EVALUATION OF BRINE SHRIMP LETHALITY OF ADIANTUM LATIFOLIUM LAM. A MEDICINAL FERN

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ABSTRACT

The Pteridophytic plant Adiantum latifolium Lam. is an important medicinal fern belongs to the family Adiantaceae. The present study was intended to determine the lethality of ethanolic extract of A. latifolium Lam. over Brine shrimp Artemia nauplii and to find out its toxicity. The studies show that the ethanolic extract of A. latifolium Lam. Expressed of mortality on Brine shrimp at higher concentration of 400 µg/ml and 600 µg/ml alone. Compared to positive and negative controls used the ethanolic extract of A. latifolium Lam. revealed lower toxicity with LD50 value at 480 µg/ml. Thus, the plant proves that it could be explored for its bioactive principles.

1. INTRODUCTION

Ferns produce a wide array of secondary metabolites endowed with different bioactivities that could potentially be useful in the treatment of many diseases. The medicinal importance of the Pteridophyte is due to the presence of some special active principle like alkaloids, flavonoids, phenols, tannins and saponins, which remain concentrated in the storage organs of the plants viz., roots, fronds, rhizome etc. In general, these secondary metabolites are an important source of drug with a variety of structural arrangements and properties (De Fátima et al., 2006).

Cancer is a major public health burden in both developed and developing countries. Plant derived agents are being used for the treatment of cancer (Shoeb Mohammad et al., 2006). Currently cancer is a common household word, among us and closely associated with at least one near and dear one, a family member or a friend, a neighbor, or a colleague, diagnosed with cancer. In 2018, an estimated 18.1 million new cases of cancer occurred Worldwide which are likely to increase to 23.6 million new cases each year by 2030 (Bray et al., 2018). Considering the high-profile nature of the disease, its treatment has been a constant struggle with relatively less success. While exploring drug, the toxicity of the drug is also to be analyzed.

The present experimental plant was explored for its phytocompounds through GCMS analysis and was found to have high medicinal value to cure various diseases and displayed phytocompound having anticancer compound. Based on the previous study of anticancer property the toxicity of the A. latifolium Lam. extract was planned to evaluate using Brine shrimp as an experimental organism.

2. MATERIALS AND METHODS

2.1: Collection of Plant materials.
Sporophytes of Adiantumlatifolium Lam. was collected from Kaniyakumari District (Tamil Nadu). These plant samples were authenticated by Dr. Raju Antony, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram, Kerala and a voucher specimen were deposited in the Department of Botany, (Voucher No. 001). The Experimental plant of A. latifolium Lam. were shade dried for two to three weeks until the moisture content of the plants was get drained.
off. After getting dried, the whole plant was ground into a fine powder. The powdered mass obtained was stored in a clean sterile bottle at room temperature.

2.1.2: Preparation of Plant extracts
The dried plant powder of A. latifolium Lam. was extracted with ethanol (Soaked with 24hrs). The ethanolic whole plant extract of A. latifolium Lam. was subjected to chromatographic techniques, which was successively eluted. The eluted experimental plant extract of A. latifolium Lam. was used for further investigation.

2.1.3: Brine Shrimp Lethality Bioassay (Meyer et al., 1982)
Brine shrimp lethality bioassay is performed for screening of general toxic properties, which also indicates a range of bioactivities such as anticancer, antiviral and pesticidal properties. The brine shrimp lethality bioassay was carried out in ethanolic extracts of Adiantum latifolium Lam. in Artemia nauplii a test organism to monitor the cytotoxicity of a compounds present in the plant. The LC\textsubscript{50} values and cytotoxicity were estimated by performing the bioassay.

Artemia nauplii were collected from the Centre for Marine Science and Technology, Rajakkamangalam, Kanya Kumari District. 38 grams sea salt (pure NaCl) was weighed, dissolved in one litre of distilled water and filtered off to get clear solution. Sea water was taken in a small tank and Artemia nauplii leach (brine shrimp eggs) was added to one side of the tank and then this side was covered. Within Two days the eggs could hatch the shrimp and to be matured as nauplii. Ten nauplii were drawn through a glass capillary and placed in each concentration such as 20, 40, 50, 200, 400 and 600\(\mu\)g/ml whole plant ethanolic extract of A. latifolium Lam. which were prepared by serial dilution using DMSO as solvent (Ananna Mahfuz et al., 2019). Each concentration was tested in triplicate, giving a total of 12 test-tubes. The final volume of the solution in each test-tube were made up to 5 ml with sea water immediately after adding shrimp larvae. As a negative control, 5 ml of DMSO alone was used.

