ANTHELMINTIC ACTIVITY OF EXTRACTS OF HOLARRHENA ANTIDYSENTERICA WALL

Kranti Satpute1*, Kaumudee Bodas2, Varsha Shende2, Wajid Chaus1, Dhumal Atul1, Galphade Amar1

1Dayanand College of Pharmacy, Latur– 413512, Maharashtra, India.
2Sinhgad College of Pharmacy, Vadgaon (BK), Pune - 411041, Maharashtra, India

ABSTRACT

Background: Helminthes have been common cause of concern and pose many problems to human beings and animals. Many medicinal plants claimed to possess anthelmintic activity and can be effectively used against these types of disorders. Holarrhena Antidysenterica, commonly called Kurchi bark is deciduous shrub belonging to Apocynaceae was traditionally used to treat Helminthiasis. Objective: To test Anthelmintic activity of aqueous and alcoholic extracts of Holarrhena Antidysenterica. Materials and Methods: Indian adult earthworms (Pheretima posthuma) were used to study anthelmintic activity. The activity was checked in aqueous and alcoholic bark extracts at three different concentration (10, 20 and 40mg/ml) and results were expressed in terms of paralysis time and death time for worms. The results were compared with standard solution, Albendazole (10, 20 and 40mg/ml). Results: Both the extract showed significant anthelmintic activity. The effect was dose dependant and shortest time taken for paralysis and death was observed in case of alcoholic extract at 40 mg/ml concentration. Conclusion: It was concluded that seeds of Holarrhena Antidysenterica have potential anthelmintic activity.

Key words: Anthelmintic, Holarrhena Antidysenterica, Alcoholic extract, aqueous extract.

INTRODUCTION

The half of world suffering from bacterial and helminthes infection, due to poor sanitation, poor family hygiene, malnutrition, and crowded living conditions. Helminthes infections are among the most widespread infections in humans, distressing a huge
population of the world. Although the majority of infections due to helminthes are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of under nourishment, anemia, eosinophilia and pneumonia. (2) Due to discovery and development of anthelmintic physician now have effective, and in some cases, broad spectrum of agents that will cure or control most infections caused by flukes or intestinal helminthes. (3) Development of resistance to most of the commercially available anthelmintic is became a severe problem worldwide. (4) The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases. Hence there is an increasing demand towards natural anthelmints. (5) There is lot of the organic & inorganic and antibiotics are antihelmintics drug which has some side effects such as constipation, vomiting, gastric irritation hypersensitivity, immune suppression and allergic reaction. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants.

Plants have provided mankind with herbal remedies for several diseases for many centuries. In India herbal medicines have been the bases of treatment and cure for various diseases in traditional methods such as Ayurveda, Unani and Sidha. Holarrhena Antidysenterica, commonly called Kurchi bark is a deciduous shrub or small tree native to India. The bark is rather rough, pale brownish or grayish. The bark has astringent, Antidyserenic, stomachic, febrifugal and tonic properties. It is used in the treatment of amebic dysentery and diarrhea. (6) Holarrhena Antidysenterica is also present in list of Anthelmintic plant in traditional remedies in India. (7)

Present work was undertaken to screen anthelmintic potential of aqueous and ethanolic bark extracts plant Holarrhena Antidysenterica in adult earth worms (Pheretima posthuma).

MATERIALS AND METHODS

Plant material: The bark of Holarrhena Antidysenterica was purchased from the local market of Latur (Maharashtra). The plant material were identified & authenticated in Department of Botany, Dayanand Science College, Latur. And voucher herbarium a specimen was deposited in Botany department of, Dayanand Science College, Latur.
Experimental worms\textsuperscript{(8)(9)(10)(11)}

Indian adult earthworms (Pheretima posthuma) were used to study anthelmintic activity. They were collected from local Nursery. The earthworms were collected from moist soil and washed with normal saline to remove all fecal matter. Adult earthworms were used for all experimental protocol. The earthworm resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings, hence can be used to study the anthelmintic activity.

Preparation of extracts

\textbf{Alcohol extract}

Bark of Holarrhena Antidysenterica were powdered and then soaked in Ethanol for 48 hrs. It was filtered through muslin cloth and filtrate was evaporated to get dry extract. It was stored in airtight container.

\textbf{Aqueous extract}

Powder of bark was soaked in water for 48 hrs day. Then it was filtered through muslin cloth and concentrated. The extract was stored in air tight container and was used for anthelmintic activity. Preliminary phytochemical analysis was carried out to find of nature of chemical constituents present in extracts.

