

Evaluation of brine shrimp lethality against methanolic extract of a medicinal fern *Phymatosorous scolopendria* (Burm. F.) Pic. Serm

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Abstract

Phymatosorous scolopendria (Burm. F.) Pic. Serm. Is a medicinal plant belonging to the family polypodiaceae. The aim of this study is to evaluate Brine shrimp lethality against whole plant methanolic extract of *Phymatosorous scolopendria* (Burm. F.) Pic. Serm. To find out its toxicity. The brine shrimp lethality test is an excellent predictive tool for the toxic potential of plant extracts. The study confirms the moderate potent cytotoxic activities of *Phymatosorous scolopendria* whole plant methanolic extract with LD50 value of 434.27 µg/ml. and therefore demands the isolation of active principles and thorough preclinical and clinical studies.

Keywords: *Phymatosorous scolopendria*, methanolic extract, brine shrimp, lethality assay

Introduction

Brine shrimp lethality assay is commonly used to check the cytotoxic effect of bioactive chemicals. It is a preliminary toxicity screening of plant extracts which was first proposed by Michael [1]. Subsequently, it was further developed by others and has been successively employed as a bioassay guide for active cytotoxic and antitumor agents [2]. This is a rapid and comprehensive test for the bioactive compound of either natural or synthetic origin. It is also an inexpensive and simple test as no aseptic techniques are required. It easily utilizes a large number of organisms for statistical validation and requires no special equipment and relatively small amount of sample is necessary.

Pteridophytic plants retain great potential because of some fascinating medicinal properties and the study of cytotoxicity is very essential to know, whether the plant has the potentiality to react with normal Cells [3]. Few Pteridophytic plants have been worked on evaluating the toxicity of extracts, and it was assessed by the brine shrimp test. *Phymatosorous scolopendria* (Burm. F.) Pic. Serm. Belongs to the family Polypodiaceae which is commonly known as musk-fern or wartfern. Ethnobotanically the plant is used for asthma, cough, Stomach ache, swollen breasts, boils, insect repellent, anti-inflammatory and bronchodilator activity [4, 5]. The preliminary phytochemical experiments strongly reveals that the plant contains phenol, steroid and terpenoid. GCMS analysis of *Phymatosorous scolopendria* revealed the presence of Bio active compounds having Anticancer, Antioxidant, antibacterial, antiviral, candidicide, hypocholesterolemic, antiarthritic, hepatoprotective properties [6, 7]. Hence preliminary toxicity screening and evaluation of lethality of *Phymatosorous scolopendria* extract over Brine shrimp is carried out.

Materials and Methods

Collection of Plant material

The sporophytic plant of *Phymatosorous scolopendria* (Burm. f.) Pic. Serm. Was collected from Kanyakumari District (Tamil Nadu). The plant was 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, Holy Cross College, Trichy (Voucher No. 001). The collected whole plant material is washed, shade dried and then powdered with the help of blender. The powder was kept in air tight bottles. The plant powder was then stored in a clean sterile bottle at room temperature.

Preparation of Plant extract

The dried plant powder of *P. scolopendria* (Burm. f.) Pic. Serm. Was extracted with methanol (Soaked with 24hrs). The methanolic whole plant extract of *P. scolopendria* (Burm. f.) Pic. Serm. Was subjected to chromatographic techniques, to elute pure compound. The eluted crude extract obtained from experimental plant extract of *P. scolopendria* (Burm. f.) Pic. Serm. Was used for further analysis towards Brine shrimp lethality assay.

Brine Shrimp Lethality Bioassay

This bioassay provides a front line screen that can be backed up by more specific and expensive bioassays, once the active compound has been isolated [8]. This *in vivo* lethality test has been successfully used as a preliminary study of cytotoxic and antitumor agents and it is a simple, cost effective method which requires small amount of test material [9].

The brine shrimp lethality bioassay was carried out in methanolic extracts of *P. scolopendria* (Burm. f.) Pic. Serm. In *Artemia nauplii* a test organism to monitor the cytotoxicity of a compounds present in the plan and to

estimate the LC50 values. *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. Aquarium air pump was fixed to the tank for uniform aeration. 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 50, 100, 200, 400 and 600 µg/ml whole plant methanolic extract of *P. scolopendria* (Burm. f.) Pic. Ser. Which were prepared by serial dilution using methanol as solvent. Each concentration was tested in triplicate, giving a total of 12 small petridish. The final volume of the solution in each petridish were made up to 5 ml with sea water immediately after adding shrimp larvae. As a negative control, 5 ml of methanol alone was used.

Determination of Lethality

In each petridish, consisting of exposing groups of 10 *Artemia nauplii* 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 (\pm)

standard deviation. LD50 values of the two plant extracts were calculated from the linear regression line in Excel, plotted with percentage of nauplii against different tested concentrations. Higher LD50 values indicate the lesser toxicity of the plant material. Podophyllotoxin (30 µg/ml) was used as a positive control in the bioassay.

