in normal conditions. For this assay, fresh human blood was collected. Then, they were taken in three different pre-weighed sterile microbes and incubated for 45 minutes at 37°C. The upper fluid was entirely dispensed from all micro-tube lines when the clot was appeared. As a standard streptokinase was used and as a negative control distilled, water was used. 100 microliter of plant extract was taken in each tube and incubated for 90 minutes at 37°C. Next, liquid that was released from the clot was removed and the tubes were weighted again to observe the weight difference when the clot disruption occurred.

Percentage of clot lysis was calculated by following equation: (%) of clot lysis = (released clot weighted) / (clot weight after clot disruption) × 100

Antimicrobial assay

Disc Diffusion Assay Method

In recent years, different studies are developing as antimicrobial agents to fight antibiotics resistance from different sources and highest concentration has given to screen and evaluate the antimicrobial activity. By using disc diffusion assay method, antimicrobial activity of *Derris robusta* was evaluated. *E. coli* bacteria (gram negative) and *Bacillus subtilis* bacteria (gram positive) were used in this study. Mular Hinton agar (MHA) was used as media in this assay. Firstly, every petri dish was autoclaved for sterilization and 20 ml of MHA was poured in every petri dish. After that, the plates were kept for a time being to be settled. With the help of cotton swab, the nutrient broth of bacterial strains was incubated in MHA. Small disc of filter paper was made by using paper punch machine and then different concentrations of plant extract (200 mg/mL and 400 mg/mL) were used to swallow that filter paper. When the discs become dry, they were transferred to the petri dishes and kept in incubator for 24 hours at 37 °C. After 24 hours the zone of inhibitions was calculated and for keeping the contamination limited, whole experiment was done under laminar flow.

Result and discussion

Antioxidant activity

Total phenolic content (TPC)

In total phenolic content test, Gallic acid was used as standard and methanol extract of leaves, which was used as a sample.

The absorbance of the sample plotted in Gallic acid calibration curve. The absorbance of the plant extract was found 0.349 and TPC value was 43.17 GAE/g against that absorbance which indicates that the plant has antioxidant activity.

Total Flavonoid content

The content of total flavonoid of the plant extracts was measured spectrophotometrically by using the aluminium chloride colorimetric assay. The flavonoid content of the extracts was expressed as mg quercetin equivalent per gram of the extract and it is 192.58 QE/g against the absorbance of 0.349 that indicates the present of flavonoid content.

DPPH free radical scavenging assay

It is known that DPPH free radical scavenging activity is increasing along with increasing concentration of the methanol extract. As the reference standard, ascorbic acid was used in this experiment for which IC₅₀ value was 75.688

µg/ml. on the other hand, the IC₅₀ value of the methanol extract of the sample plant was 91.45 µg/ml. this result indicates the presence of antioxidant activity which is less significant.

Table 1: Evaluation of DPPH free radical scavenging activity of methanol extract of *Derris Robusta*.

	R ² value	IC ₅₀
Standard	0.6277	75.688
Sample (methanol extract)	0.7084	91.45

Cytotoxic activity: This brine shrimp lethality assay was used to assess the cytotoxic property of methanol extract of plant material. Here, different concentrations standard and sample were plotted that provided different percentages of mortality. Percentage of mortality was found to increase along with the increasing concentrations of standard and methanol extract. This study indicates the methanol extract of plant material has cytotoxic activity.

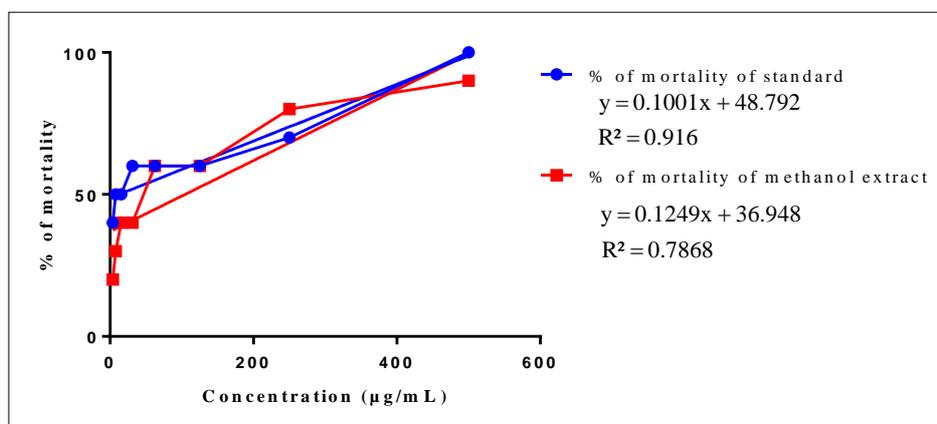


Fig 1: Cytotoxic Activity of *Derris roobusta*.

Thrombolytic activity

Table 2: Evaluation and results of the thrombolytic activity.

Name of the sample	W1	W2	W3	W4	W5	% of clot lysis
Plant extract	0.734	1.491	1.279	0.545	0.212	38.90
Standard	0.781	1.532	1.249	0.468	0.283	60.47
Blank	0.820	1.748	1.613	0.793	0.135	17.02

Here, W1 = Micro-tube weight, W2 = Clot with micro-tube weight, W3 = Clot with micro-tube weight after clot disruption, W4 = Clot weight after clot disruption, W5 = Released clot weight.

Plasminogen enzyme is usually activated by thrombolytic agents and it also removes fibrin bonds in blood, as a result, the clot becomes soluble and blood flow is restored. Here, methanol extract showed much lower level of thrombolytic activity than standard. Standard gave 60.47% clot lysis, distilled water was used as a negative control, which provided 17.02% clot lysis and methanol extract of plant leaves showed 38.90% clot lysis. After comparing the clots lysis value of plant extract with the positive control value, it was observed that plant material revealed thrombolytic activity but less than standard.

Antimicrobial assay

Table 3: Antimicrobial activity of the leaves of *Derris Robusta*.

Group	Inhibition zone (mm)	
	Gram (+ve) bacteria (<i>E.coli</i>)	Gram (-ve) bacteria (<i>B. subtilis</i>)
Control	0.00	0.00
Standard	15.12±1.09	11.6±0.67
Plant extract (200mg/mL)	14.43±0.29	0.00
Plant extract (400mg/mL)	17.91±1.21	0.00

The plant extract showed antimicrobial activity at all concentrations tested with a broad spectrum of activity, inhibiting against the growth of both Gram positive and Gram-negative bacteria. The antimicrobial potential was especially showed against *E. coli* and *B. subtilis*, when exposed to 400 mg/mL of methanol extract of plant and made it impossible when exposed to 200 mg/mL of methanol

extract of dried leaves as shown in the table. These results indicate that the antimicrobial activity of the plant extract is not as significant as standard.

Conclusion

The methanol extract of the *Derris robusta* leaf was investigated to evaluate the therapeutic properties. In this

study, it was clearly observed that this plant has various therapeutic potentials. The findings of the present study provide convincing evidence that methanol extract of *Derris robusta* leaves possesses remarkable cytotoxic effect, antioxidant activity, thrombolytic activity, antimicrobial activity. However, further chemical and pharmacological studies are required to isolate the bioactive compounds and elucidate the precise mechanisms responsible for the observed pharmacological activities of this plant.

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