Phytochemistryical & Anthelmintic Studies on Blumea Lacera


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ABSTRACT: Purpose – The burden of ill health due to anthelmintic infection increasing day by day, in addition to this present anthelmintics are suffering from the problems of resistance and side effects, so it is strictly recommended to find out the anthelmintic drug with assured safety and less chances of resistance. Ayurweda is considered as one of the safest system of medicine and number of plant has reported for anthelmintic use, but activity of those plants is not proved scientifically in case of number of plants. Present study is based on the same objective so as to make an attempt to find out, to evaluate and to prove the anthelmintic activity of ayurvedic plant Blumea lacera scientifically. Methods - Extraction was made by soxhilation and maceration for extraction of alcoholic and aqueous extract respectively, freshly prepared extract was first used for preliminary phytochemical investigation then screened for in-vitro anthelmintic activity against Ascaris lumbricoides and Pheritema postuma by using piperazine citrate as standard. Results and Conclusions – The results of study found that the plant Blumea lacera possess good anthelmintic activity in dose dependent manner. © 2011 IGJPS. All rights reserved.

KEYWORDS: Blumea lacera; Anthelmintic activity; Ethanolic extract; Aqueous Extract; Pheritema postuma; Ascaris lumbricoides; Piperazine citrate.

INTRODUCTION

Helminthiasis a parasitic infection still considered as the major cause of ill health of number of peoples throughout the world especially peoples from deprived communities of undeveloped countries with poorer sanitary and health facilities, because it is mostly caused and sprayed through to environmental contamination and transmission. [1, 2] This parasitic infection is also responsible for increasing the mortality and morbidity day by day all over the world. The worms which are associated with problem of ill health include the trematodes (flukes), cestodes (tapeworms) and intestinal nematodes (roundworms). Recent estimates suggest that Ascaris lumbricoides infects more than 1 billion people and hookworms (Ancylostoma duodenale and Necator americanus combinely) infect about 740 million peoples. [3, 4] The condition is more serious in case of countries with low income which affected worstly and
having high risk of morbidity. The problem associated with ill health is getting more and more day to day and approximately 2.9 billion people are infected with different forms of Helminthiasis throughout the world and in case of China condition is more serious. In China 63% population is infected with either one of the nematode species [2, 5]. Though the mortality is considerably low as compared to huge amount of infections, still it causes more than 500,000 deaths annually. [1, 3, 5]

This huge burden of ill health is acknowledged by health organizations of some countries, but need to take more steps in more serious manner because most of the research organization and countries are not taking interest in anthelmintic drug as they are busy on working with more glamorous drugs for treating diseases associated with cardiac system, central nervous system, cancer, etc. So it is strictly recommended to take steps in serious manner so as to control the enormous health burden caused by these parasites. In addition to this the present anthelmintics are suffering from the problem of resistance, side effects and reinfection after removal of drug therapy. Side effects of anthelmintic commonly include intestinal gastro-intestinal disturbances nausea and giddiness, while various studies and reviews have showed the resistance to anthelmintic is increasing day to day. [6-11]

So to it is recommended to develop new alternatives which can treat helminthiasis completely with giving assurance about the safety in significant manner. In the recent years, the importance of Herbal drugs in the field of Medicine has greatly increased because of their assurance about safety and complete cure. Therefore the demand for the herbal formulation is also increasing accordingly. But the major problem associated with herbal drugs was lack of quality control and standardization methods, so standardization and quality control of phytochemical constituents is accelerated now days greatly with the development of different instrumental methods of analysis and this field becomes the center of attraction and interest for number of researcher and organizations working to develop the new drugs with sufficient safety profile. [5, 11]

Nature has provided a complete store-house of remedies to cure almost all the diseases of mankind and other animals too. Number of plant has reported anthelmintic activity but they are considered as underutilized because of lack of scientific proofs and records. Number of plants have reported and scientifically proved anthelmintic activity and use as well. While some plants were used as anthelmintics in ancient days but now days they are underutilized because of lack of scientific data because their anthelmintic activity is not proved scientifically. The plant Blumea lacera belongs to family Compositae had been reported for its anthelmintic use in ancient literature, but its anthelmintic activity was not scientifically proved, so through this study we had evaluated and proved the anthelmintic activity of Blumea lacera scientifically. [12-14]

**MATERIALS & METHODS**

The whole plant of Blumea lacera belongs to family Compositae was collected from rural areas of Latur district of Marathwada region, the collected plant material was confirmed and authenticated by the Botany department of School of Life Science, S.R.T.M. University Nanded.

