IN VITRO ANTHELMINTIC ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF AGLAIA LAWII LEAVES

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ABSTRACT

Aim of our study is to evaluate the anthelmintic activity of different solvent extracts of Aglaia lawii leaves. Natural products play an important role in the discovery and development of new pharmaceuticals. The development of anthelmintic resistance, side effects and the cost of conventional anthelmintic drugs led to the evaluation of medicinal plants as an alternative source of anthelmintics. Aglaia species has a long history of traditional use as an anthelmintic. So the present study is aimed to carry out the anthelmintic activity of various extracts of Aglaia lawii. Air shade dried and pulverized plant material was refluxed with ethyl acetate, acetone, ethanol and methanol solvents and respective extracts were screened for activity using Eicinia foetida. Albendazole is used as standard. The anthelmintic assay was carried out as per the method of Nargund with minor modifications. It was observed that all the extracts of Aglaia lawii leaves have exhibited positive response to certain degree of anthelmintic activity in dose dependent manner. Acetone and methanol extracts of plant material exhibits significant activity at all dilutions as compared to the standard. Ethyl acetate extract is the least active among four extracts. The rate of paralysis as well as death is higher at more concentrated extracts. The results indicate that acetone and methanol extracts are more potent than ethyl acetate and ethanol extracts. It is concluded based on the findings of the present study that the leaves of Aglaia lawii possess varying degree of anthelmintic activities. The dose of the extract is increased, a gradual increase in the activity of extracts. Acetone and methanol extracts exhibited significant anthelmintic activity at highest concentration of 30 mg/ml. This study strongly supports the traditional use of leaves as anthelmintic.

Keywords: Aglaia lawii, Albendazole, Anthelmintic activity, Eicinia foetida, Leaves extracts.

INTRODUCTION

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. Lack of adequate sanitary facilities, supply of pure water, poverty and illiteracy are some factors responsible for widespread nature of this disease in the developing countries. The common parasites observed in India are round worms, hookworms, thread worms, tapeworms, guinea worms and filarial worms. They can cause loss of blood, nutritional deficiencies, urticaria and other conditions. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of under nourishment, anaemia, eosinophilia and pneumonia. 1 Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas. 2 The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore; there is a foremost problem in treatment of helminthes diseases 1. Hence, there is an increasing demand towards natural anthelmintics. An ideal anthelmintics must have a wide margin between its toxicity to the worm. The drug must be effective in one dose. Anthelmintic drugs can be classified according to their structure as well as to their action against the specific types of helminthes. 3 As per WHO, only few drugs are frequently used in the treatment of these parasite infections. 4 Aglaia is a genus of more than 100 species belonging to the Mahogany family (Meliaceae). These trees occur in the tropical and subtropical forests of Southeast Asia, Northern Australia and the Pacific. Some are important trees; others have edible fruits, scented flowers or medicinal properties. Many have complex biological relationships with their dispersal agents. Some show insecticidal bioactivity. 5 Certain species of Aglaia have traditionally been used for their medicinal and healing properties such as the treatment of fever, diarrhea, inflammation and wounds. Extracts have also been used as bactericides, insecticides and in perfumery. 6 Aglaia lawii is distributed from India, through Burma (Myanmar), Thailand, Indo-China and throughout Malaysia towards the Solomon Islands. 7,8 It is a medium sized to fairly large tree which can reach up to 40 m tall. The wood is reported as hard and durable. 9 It is a traditional medicinal plant having been used for the treatment of bacterial infection, liver, tumor diseases and headaches. 10 The presence of phytoconstituent was detected by standard protocol 11,12. Chemically polyphenolic compounds are tannins which accomplish anthelmintic activity. 13 The presence of flavonoids and polyphenolic components responsible anthelmintics interfered with energy generation helminth parasites by uncoupling oxidative phosphorylation 14. Tannins can bind to free protein in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death 15.

Experimental

Plant material

The plant material was collected from Mulshi, Pune, Maharashtra, India. It was authenticated from Botanical survey of India, Pune, Maharashtra, India. Its Authentication no. is BSI/WRC/Tech/2010/1028. Pune, India. Air shade dried and pulverized plant material was refluxed with ethyl acetate, acetone, ethanol, methanol solvents and respective extracts were screened for activity.

Screening for phytochemicals

Qualitative assay of the extracts for the presence of phytoconstituents such as carbohydrates, alkaloids, glycosides, flavonoids, tannins etc were performed following Standard procedure 11,12.
Anthelmintic Assay

The anthelmintic assay was carried out as per the method of Indian earthworm Albendazole, normal saline were purchased from authorized and lethal time was ascertained by death of motionless worm. The paralysis time of Albendazole with normal saline were used as standard. Same parasite of human being physiological resemblance with the intestinal round worm earthworms were of approximately equal size (14 cm).

Animal

Indian earthworm species Eicinia foeitida was collected from Mahatma Phule Agriculture University, Pune, India. All earthworms were of approximately equal size (14 cm).

Anthelmintic Assay

The anthelmintic assay was carried out as per the method of Nargund and Ajaiyeoba EO et al. with minor modifications. The assay was performed on adult Indian earthworm Eicinia foeitida due to its anatomical and physiological resemblance with the intestinal round worm parasite of human being. Different dilutions of Albendazole with normal saline were used as standard. Same dilutions of ethyl acetate, acetone, ethanol and methanol extracts in normal saline solution were used for the assay and normal saline served as control. The time taken for complete paralysis and death was recorded. External stimuli were applied to ascertain the paralysis time. The time taken by worm to become motionless was considered as paralysis time and lethal time was ascertained by death of motionless worm followed by fading away of their body color.