Results and Discussion

Cytotoxic activity of whole plant methanolic extract *P. scolopendria* (Burm. f.) Pic. Ser. Over 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. Brine shrimp cytotoxic assay of whole plant methanolic extract of *P. scolopendria* (Burm. f.) Pic. Ser. Was performed to evaluate its cytotoxic activity. In the assay, the degree of mortality of hatched nauplii were directly, related to the concentration of the extract. The *P. scolopendria* (Burm. f.) Pic. Ser. Whole plant extract showed low toxicity when compared to the positive control Podophyllotoxin. The toxic activity of *P. scolopendria* (Burm. f.) Pic. Ser. Whole plant extract against brine shrimp larvae was tabulated (Table-1). In 30 µg/ml concentration, the lethality noticed was 50 % under control. The lethality rate of larva was found to be dose dependent. The whole plant methanolic extract of *P. scolopendria* (Burm. f.) Pic. Ser. Showed 0, 10, 20, 30, 40, 70 percentage of mortality at 50, 100, 200, 400 and 600 µg/ml concentration respectively. The LD50 value of *P. scolopendria* (Burm. f.) Pic. Ser. Extract was noticed at 434.27 µg/ml (Figure -1). So it clearly shows that the *P. scolopendria* (Burm. f.) Pic. Ser. Extract is comparably much lower in toxicity than the Podophyllotoxin. So the extract of *P. scolopendria* (Burm. f.) Pic. Ser. Can be used as a medicine.

Table 1: Showing mortality percentage of whole plant methanolic extract of *P. scolopendria* (Burm. f.) Pic. Ser. Over Brine Shrimp

S.No	Conc. of Control and extract (µg/ml)	No. of Brine shrimps per test sample	Average number of survivors	Average number of deaths	Percentage of mortality
1.	Podophyllotoxin (P.C) 30	10	5	5	50
2.	Methanol (N.C)	10	10	0	0
3.	50	10	10	0	0
4.	100	10	8	2	20
5.	200	10	7	3	30
6.	400	10	6	4	40
7.	600	10	3	7	70

P.C – Positive Control; N.C – Negative Control

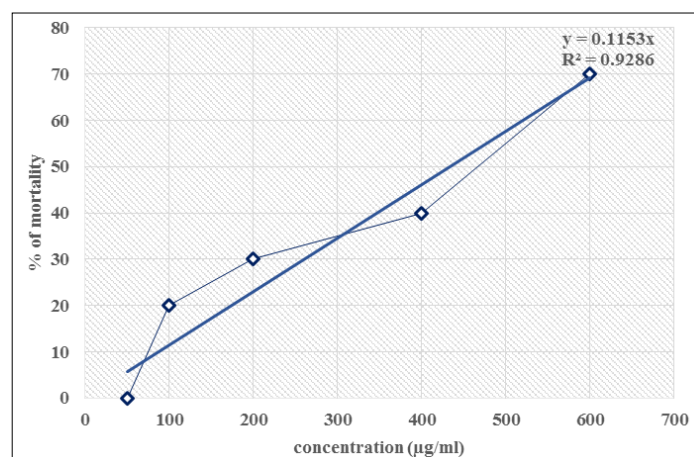


Fig 1: Cytotoxic activity of whole plant methanolic extract of *P. scolopendria* (Burm. f.) Pic. Ser. Over Brine Shrimp

The assessment of the toxicity of plant extracts is essential for safe treatment. It consent the identification of the principal toxicity of the plant and the effects of acute overdose. Bioactive compounds are almost always toxic in high doses. Thus determination of *in vivo* lethality in a simple zoological organism like brine shrimp, is essential and it can be used as a convenient monitor for screening and fractionation in the discovery of and monitoring bioactive natural Compounds ^[10]. The brine shrimp lethality test is considered to be very useful in determining various biological activities such as cytotoxic, phototoxic, pesticidal, trypanocidal, enzyme inhibition and ion regulation Activities ^[11]. In the present study, the whole plant methanolic extracts of *P. scolopendria* (Burm. f.) Pic. Serm. Showed a marked activity in terms of brine shrimp lethal effect. The lethality of whole plant methanolic extracts of *P. scolopendria* (Burm. f.) Pic. Serm. Was found to be most effective which might be due to the toxic compounds present in the crude extracts.

Works on whole plant ethanolic extract of *Angiopteris evecta* (G.Forst.) Hoffm. *Pyrrosia lanceolata* (L.) Farwell. And *Adiantum latifolium* Lam. was found to be more effective against brine shrimp with LD50 value of 357.14 µg/ml ^[12], 464.95 µg/ml ^[12] and 480 µg/ml ^[13] respectively. Similar observation was found in our studies that, the whole plant methanolic extract of experimental plant *P. scolopendria* (Burm. f.) Pic. Serm. With LD50 value of 434.27 µg/ml. Lethality frequency of brine shrimp which increased proportionally as the concentration of the extract increases. Brine shrimp lethality assay on *Cyathea* species and *Acrostichum heterophyllum* also revealed the nontoxic nature of the extracts upto concentrations of 1000 µg/ml. The results indicate the suitability of *A. heterophyllum* as herbal drug resource ^[14, 15]. The results of our experimental plant *P. scolopendria* (Burm. f.) Pic. Serm. Also coincide with the above result and found non-toxic with concentration below 1000 µg/ml and hence it is can be used as herbal drug.

Conclusion

The results indicate that higher doses of plant extract causes less lethality when compared to positive and negative control. The study reveals that, the experimental plant *P. scolopendria* (Burm. f.) Pic. Serm. Could be less lethal for human being and therefore the plant can be recommended as an isocratic medicinal form for pharmacological evaluations. Based on this toxicological study it can be recommended for anti-cancer study.

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