Test parasites (worms) used for the study were Ascaris lumbricoides belongs to family Ascarididae, (a type of round worm) and Pheritema posthuma belongs to family Megascolecida. Parasites used under study were collected and authenticated from School of Life Science, S.R.T.M. University, Nanded. The worms used for the study were uniform in size. [15-17] We had used these two parasites because of their anatomical and physiological similarities with human parasite responsible for causing helminthiasis. Numbers of past studies involving evaluation of anthelmintic activity recommends the use of Pheritema posthuma and Ascaris
\textit{lumbricoides} as model test parasites for screening anthelmintics, [16-23] and it has been reported that substances which have toxic effect against earthworms also have toxic effects against most of the worms by causing primary irritation which leads to paralysis to the worms that results in the withdrawal of the worm outside the body. [15,19-24]

\textbf{Preparation and Preliminary Phytochemical Investigation of extract}

Well dried collected plant was ground in to powder form.500g of powder was used for extraction made by using maceration and soxhilation separately; aqueous extract was made by maceration method while soxhilation was used for preparing alcoholic extract. Solvents used were water for maceration and ethanol for soxhilation. Freshly collected extract of plant was first divided in two groups as aqueous and alcoholic, third group was made by mixing both extract together in 1:1 proportion. Each of this group was subdivided in to four subgroup of different concentration of 10%, 25%, 50% and 100% as shown in Table – 1. Solvent used for dilution was distilled water. These freshly prepared solutions were used for evaluation of anthelmintic activities. In one group water was used, it termed as controlled group, while one group was consist of piperazine citrate which was used as standard at concentration of 10%.[5, 21-24]

\begin{table}[h]
\centering
\caption{Composition and Coding of Final Extract Dilutions -}
\begin{tabular}{ |c|c|c|c|c|c|c|c|}
\hline
\textbf{Aq. Extract of Blumea lacera (A)} & \textbf{Alco. Extract of Blumea lacera (B)} & \textbf{Combination (Alcoholic : Aqueous Extract, 1:1) (C)} \\
\hline
\textbf{Code} & \% Concentration & \textbf{Code} & \% Concentration & \textbf{Code} & \% Concentration \\
\hline
A1 & 10\% & B1 & 10\% & C1 & 10\% \\
A3 & 50\% & B3 & 50\% & C3 & 50\% \\
A4 & 100\% & B4 & 100\% & C4 & 100\% \\
\hline
\end{tabular}
\end{table}

Preliminary phytochemical evaluation of extract was performed so as to confirm the presence of different chemical constituent present in plant. The presence of different constituent in prepared extract were confirmed by doing general chemical tests for identification of respective chemical constituents as shown in Table -2. [21, 23]

\textbf{Evaluation of anthelmintic activity}

Anthelmintic activity was evaluated by using the common method of evaluation of anthelmintic activity as used in number of previous studies [5, 16, 18, 19] baed on the evaluation of anthelmintic activity of plant extract. First petri plates were named and grouped as per dilution codes for different extract as shown in Table -1; then one worm is placed in each plate after that respective dilution of extracts was poured in that plates. After pouring the extract worms were placed in plates and plates were keep under observation until the death of worms. Observations were recorded in the form of time required for consecutive attacks of paralysis till the death of earthworm and at the end time required for complete death of worm was noted, death of worm has been confirmed by pointing with needle. [21-24]
Preliminary phytochemical Investigation

The preliminary phytochemical investigation of prepared plant extract was performed and presence of, tannins, alkaloids, saponin anthraquinone glycoside, steroids, flavonoids, phenolics and terpenoids in prepared plant extract was confirmed. The result of phytochemical extraction was as shown in Table – 2.

