SCREENING THE INVITRO ANTHELMINTIC ACTIVITY OF
ALTERNANTHERA SESSILIS LEAVES

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ABSTRACT

Objective: The present study aimed at the in-vitro evaluation of anthelmintic activity of aqueous, methanol and acetone extracts of the leaves of Alternanthera sessilis using Pheretima posthuma. Methods: The parameters like the time of paralysis and the time of death of Pheretima posthuma were determined by using the different solvent extracts of A. sessilis at the concentrations of 25, 50, 75 and 100 mg/ml. Results: All the extracts of A. sessilis exhibited significant anthelmintic activity at 25, 50, 75 and 100 mg/ml as compared with standard drug - albendazole (15 mg/ml). The methanolic extract showed greater activity at each concentration than aqueous and acetone extracts. Conclusion: In conclusion, the use of leaves of A. sessilis as an anthelmintic have been confirmed and further studies are suggested to isolate the active principles responsible for the activity.

KEYWORDS: Alternanthera sessilis, Anthelmintic activity, Pheretima posthuma, methanolic extract, Albendazole.

INTRODUCTION

Helminthes effecting man and cattle: Helminthes are recognized as a major problem to livestock’s throughout tropics. In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia.[1] The helminthes parasites mainly subsist in human body in intestinal tract, but they are also found in tissue, as their larvae migrate towards them.[2] Helminthes are divided into three groups based on their body segmentations, namely: Trematodes (flukes), Nematodes (roundworms) and Cestodes (tapeworms). Helminthes have multi-cellular bodies.
and complex life cycles involving maturation in a host organism. In most developing countries, intestinal helminth infections are a major health concern because factors that pre-dispose humans to these infections abound in these areas.\textsuperscript{[3]} Estimated that the global burden of helminth infections, in terms of disability-adjusted life years (DALYs), is 39 million life years which was comparable to that of tuberculosis (34.7 million DALYs) or malaria (46.5 million DALYs), the two major human infectious diseases associated with a high mortality rate. Factors that sustain the parasite life cycles and favour the proliferation of the disease vectors include poor sanitation, poverty, unsafe water, malnutrition and ignorance.\textsuperscript{[4]} Gastrointestinal parasite becomes a serious threat to the livestock production in the developing nations. Even most common drugs like piperazine salts have been shown to have side effects like nausea, intestinal disturbances and giddiness.\textsuperscript{[5]} Inspite of the development of anthelmintic resistance in the parasites of higher economical significance, chemotherapy is still used widely for the purpose of controlling the helminthes.\textsuperscript{[6]} Helminthiasis which is caused by the helminthes infection is proved to be a major constraint in the livestock production all around the globe. As mentioned above, chemotherapeutics remain the corner stone for treating the helminthiasis by overcoming certain factors such as chemical residues and toxicity, increased cost, non-adaptability of drugs and non-availability in the remote areas.\textsuperscript{[7]} Among the natural sources, medicinal plants play an important role to most of the medicinal preparations as raw plant materials, refined crude extracts and mixtures etc. Even in recent times, majority of the people are still depending on the traditional medicine for their primary health care. According to the World Health Organization, almost 80% of the world’s population is still relying on traditional plant-based medicines.\textsuperscript{[8]} It has been studied that fruits and herbs containing phytochemicals and non-nutritives may protect human from a host of diseases for their biological activities.\textsuperscript{[9]} The history of herbal medicine is almost as old as human civilization. The plants are known to provide a rich source of botanical anthelmintics, antibacterials and insecticides.\textsuperscript{[10,11]} A number of medicinal plants have been used to treat parasitic infections in man and animals.\textsuperscript{[12-16]} \textit{Alternanthera sessilis} (L.) (Amaranthaceae) is known in english as sessile joy weed or dwarf copper leaf and in Bangladesh as Chanchishak. It is an aquatic plant and can be commonly observed in marshy areas and wetlands of Bangladesh. Folk medicinal practitioners of Bangladesh consider the plant to possess medicinal properties. In Bangladesh, the plant is used to treat gonorrhoea, low sperm count and leucorrhrea in Noakhali district\textsuperscript{[17]} and in several areas of Faridpur and Rajbari districts, the plant is used by folk medicinal practitioners for treatment of severe pain.\textsuperscript{[18]} In India the tribals of Bargarh district use the plant to treat blood dysentery.\textsuperscript{[19]} Different communities of
Uttara Kannada district of Karnataka use the plant for treatment of ulcers, cuts and wounds.[20] The plant is used by local tribals (Santals, Gonds, Kotha, Bathudi) and inhabitants of Kaptipada Forest Range in Orissa for treatment of fevers, ophthalmia, gonorrhoea and pruritis.[21] The local people of Amarkantak region, Madhya Pradesh have multiple uses for the plant including treatment of burning sensations, diarrhoea, skin diseases, dyspepsia, haemorrhoids, liver and spleen diseases and fever.[22] The Irulatribals of Kalavai, Vellore district treat headache, hepatitis and asthma with the plant.[23] Anti-bacterial activity and possible cytotoxicity as demonstrated by brine shrimp lethality assay has been reported for A. sessilis.[24] According to Tan and Kim et al., ethyl acetate fraction of A. sessilis Red to reduce fasting blood glucose level, triglyceride level and free fatty acid level when administered to obese type 2 diabetic rats induced by high fat diet and streptozotocin.[25] Anti-allergic effect of ethanolic extract of the plant has also been reported by Rayees et al.[26] Hence the present study of aims to evaluate the anthelmintic activity of various extracts of A. sessilis leaves.