2.1.4: Determination of Lethality
In each test tube, consisting of exposing groups of 10 Artemia nauplii aged 12 to various concentrations of the drug extract, the numbers of survivors were counted, and percentages of deaths were calculated. The toxicity was determined after 12 hours of exposure. Larvae were considered dead if they did not exhibit any internal or external movement during several seconds of observation. The number of dead and live nauplii in each tube was counted using stereomicroscope.

The percentage of mortality (% M) = percentage of survival in the control - percentage of survival in the treatment.

The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes.

Experiments were done in triplicates and the results were calculated as mean (±) standard deviation. Best fit line linear regression analysis was carried out using SPSS Package. LD\textsubscript{50} values of the two plant extracts were calculated from the best fit linear regression line plotted with percentage of nauplii against different tested concentrations. Higher LD\textsubscript{50} values indicate the lesser toxicity of the plant material. Podophyllotoxin (30 \(\mu\)g/ml) was used as a positive control in the bioassay.

3. RESULTS AND DISCUSSION
3.1: Cytotoxic activity of frond ethanolic extract of Adiantum latifolium Lam. over Brine Shrimp
Brine shrimp lethality assay reveals that the degree of mortality of hatched nauplii was directly related to the concentration of the extract. The present assay shows a moderate cytotoxicity activity at high concentration.
The whole plant ethanolic extract of A. latifolium Lam. showed low toxicity when compared to the positive control Podophyllotoxin. Using positive control, podophyllotoxin, 30μg/ml concentration showed 50% lethality. The lethality rate of larva was found to be dose dependent. The whole plant ethanolic extract of A. latifolium Lam. showed 0, 0, 10, 10, 30, 40 and 60 percentage of mortality at 20, 40, 50, 100, 200, 400 and 600 µg/ml concentrations respectively (Table -1). The LD50 value of whole plant ethanolic extract of A. latifolium Lam. was noticed at 480 µg/ml (Figure -1). Hence the study clearly indicates that the extract of A. latifolium Lam. is comparably much lower toxic than the Podophyllotoxin. Hence the whole plant ethanolic extract of A. latifolium Lam. is low toxic and it can be used as a drug in future.

**Table – 1 Showing mortality percentage of whole plant ethanolic extract of Adiantum latifolium. over Brine Shrimp**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Conc. of control and extract (µg/ml)</th>
<th>No. of brine shrimp per test sample</th>
<th>Average number of survivors</th>
<th>Average number of deaths</th>
<th>Percentage mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Podophyllotoxin (P.C) 30</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>DMSO (N.C)</td>
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<td>0</td>
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<tr>
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<td>600</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>60</td>
</tr>
</tbody>
</table>

PC- Positive Control NC- Negative Control

**Fig - 1: Cytotoxic activity of frond ethanolic extract of Adiantum latifolium Lam. over Brine Shrimp**

Brine shrimp lethality bioassay (BST) is the rapid, inexpensive, and simple bioassay for testing plant extracts bioactivity which in most cases correlates with cytotoxic and antitumor properties (Krishnarajuet al., 2005). Bioactive compounds are almost always toxic in high doses. In the present
study, the whole plant ethanolic extracts of A. latifolium Lam. showed a marked activity in terms of brine shrimp lethal effect. The lethality of whole plant ethanolic extracts of A. latifolium Lam. was found to be most effective which might be due to the toxic compounds present in the crude extracts. George et al., (2016), reported that the brine shrimp lethality assay on Acrostichumheterophyllum also revealed the nontoxic nature of the extracts up to concentrations of 1000 µg/ml. Our results also indicate the suitability of A. latifoliumLam. as herbal drug resource based on the above reference.

4. CONCLUSION

The results indicate that higher doses of plant extract causes less lethality when compared to positive and negative control. This study reveals that, the experimental plant A. latifolium Lam. could be less lethal for human being and therefore the plant can be recommended as an isocratic medicinal form for pharmacological evaluations.

5. ACKNOWLEDGEMENT

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REFERENCES