Phytochemical Screening

The bark ethanol extract was tested and it revealed that the positive result of Steroids, Alkaloids, Terpenoids, Saponins, Tannins, Flavonoids. Bark aqueous extract was tested and it revealed that the positive result of Carbohydrates, proteins, Flavonoides, Saponins, Glycosides, Amino acids. Phytochemical screening of the extract was carried out according to the standard method.\textsuperscript{(12)}

\textbf{Standard solution}

Albendazole (10 mg/ml 20 mg/ml & 40mg/ml) was administered as standard solution.

\textbf{Test solution}

The different concentration (10, 20 and 40mg/ml) of bark alcohol Extracts (ALE) and aqueous Extract(AQE) of concentration (10, 20 and 40mg/ml) were also prepared. All the extracts and the standard drug solution were freshly prepared before starting the experiments.
Experimental design

The anthelmintic activity was carried out in 10 groups; each group consists of 6 worms and worms were released into 10 ml of desired formulations. Group 1 were the control worms placed in normal saline; Group 2-4 received bark alcohol Extracts of Holarrhena Antidysenterica at 10, 20 and 40mg/ml concentrations; Group 5-7 were treated with bark aqueous Extract of various (10, 20 and 40mg/ml) concentrations. Group 9-10 serves as standard and was treated with Albendazole (10, 20 and 40mg/ml). The observations were made for the time taken for paralysis and Death of worm. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with their body colors fading away.

RESULTS AND DISCUSSION

In vitro Anthelmintic activity

In anthelmintic assay, the alcoholic and aqueous bark extracts showed paralysis and death of worms. The effect was dose dependant and shortest time taken for paralysis and death was observed in case of alcohol extract at 40 mg/ml concentration with potent activity against Indian adult earthworms (Pheretima posthuma). Both the extracts showed comparable results as that of standard drug as displayed in Table 1.

Table 1. Anthelmintic Activity of Alcoholic and aqueous Extracts of Holarrhena Antidysenterica

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration mg/ml</th>
<th>Time taken for paralysis (min)</th>
<th>Time taken for death (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normal Saline)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bark alcoholic extract</td>
<td>10</td>
<td>49.00±0.73</td>
<td>79.67±0.67</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>30.83±1.01</td>
<td>57.67±0.78</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>25.00±0.73</td>
<td>47.50±0.76</td>
</tr>
<tr>
<td>Bark aqueous extract</td>
<td>10</td>
<td>56.33±1.14</td>
<td>58.67±1.03</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>45.67±1.20</td>
<td>43.17±1.53</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>30.50±0.76</td>
<td>30.17±1.10</td>
</tr>
<tr>
<td>Albendazole</td>
<td>10</td>
<td>69.83±0.60</td>
<td>77.50±1.52</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>47.00±0.57</td>
<td>54.83±1.51</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>28.83±1.22</td>
<td>40.00±0.57</td>
</tr>
</tbody>
</table>

All Values represents Mean± SEM; n=6 in each group
Graph-1. Time taken for paralysis after treatment with different extract.

Where ALE - Alcoholic Extracts, AQE - Aqueous Extract, ALB - Albendazole.
The number represents concentration.

Graph-2. Time taken for death after treatment with different extract.

Where ALE - Alcoholic Extracts, AQE - Aqueous Extract, ALB - Albendazole.
The number represents concentration.

As displayed in Graph-1 and Graph-2, alcoholic and aqueous extract of Holarrhena Antidysenterica exhibited significant anthelmintic activity in dose dependent when compare with reference standard Albendazole. Alcoholic extract in concentration 40mg/ml was found
to be paralysis and death of warm in 25.00 and 47.50 respectively which is potentially more effective as compare with aqueous extract with standard reference drug Albendazole.

The compound constituents responsible for anthelmintic activity were not investigated, however preliminary phytochemical screening of alcoholic extract give positive test for Steroids, Alkaloids, Terpenoides, Saponins, Tannins, Flavonoides. The role of Flavonoides, Phenolic compound and steroids as anthelmintic activity.\(^{(14)}\)(\(^{(15)}\)(\(^{(16)}\))

The activity of bark alcoholic extract may attribute due to presence of Flavonoides, alkaloids and steroids which is reported of Holarrhena Antidysenterica.

The comparison between treated groups with standards was carried out using one way ANOVA test. All the results were found to be significant with P value less than 0.0001 (P<0.0001)

CONCLUSION
It was concluded that seeds of Holarrhena Antidysenterica showed potential anthelmintic activity. Further research work is needed to isolate phytoconstituent responsible for anthelmintic activity.

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