MATERIALS AND METHODS

Collection of plant material

The plant A. sessilis was collected from field areas around Mayiladuthurai, Tamilnadu, India, where it was found naturally in wetlands. The leaves were separated and washed thoroughly in running tap water to remove soil particles and other adhered debris and then finally washed with sterile distilled water. The leaves were shade dried and ground well into fine powder. The powdered materials were stored in air tight container until the time of use.

Phytochemical screening

10 grams of A. sessilis powder was soaked in 50 ml of various solvents (aqueous, methanol and acetone) separately and kept at room temperature for 12 hours.[27,28] The samples were filtered and used for phytochemical screening. Phytochemical screening the aqueous, methanol and acetone extracts of this plant were screened for the presence of various phytochemical constituents such as alkaloids, flavonoids, phlobatannins, anthroquinones, steroids, tannins, phenols, terpenoids, saponins, resins, carbohydrate, protein and amino acid compounds.[29,30,31]

Test for alkaloids

Crude extract was mixed with 2 ml of Wagner’s reagent. Reddish brown colored precipitate indicates the presence of alkaloids.
Test for flavonoids
5 ml of dilute ammonia solution was added to a portion of the crude extract followed by addition of concentrated $\text{H}_2\text{SO}_4$. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Test for resins
To 1 ml of crude extract was treated with few drops of acetic anhydride solution followed by 1 ml of concentrated $\text{H}_2\text{SO}_4$. Resins give colouration ranging from orange to yellow.

Test for saponins
Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam was taken as an indication for the presence of saponins.

Test for glycosides
5 ml of crude extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated $\text{H}_2\text{SO}_4$. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for steroids
2 ml of acetic anhydride was added to 0.5 ml crude extract of plant sample with 2 ml $\text{H}_2\text{SO}_4$. The colour changed from violet to blue or green in samples indicates the presence of steroids.

Test for tannins
1 ml of the sample was taken in a test tube and then 1 ml of 0.008 M potassium ferric cyanide was added. 1 ml of 0.02 M Ferric chloride containing 0.1 N HCl was added and observed for blue-black coloration.

Test for phenols
To 1 ml of various solvent extract of sample, 2 ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution was added. Formation of blue or green colour indicated the presence of phenols.
Test for terpenoids
5 ml of crude extract was mixed with 2 ml of chloroform and 3 ml of concentrated H$_2$SO$_4$ was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for anthroquinones
Dilute NaOH was added to the 1 ml of crude extract. Blue green or red coloration indicates the presence of anthroquinones.

Evaluation of invitro anthelmintic activity$^{[32]}$
Experimental procedure
Test drug
The plant leaf powder was subjected to hot water maceration to obtain aqueous extracts. The dry powder is extracted with acetone and methanol using maceration process for 48 hours. The powdered A. sessilis was extracted exhaustively with increasing polarity solvents (methanol, acetone and water) for 72 hours followed 48 hours and 24 hours. The solvents were pooled, distilled under vaccum and dried under vaccum dessicator. Different concentrations (25, 50, 75 and 100mg) of each extract solution were prepared by diluting the stock solution, in propylene glycol, using normal saline.

Reference drug
Albendazole was prepared by dissolving them in normal saline at a concentration of 15mg/ml.

Experimental control treatment
A 10% propylene glycol in normal saline was used as experimental control treatment.

