Screening of Anthelmintic Activity of Various Extracts of Zizyphus Jujuba Bark

Dhanapal Venkatachalam
Principal, Sree Sastha Pharmacy College, Sree Sastha Nagar, Chembarambakkam, Chennai-600123

ABSTRACT

The objective of the study was to evaluate anthelmintic activity on adult Indian earthworm Phertima posthuma in various extracts from the bark of Zizyphus jujuba. Z. jujuba commonly called, Red Date or Chinese date or Bera (Pushto), belonging to family Rhamnaceae, is used primarily for its fruits. Jujube, a delicious fruit, is an effective herbal remedy improving stamina and muscular strength and aids weight gain, strengthens liver function and increases immune system resistance. The dried bark of Zizyphus jujuba was powdered and extracted with various solvents by successive soxhlet hot extraction process with increasing order of polarity. The various extracts were screened for anthelmintic activity on adult earthworms Phertima posthuma, using piperazine citrate as standard drug. The results revealed that the methanol extract of Zizyphus jujuba bark showed anthelmintic activity at a concentration of 20 mg/ml, whereas the aqueous extract of Z. jujuba bark also showed paralysis and death at similar concentrations. The other test concentrations of both the extracts showed marked degree of anthelmintic activity. The anthelmintic effect of methanol extract at 40 mg/ml concentration is comparable with that of the effect produced by the standard drug piperazine citrate. The methanol extract showed the effect beyond 80 mg/ml concentration that is comparable with the standard drug Piperazine citrate. The methanol extract was more effective than the other extracts. The activity of the extracts was found to be inversely proportional to the time taken for paralyse or death of the earth worms. The active principles responsible for anthelmintic activity is due to the presence of flavonoids, steroids and tannins in the extracts. The results concludes the methanol extract was more anthelmintic activity than the other extracts.

Keywords: Zizyphus jujuba, Anthelmintic activity, Phertima posthuma, Piperazine citrate

*Corresponding Author Email: vddpaul@gmail.com
Received 12 November 2019, Accepted 19 November 2019
INTRODUCTION

Helminthiasis is a worldwide and one of the common diseases of all ages especially in third world countries. The parasitic diseases cause severe morbidity by affecting population in endemic areas with major economic and social consequences. Helminth infections are among the most common infections in man, affecting a large proportion of the world’s population. In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anaemia, eosinophilia, and pneumonia. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travelers who have visited those areas and some of them can develop in temperate climates. Helminthiasis is a disease in which a part of the body is infested with worms such as pinworm, roundworm or tapeworm. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver and other organs, infected people excrete helminth eggs in their faeces, which then contaminate the soil in areas with inadequate sanitation. Other people can then be infected by ingesting eggs or larvae in contaminated food, or through penetration of the skin by infective larvae in the soil (hookworms). Parasitic diseases cause severe morbidity, including filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis. As per WHO only synthetic drugs are frequently used in the treatment of helminth infestations in human beings but these synthetic drugs are out of reach of millions of people and have a lot of side effect. In view of this, an attempt has been made to study the anthelmintic activity of herbal drug. Zizyphus jujuba is also called as Badari, Baer, Bogari, Barihannu belonging to family Rhamnaceae. The plant is distributed throughout India, Iran, Afganistan, and in China. It is a small subdeciduous tree with dense spreading crown, commonly 0.6 m. girth and 6 m. high. The bark is blackish to grey or brown, rough, regularly and deeply furrow, the furrowed, the furrows are at about 1.2 cm apart. Blaze 9-13mm., Branches usually armed with spines, mostly in pairs, one straight, the other curved. Leaves 3-6.3 by 2.5-5 cm., oblong or ovate, usually minutely serrulate or apex distinctly toothed, obtuse, base oblique and 3nerved, nerves depressed on the glabrous shining upper surface. Petiole 2.5-10 mm long. Flowers 3.8-5 mm. In Diam., greenish, in dense axillary tomentose cymes or fascicles 1.2-1.9 cm Long. Drupe 1.2-2.5 cm. Diam., globose, first yellow then orange and finally reddish brown, containing a single stone surrounded by fleshy pulp. It is used primarily for its fruits. Jujube, a delicious fruit, is an effective herbal remedy improving stamina and muscular strength and aids weight gain. It strengthens liver function and increases immune system resistance. It functions as antidote, diuretic, emollient and expectorant. The leaves are febrifuge, astringent and said to promote the
hair growth. In the treatment of strangury they are used to form a plaster. The dried fruits are anticancer, anodyne, refrigerant, sedative, styptic, pectoral, tonic and stomachic. They help in digestion and blood purification.\textsuperscript{7-12} The present study deals with the anthelmintic effect of \textit{Zizyphus jujuba} using a standard laboratory procedure.

**MATERIALS AND METHOD**

**Collection and authentication:**
\textit{Zizyphus jujuba} was collected from in and around Chembarambakkam, Chennai India. The plant was identified and authenticated by the taxonomist. The authenticated specimen was deposited in the Department of Pharmacognosy, Sree Sastha Pharmacy College. The authentication specimen number is SSPC/P.COG/001/2019. The bark was dried in room temperature for 2 months. Dried specimen was powdered using mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

**Preparation of extract:**
The powdered plant material was extracted with successive solvent extraction ranging from non-polar to polar using soxhelet hot extraction process. The solvent was than distilled under reduced pressure, which gave brownish-black colored residues. The dried extracts were suspended in 0.1\% Tween 80 in normal saline (vehicle) and used for anthelmintic activity.

