In vitro anti-arthritic activity of cnidoscolus aconitifolius latex extract by protein denaturation method

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ABSTRACT:

The study is intended to gauge the Latex of *Cnidoscolus aconitifolius* for commended anti-arthritic activity using *in vitro* approach. These were analyzed by inhibition of protein denaturation method. Acetyl salicylic acid was used as a standard drug. Results revealed that the petroleum ether extract of *Cnidoscolus aconitifolius* latex at four different concentrations influenced significant anti-arthritic activity as compared to standard used drug. The latex petroleum ether extract showed better activity. In the study, petroleum ether extract of *Cnidoscolus aconitifolius* latex inhibited heat induced protein denaturation and may be one of the reasons of possessing anti-arthritic activity. It can be concluded that petroleum ether extract of *Cnidoscolus aconitifolius* latex possess good anti-arthritic activities. The medicinal value of *Cnidoscolus aconitifolius* latex and make reasons why this is used traditionally for various diseases. From this study we conclude that it is promising to develop a latex based anti-arthritic drug for the ailments of rheumatic diseases and other associated diseases.

Keywords: Latex, *Cnidoscolus aconitifolius*, Anti-arthritic, Petroleum ether, Protein denaturation


INTRODUCTION

Throughout the history, humans have been using medicinal plants. These plants contain many chemical compounds which are effective against fungal infections, insect bites and even animal bites. More than 12,000 active compounds are known to man. Like pharmaceutical drugs, these herbs can have medicinal as well as adverse effects. A single herb may contain many chemical substances and taking the whole herb can lead to complications [1]. The global demand of herbal medicine is constantly increasing and I expected to reach US$ 7 trillion by 2050. The Indian share in the world trade is comparatively quite low [2]. There are several methods to selecting higher plants as contenders for drug improvement with the greatest prospect of success. The role of information derived from various systems of traditional medicine such as ethnomedicine and its utility for drug discovery purposes [3]. *Cnidoscolus aconitifolius*, commonly known as chaya or tree spinach belongs to the family Euphorbiaceae. It is a large, fast-growing leafy perennial shrub that is believed to have originated in the Yucatán Peninsula of Mexico. The specific epithet,
**MATERIALS AND METHODS**

**Sample Collection**

The plant material used in this study was *Cnidoscolus aconitifolius* latex. *C. aconitifolius* latex were collected from Kanyakumari district during the month of January - February in the year 2019. The latex was collected from the leaf nodes, stem etc. using filler.

**Preparation of Powder from Latex**

The latex was allowed to dry in eppendorf tube. The dried latex was separated and cleaned well. Cleaned latex were then allowed to dry and crushed using a mortar and pestle. After grinding, the latex was transferred into air tight container with proper labelling for further use.

**Preparation of Extract from Latex**

The dried and powdered *Cnidoscolus aconitifolius* latex were extracted sequentially with Ethanol, Methanol, Chloroform, Petroleum ether, Ethyl acetate and Aqueous using Soxhlet apparatus. Petroleum ether extract was used for further studies.

**Anti-arthritic Activity**

*In vitro* anti-arthritic activity of *Cnidoscolus aconitifolius* latex (petroleum ether extracts) were analyzed by inhibition of protein denaturation method.
The test solution consists of 0.45 ml of bovine serum albumin and 0.05 ml of test solution. The test control consists of bovine serum albumin and 0.05 ml of distilled water. Product control consists of 0.45 ml of distilled water and 0.05 ml of test solution. Standard solution consists of 0.45 ml of Bovine Serum Albumin and 0.05 ml of Diclofenac sodium. All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37°C for 20 minutes and the temperature was increased to 57°C for 3 minutes. After cooling, added 2.5ml of phosphate buffer to the solutions. The absorbance was measured using uv-visible spectrophotometer at 416nm (SL119, Systronics).

The percentage inhibition of protein denaturation can calculated as:

\[
\% \text{ Percentage inhibition} = 100 - \left( \frac{\text{OD of test solution} - \text{OD of product control}}{\text{OD of test control}} \right) \times 100
\]

RESULTS AND DISCUSSION

The in vitro anti-arthritis activity by Bovine Serum Albumin denaturation method at concentration of 62.5µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL showed 33.10 %, 57.86 %, 63.57 %, and 67.38 % inhibition of denaturation of bovine serum were tabulated in Table 1. The knowledge standard diclofenac at 62.5µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL which showed 50.77 %, 74.27 %, 81.13 % and 89.36 % inhibition of denaturation biological activities were tabulated in Table 2. The optical density of the standard test control was 0.0583, whereas the optical test control of sample was 0.0420. Some literature reported that denaturation of protein is one of the cause of rheumatoid arthritis. The comparative protein denaturation of standard diclofenac sodium and C. aconitifolius latex petroleum ether extract was graphically shown in Fig 1.

<table>
<thead>
<tr>
<th>Concentrations (µg/mL)</th>
<th>OD of test solution</th>
<th>OD of product control</th>
<th>Percentage of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>62.5</td>
<td>0.227</td>
<td>0.1983</td>
<td>50.77</td>
</tr>
<tr>
<td>125</td>
<td>0.0311</td>
<td>0.0161</td>
<td>74.27</td>
</tr>
<tr>
<td>250</td>
<td>0.0553</td>
<td>0.0443</td>
<td>81.13</td>
</tr>
<tr>
<td>500</td>
<td>0.0876</td>
<td>0.0814</td>
<td>89.36</td>
</tr>
</tbody>
</table>
Table 2. Effect on protein denaturation (*C. aconitifolius* latex petroleum ether extract)

<table>
<thead>
<tr>
<th>Concentrations (µg/mL)</th>
<th>OD of test solution</th>
<th>OD of product control</th>
<th>Percentage of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>62.5</td>
<td>0.0391</td>
<td>0.011</td>
<td>33.10</td>
</tr>
<tr>
<td>125</td>
<td>0.0475</td>
<td>0.0298</td>
<td>57.86</td>
</tr>
<tr>
<td>250</td>
<td>0.0649</td>
<td>0.0496</td>
<td>63.57</td>
</tr>
<tr>
<td>500</td>
<td>0.0895</td>
<td>0.0758</td>
<td>67.38</td>
</tr>
</tbody>
</table>

Fig. 1. Percentage of inhibition vs. Concentration *in vitro* anti-arthritic activity (*Diclofenc Sodium* and *C. aconitifolius*)

Production of auto-antigens in certain rheumatic diseases may be due to denaturation of proteins. Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding [6]. In the present study, petroleum ether extract of *C. aconitifolius* latex inhibited heat induced protein denaturation and may be one of the reason of possessing anti-arthritic activity [7].

CONCLUSION

It has been foreseeable that 80% of the populations of developing countries rely on traditional medicines. The improved popularity of herbal suppositories for cancer healing possibly can be attributed to the belief that herbal drugs provide benefit over that of allopathic medicines while being less toxic [8]. Finally, it can be concluded that
petroleum ether extract of *C. aconitifolius* latex possess good in-vitro anti-cancer and anti-arthritic activities. By further extensive research, we can explore the medicinal value of *C. aconitifolius* latex and make reasons why this is used traditionally for various diseases. From this study we conclude that it is possible to develop a latex based anti-arthritic drug for the ailments of rheumatic diseases and other associated diseases [9].

**REFERENCE**