Normal control
Saline was prepared and used to treat the normal control group.

Experimental worms
All the experiments were carried out in Indian adult earthworms Pheretima posthuma due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collected from moist soil and washed with water to remove all fecal matters.
Anthelmintic activity

The anthelmintic activity was performed according to the method Ghosh et al. on adult Indian earth worm *Pheretima posthuma* as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. *Pheretima posthuma* was placed in petridish containing four different concentrations (25, 50, 75 and 100mg) each of *A. sessilis* (methanol, acetone and water) extract solutions. Each petridish was placed with 3 worms and observed for paralysis and death. The mean time for paralysis was noted when no movement of any sort could be observed, except when the worm as shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. In the same manner albendazole was included as reference compound. The test results were compared with reference compound albendazole (15mg/ml) treated samples.

RESULTS

Table-1 Qualitative evaluation of phytochemical constituents of aqueous, methanol, and acetone extract of *A. sessilis*.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Phytochemical constituents</th>
<th>Aqueous extract</th>
<th>Methanolic extract</th>
<th>Acetone extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Resins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>Anthroquinones</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td>Phlobatannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12.</td>
<td>Carbohydrate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13.</td>
<td>Protein and amino acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+)Present and (-) Absent

Table-1 indicates the phytochemical constituents of aqueous, methanol and acetone extracts of *A. sessilis* leaves when subjected to qualitative analysis for alkaloids, flavonoids, resins, saponins, glycosides, tannins, phenols, terpenoids, anthroquinones, phlobatannins, carbohydrate, protein and amino acid. By preliminary phytochemical screening it was found that all the three extracts of plant contain carbohydrates, proteins, alkaloids, steroids, terpenoids, saponins, glycosides, tannins and phenols.
Table-2 *Invitro* anthelmintic activity of various extracts *A. sessilis*.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment of extracts</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>1.</td>
<td>Normal control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Experimental control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Albendazole (Reference)</td>
<td>15</td>
<td>38±0.4</td>
<td>58±0.4</td>
</tr>
<tr>
<td>4.</td>
<td>Aqueous</td>
<td>25</td>
<td>35±0.6</td>
<td>54±0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>32±0.3</td>
<td>49±0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75</td>
<td>29±0.9</td>
<td>44±0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>18±0.9</td>
<td>30±0.9</td>
</tr>
<tr>
<td>5.</td>
<td>Methanol</td>
<td>25</td>
<td>29±0.1</td>
<td>42±0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>23±0.4</td>
<td>39±0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75</td>
<td>17±0.3</td>
<td>28±0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>10±0.1</td>
<td>23±0.3</td>
</tr>
<tr>
<td>6.</td>
<td>Acetone</td>
<td>25</td>
<td>30±0.4</td>
<td>44±0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>25±0.5</td>
<td>42±0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75</td>
<td>22±0.4</td>
<td>38±0.4</td>
</tr>
</tbody>
</table>

All values represent Mean ± SD; n=3 in each group. Comparisons made between standard versus treated groups.

Table -2 Shows higher concentration of extract produced paralytic effect much earlier and taken for death was shorter for worms. Aqueous, methanol and acetone extracts of *A. sessilis* exhibited anthelmintic activity in dose-dependent manner and showing maximum efficacy at 25, 50, 75, 100 mg/ml concentration for worms than albendazole.

Figure 1. Time taken for paralysis of *Pheretima posthuma* by various solvent extracts of *A. sessilis*.
Figure 2. Time taken for death of *Pheretima posthuma* by various solvent extracts of *A. sessilis*

Figure 1 and 2- shows higher concentration of extract produced paralytic effect much earlier and time taken for death was shorter for worms respectively. methanolic extract of *A. sessilis* exhibited anthelmintic activity in dose-dependent manner and showing maximum efficacy at 25, 50, 75, 100 mg/ml concentration for worms than acetone and aqueous extracts of *A. sessilis* leaves.

**DISCUSSION**

In the present study all the tests were performed *invitro*. One of the main advantages of analyzing the biological properties of plant extracts *invitro* is that, the process is cost effective and includes rapid turnover allowing the screening of plants at large scale.\(^{[33]}\)

Preliminary phytochemical studies on *A. sessilis* exposed the presence of carbohydrates, proteins, alkaloids, flavanoids, steroids, trepenoids, saponins, glycosides, tannins and phenols etc.