Table 2 - Preliminary Phytochemical Evaluation:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Phytochemical Test</th>
<th>Aqueous Extract (A)</th>
<th>Alcoholic Extract (B)</th>
<th>Alcoholic + Aqueous Extract (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Tannins</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2.</td>
<td>Alkaloids</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>3.</td>
<td>Saponin</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>4.</td>
<td>Anthraquinone glycoside</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>5.</td>
<td>Steroids</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>6.</td>
<td>Flavonoids</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>7.</td>
<td>Phenolics</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>8.</td>
<td>Terpenoids</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Evaluation of anthelmintic activity

Results were recorded as shown in table-3 as in the form of time required for the consecutive attacks of paralysis till the death of parasite; generally death of parasite was observed after three attacks of paralysis. The time required for death of parasite were also recorded which was the endpoint of observations. At lower concentration range of 10% the death of parasite was not observed which may be because of low potency of extracts; but further increase in concentration were found to possess good anthelmintic potential in dose dependent manner as shown in observation table.

Results were also plotted as shown in graph for comparison of potency of both extracts. Both extracts were showing good anthelmintic activity when compared with piperazine citrate as standard, particularly at dose of 100% concentration. But concentration of extract required to show equal potency with standard was very high. Combination of extracts was found more potent as compared to both extract when they used separately, it may be because of presence of all chemical constituent which get extracted with either aqueous or alcoholic extract. The death of parasite was observed after three attacks mostly but some time attacks of paralysis was not observed as in case of dilution of 10% concentration, some time parasite get recovered after one or two attacks of paralysis as like in case of dilution of dose of 25% concentration, While sometime the death of parasite was observed after two attacks of the paralysis only which may be because of the high potency of the extract.

The results of study shows the extracts of plant were showing very significant anthelmintic activity in dose dependent manner. Alcoholic extract was found to possess slight more potency when compared to aqueous.
### Table 3–Observation and Result -

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample Code</th>
<th>Pheritema postuma</th>
<th>Ascaris lumbricoides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Time of Attacks of paralysis (S)</td>
<td>Time req. for Death (S)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ist</td>
<td>IIrd</td>
</tr>
<tr>
<td>1)</td>
<td>Controlled (Water)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2)</td>
<td>Piperazine Citrate (S)</td>
<td>1112</td>
<td>1857</td>
</tr>
<tr>
<td>3)</td>
<td>A1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4)</td>
<td>A2</td>
<td>3023</td>
<td>4945</td>
</tr>
<tr>
<td>5)</td>
<td>A3</td>
<td>1909</td>
<td>3198</td>
</tr>
<tr>
<td>6)</td>
<td>A4</td>
<td>1110</td>
<td>1953</td>
</tr>
<tr>
<td>7)</td>
<td>B1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8)</td>
<td>B2</td>
<td>2132</td>
<td>3016</td>
</tr>
<tr>
<td>9)</td>
<td>B3</td>
<td>1319</td>
<td>1829</td>
</tr>
<tr>
<td>10)</td>
<td>B4</td>
<td>756</td>
<td>1368</td>
</tr>
<tr>
<td>11)</td>
<td>C1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12)</td>
<td>C2</td>
<td>1764</td>
<td>3808</td>
</tr>
<tr>
<td>13)</td>
<td>C3</td>
<td>812</td>
<td>1432</td>
</tr>
<tr>
<td>14)</td>
<td>C4</td>
<td>569</td>
<td>1087</td>
</tr>
</tbody>
</table>
Finally study concludes that the plant under study has found to possess significant anthelmintic activity in dose dependent manner and has potential to develop as useful and safe anthelmintic alternative, but it demands more thorough study to find out the exact chemical responsible for anthelmintic activity of plant so as to isolate and extract it separately so as to improve the potency. The phytochemical evaluation of plant shows that plant bear vast chemical composition, but tannins, alkaloids and phenolics present in plant may be responsible for anthelmintic activity of plant, because they have already reported responsible for anthelmintic activity of various plants. [25-30]

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