**Experimental procedure**
Benzene, Chloroform, Acetone, Methanol, and aqueous extracts from the bark of \textit{Zizyphus jujuba} were investigated for anthelmintic activity against \textit{Pheretima posthuma}. Various concentrations 20, 40, 60 and 80 mg/ml of methanolic and aqueous extracts, 40mg/ml of Benzene, Chloroform and Acetone extracts were tested by bioassay, which involved determination of time of paralysis and time of death of the worms. Piperazine citrate was used as standard reference and 0.1\% Tween 80 in normal saline as control. The Anthelmintic assay was carried as per the method followed by Ajaiyeoba et al with minor modifications.\textsuperscript{13} The assay was performed on adult Indian earthworms, \textit{Pheretima posthuma} due to its anatomical and physiological resemblance with that of intestinal round worm parasite of human beings.\textsuperscript{14-16} Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds \textit{in vitro}.\textsuperscript{17-19} The earthworms were collected from moist soil and washed with normal saline to remove all faecal matter and were used for the anthelmintic study. The earthworms of 6-8 cm in length and 0.2-0.3 cm in width were used for all experimental protocol. The earthworms were divided into thirteen groups containing six earthworms in each group. All the extracts and standard drug solution were freshly prepared in
0.1% Tween 80 in normal saline before starting the experiments. Different extracts and standard drug solutions were poured in different petri plates. All the earthworms were released into 10ml of formulation as follows: Benzene, Chloroform, Acetone, Methanol, Aqueous extracts and Piperazine citrate in different concentrations. Observations were made for the time taken to paralysis and death of worms. 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 when dipped in warm water (50ºC) followed with fading away of their body colours.

RESULTS AND DISCUSSION

Table 1: Anthelmintic activity of various extracts of Zizyphus jujuba bark

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (mg/ml)</th>
<th>Paralysis Time (Min)</th>
<th>Death Time (Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene extract</td>
<td>40mg/ml</td>
<td>79.66 ± 0.50</td>
<td>302.02 ± 0.58</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>40mg/ml</td>
<td>75.76 ± 0.42</td>
<td>262.50 ± 0.76</td>
</tr>
<tr>
<td>Acetone extract</td>
<td>40mg/ml</td>
<td>148.71 ± 0.51</td>
<td>79.75 ± 0.85</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>20mg/ml</td>
<td>147.25 ± 0.85</td>
<td>194.5 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>40mg/ml</td>
<td>36.5 ± 2.02</td>
<td>116.6 ± 0.74</td>
</tr>
<tr>
<td></td>
<td>60mg/ml</td>
<td>27.3 ± 1.52</td>
<td>48.3 ± 1.76</td>
</tr>
<tr>
<td></td>
<td>80mg/ml</td>
<td>10 ± 0.57</td>
<td>23.6 ± 1.14</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>20mg/ml</td>
<td>175.6 ± 0.76</td>
<td>250.02 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>40mg/ml</td>
<td>165.7 ± 1.15</td>
<td>240.02 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>60mg/ml</td>
<td>45.75 ± 0.52</td>
<td>117.6 ± 0.80</td>
</tr>
<tr>
<td></td>
<td>80mg/ml</td>
<td>36.62 ± 0.80</td>
<td>302.02 ± 0.58</td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td>40mg/ml</td>
<td>42.0 ± 1.26</td>
<td>59.4 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>60mg/ml</td>
<td>33.4 ± 0.60</td>
<td>55.6 ± 0.24</td>
</tr>
</tbody>
</table>

Figure 1: Anthelmintic activity of Zizyphus jujuba bark
CEZJ- Chloroform Extract of Zizyphus jujuba, AEZJ-Acetone Extract of Zizyphus jujuba, MEZJ- Methanol Extract of Zizyphus jujuba, AEZJ-Aqueous Extract of Zizyphus jujuba, BEZJ-Benzene Extract of Zizyphus jujuba

Preliminary phytochemical analysis showed the presence of flavonoids, steroids and tannins like phytoconstituents in the extracts of *Zizyphus jujuba* bark. Some of these phytoconstituents may be responsible to show a potent anthelmintic activity. The data revealed that the methanol extract showed anthelmintic activity at a concentration of 20 mg/ml, whereas the aqueous extract also showed paralysis and death at similar concentrations. The other test concentrations of both the extracts showed marked degree of anthelmintic activity. The anthelmintic effect of methanol extract at 40 mg/ml concentration is comparable with that of the effect produced by the standard drug piperazine citrate. The methanol extract showed the effect beyond 80 mg/ml concentration that is comparable with the standard drug Piperazine citrate. The present study suggested that the methanol extract was more effective than the other extracts, even though all the extract were endowed with anthelmintic property. The activity was concentration dependent of the different extracts. The activity of the extracts was found to be inversely proportional to the time taken for paralysis / death of the earth worms. Phytochemical screening of the extracts revealed the presence of flavonoids, tannins and steroids. Tannins chemically polyphenolic compounds, were shown to produce anthelmintic activities. Reported anthelmintic effect of tannins, can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death. Further studies are under process to identify the possible phytoconstituents responsible for anthelmintic activity.

**CONCLUSION:**

The traditional use of the leaves of *Zizyphus jujuba* as anthelmintic has been confirmed using the different extracts and showed significant anthelmintic activity. Further it would be interesting to isolate the responsible phytoconstituents, which are responsible for the anthelmintic activity and the mechanism of action, which is being attempted in the laboratory.

**ACKNOWLEDGEMENT:**

The author was thankful to the Chairman of Sree Sastha Pharmacy College, Chennai-Bangalore Highway, Chembarambakkam, Chennai, Tamilnadu for providing facilities to carry out the present research work

**REFERENCES:**


AJPTR is

- Peer-reviewed
- bimonthly
- Rapid publication

Submit your manuscript at: editor@ajptr.com