The crude extract samples, which were used to evaluate anthelmintic activity, showed variable times at different concentrations and the mean time values were calculated for each parameter. The crude extracts of *A. sessilis* showed the significant anthelmintic effect causing death of the worm at all the concentrations but the time of death was different in each case compared to albendazole. However, when observed the response of worms in case of paralysis, there was significant variation among the results produced by the different extracts at different concentrations like 25, 50, 75 and 100mg/ml. The methanolic extract showed
more significant effect on paralyzing the worms at every concentration compared to that of acetone and aqueous extracts. The effect of extracts on the paralysis (or) helminthiasis of the worm, according to the results (Figure 1) may be indicated as methanol>acetone>aqueous extracts. This may be due to the increased level of extraction of tannins in methanolic extract followed by acetone>aqueous extracts. The data presented in the table and observations made thereof, lead to the conclusion that the different degree of helminthiasis of the different extracts are due to the level of tannins present in it. Some of these phytoconstituents like alkaloids, tannins, phenols etc. may be accountable to have a significant anthelmintic activity.[34]

Although the plants have the anthelmintic activity mainly due to their phytoconstituents specially due to secondary metabolites it has not be understand clearly the mechanism of action of herbs for their anthelmintic activity. Phytoconstituents, jointly or separately may act by inhibition of tubulin polymerization and blocking glucose uptake.[35] Any damage to the mucopolysaccharide membrane of worms will expose the outer layer restricting their movement which finally may cause paralysis and ultimately death of parasite.[36]

The anthelmintic effects of tannins may be attributed to its capacity to bind free protein available for larval nutrition and thus reducing the nutrient availability resulting in larval starvation or decrease in gastrointestinal metabolism directly through inhibition of oxidative phosphorylation causing larval death.[37,38] According to Roy et al., alkaloids may act on central nervous system and caused paralysis of the earthworm.[39] The effect can be due to presence of the steroidal alkaloid and oligoglycosides which may suppress the transfer of sucrose from the stomach to the small intestine together with their antioxidant effect which is capable of reducing the nitrate generation which can interfere in local homeostasis that is essential for the development of helminthes.[40]

Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation or, binds to the glycoprotein on the cuticle of parasite and cause death.[41] Coming to the chemistry of nematode surface, it is a collagen rich extracellular matrix (ECM) providing protective cuticle that forms Exoskeleton and is critical for viability, the collagen is a class of proteins that are modified by a range co- and post-translational modification prior to assembly into higher order complexes or ECMS.[42] The mammalian skin also consists largely of collagen in the form of fibrous bundles. In leather making industry, vegetable tannins are commonly used in the
tanning operation of leather processing that imparts stability to collagen of skin matrix through its reactivity and hence make the collagen molecule aggregate into fibres this results in the loss of flexibility in the collagen matrix and gain of mechanical property with improved resistance to the thermal or microbial/enzymatic attack.[39] Similar kind of reaction is expected to take place between the *Pheretima posthuma* (the earth worm) and the tannin of *A. sessilis*, possibly by linking through hydrogen bonding, as proposed in this study. This form of reactivity brings toughness in the skin and hence the worms become immobile and non-functional leading to paralysis followed by death. In another study, alkaloids were reported to cause paralysis of the worms by acting on its central nervous system.[39] The prime effect of albendazole is to cause a flaccid paralysis of the worm which results in expulsion of the worm by peristalsis. Albendazole acts to increase chloride ion conductance of worm muscle membrane which produces hyperpolarization and excitability reduction that leads to muscle relaxation and flaccid paralysis of worms.[43] It is expected that the phytochemicals present in the extract of *A. sessilis* may have produced similar effects, causing death of the worms. Therefore, the usual claim of leaves of *A. sessilis* as an anthelmintic has been confirmed as the extracts shown significant activity against *Pheretima posthuma*.

**CONCLUSION**

*A. sessilis* was used from the ancient period for the treatment of the above anthelmintic diseases but without knowing their actual mechanism and the compound that is responsible for the curing action. The present report confirms that the various solvent extracts of *A. sessilis* shows anthelmintic activity. The results of paralysis time and death time of methanolic extract of this plant leaf was showing greater activity at concentration–dependent when compared with other extracts and standard drug-albendazole. The chemically provided drugs are more costly and provide higher side effects when compared to the natural drugs that are obtained from the plant source were cheaper and provide lesser on the host organism. Hence, further study must be carried out to explore the *A. sessilis* of higher efficiency and lesser side effect.

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