Chemicals

Albendazole, normal saline were purchased from authorized pharmaceuticals. The solvents and other chemicals used during experimental protocol were of analytical grade. These extracts of ethyl acetate, acetone, ethanol and methanol are effective in a broad range of helminth infections, including round worms, hook worms etc. In the laboratory model could provide a rational for the traditional use of these plants as anthelmintic. The present study it was observed that all the extracts of Aglaia lawii leaves have exhibited positive response to certain degree of anthelmintic activity in dose dependent manner. Results are expressed in terms of time of paralysis and time of death of worms. These extracts of ethyl acetate, acetone, ethanol and methanol are effective in a broad range of helminth infections, including round worms, hook worms etc. In the laboratory model could provide a rational for the traditional use of these plants as anthelmintic. In the present study it was observed that all extracts of Aglaia lawii have exhibited higher activity response to certain degree of anthelmintic activity than standard. Acetone and methanol extracts of plant material exhibits significant activity at all dilutions as compared to ethyl acetate and ethanol extracts. The rate of paralysis as well as death is higher at more concentrated extracts. It means at higher concentrations time of paralysis and death is less as compared to lower concentrations. It concludes that acetone and methanol extracts have displayed profound anthelmintic activity. The extracts demonstrated the trend for activity as Acetone > Methanol > Ethanol > Ethyl acetate

RESULTS AND DISCUSSION

This study suggests that the plants used by tribal’s traditionally to treat intestinal worm infections, showed significant anthelmintic activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of these plants as anthelmintic. In the present study it was observed that all the extracts of Aglaia lawii leaves have exhibited positive response to certain degree of anthelmintic activity in dose dependent manner. Results are expressed in terms of time of paralysis and time of death of worms. These extracts of ethyl acetate, acetone, ethanol and methanol are effective in a broad range of helminth infections, including round worms, hook worms etc. In the laboratory model could provide a rational for the traditional use of these plants as anthelmintic. In the present study it was observed that all extracts of Aglaia lawii have exhibited higher activity response to certain degree of anthelmintic activity than standard. Acetone and methanol extracts of plant material exhibits significant activity at all dilutions as compared to ethyl acetate and ethanol extracts. The rate of paralysis as well as death is higher at more concentrated extracts. It means at higher concentrations time of paralysis and death is less as compared to lower concentrations. It concludes that acetone and methanol extracts have displayed profound anthelmintic activity. The extracts demonstrated the trend for activity as Acetone > Methanol > Ethanol > Ethyl acetate

Table 1: Anthelmintic Activity of Aglaia lawii Leaves Extracts

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample</th>
<th>Concentration (mg/ml)</th>
<th>Time for paralysis (P) min</th>
<th>Time for death (D) min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (Normal saline)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Albendazole (Standard)</td>
<td>10</td>
<td>140 ± 0.182</td>
<td>225 ± 0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>130 ± 0.18</td>
<td>200 ± 0.210</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>110 ± 18</td>
<td>140 ± 0.23</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl acetate extract</td>
<td>10</td>
<td>131 ± 0.16</td>
<td>137 ± 0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>95 ± 0.150</td>
<td>100 ± 0.160</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>85 ± 0.160</td>
<td>89 ± 0.170</td>
</tr>
<tr>
<td>4</td>
<td>Acetone extract</td>
<td>10</td>
<td>91 ± 0.170</td>
<td>97 ± 0.181</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>71 ± 0.175</td>
<td>86 ± 0.180</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>61 ± 0.330</td>
<td>78 ± 0.179</td>
</tr>
<tr>
<td>5</td>
<td>Ethanol extract</td>
<td>10</td>
<td>101 ± 0.134</td>
<td>106 ± 0.160</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>92 ± 0.122</td>
<td>97 ± 0.162</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>83 ± 0.133</td>
<td>88 ± 0.125</td>
</tr>
<tr>
<td>6</td>
<td>Methanol extract</td>
<td>10</td>
<td>95 ± 0.160</td>
<td>100 ± 0.135</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>87 ± 0.155</td>
<td>92 ± 0.120</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>77 ± 0.160</td>
<td>82 ± 0.139</td>
</tr>
</tbody>
</table>

P < 0.05 when compared to control. Values are expressed as mean ± SEM

Graph 1: Shows Comparative Study on Anthelmintic Activity of Aglaia lawii Extracts on Adult Indian Earthworm

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It clearly indicates that the crude acetone and methanol extracts significantly demonstrate paralysis and cause death of worms in dose dependant manner while, ethyl acetate and ethanol show quite weak anthelmintic effect as compared to above extracts. These extracts get highly absorbed in the body. The efficacy of an extract depends on transit time in the gastro intestinal tract (GIT). The potency is extremely high as compared to standard Albendazole. Phytochemical screening of crude extracts reveals the presence of flavonoids, poly phenolic compounds and tannins as major constituents. These are responsible for the said activity. The gradual increase in a dose exhibited a stepwise increase in the said activity. Acetone and methanol extracts display very powerful anthelmintic activity at all concentrations that is from 10 mg to 30 mg / ml than frequently used standard albendazole. The consequential and conscientious part of the experiment demonstrates that the results are very prominent at all concentrations of ethyl acetate, acetone, ethanol and methanol extracts. The results are presented (Table 1) and graphically illustrated (Graph 1).

CONCLUSION
It is concluded from the results that, Aglaia lawii leaves have powerful anthelmintic potential. It posses varying degree of anthelmintic activities. The activity of extracts is dose dependent. Acetone and methanol extracts display higher anthelmintic activity than standard. This study strongly supports the traditional use of leaves as anthelmintic. Therefore, in-vivo trials may be conducted for the use in livestock on scientific basis. Use of herbal products as antimicrobial agents may provide the best alternative to the wide and injudicious use of synthetic antibiotics.

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