

Cytotoxic activity of *Parmelia perlata* extracts against *Artemia salina*

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ABSTRACT

The brine shrimp lethality bioassay represents a rapid, inexpensive, and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and antitumor properties. The assay is considered to be a very useful tool for preliminary assessment of toxicity. In this study, the lichen *Parmelia perlata* was extracted with solvents of varying polarity such as hexane, ethyl acetate, acetone, and methanol and tested for hatch inhibition of cysts of *Artemia salina* and cytotoxic activities against *A. salina* naupilii. The highest cytotoxic potential among all the plant extracts tested was explored from the hexane extract of *P. perlata* which showed 100% brine shrimp mortality at 100 ppm. The least activity among all the plant extracts was found in the methanol extract of *P. perlata*.

KEY WORDS: *Parmelia perlata*, crude extract, *Artemia salina*, cytotoxic activities

INTRODUCTION

Natural therapies, such as the use of plant-derived products in cancer treatment, may reduce adverse side effects. Currently, a few plant products are being used to treat cancer. However, a myriad of many plant products exists that have shown very promising anti-cancer properties *in vitro* but have yet to be evaluated in humans (Desai *et al.*, 2008).

The plants have been used for medicinal purposes throughout human history, and the first pharmaceuticals were derived from medicinal plants (McRae *et al.*, 2007). Globally, there is a positive trend in favor of traditional medicine and ethnopharmacology. The brine shrimp lethality bioassay represents a rapid, inexpensive, and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and antitumor properties. The assay is considered to be a very useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, toxicity of plant extracts, heavy metals, and cytotoxicity testing of dental materials (Sharma *et al.*, 2013).

Various active compounds derived from medicinal plants have been assessed for their efficacy and tolerability

in the treatment of breast cancer. Some of these plant species including *Taxus baccata* (paclitaxel, docetaxel), *Podophyllum peltatum* (etoposide), *Camptotheca acuminata* (camptothecin), and *Vinca rosea* (vinblastine, vinorelbine) have well recognized antitumor activity in breast cancer and have been evaluated in clinical trials. The plants are good and cheap sources for the prevention and treatment of oxidative stress and cancer (Richard *et al.*, 2015). The treatment of cancer by use of natural products and traditional medicine by applying the concepts of Ayurveda is attaining a great significance scope of cancer research. Medicinal plants contain good immunomodulatory and antioxidant properties which lead them to be an anticancer drug (Priya *et al.*, 2015).

The lichen *Parmelia perlata* is used as one of the important spices in India. It is liked for its flavor. The *P. perlata* is an anti-emetic substance, i.e. used to stop vomiting (Rao *et al.*, 2011) and also used in traditional medicine for rapid wound healing (Vidyalakshmi and Kruthika, 2012). In this study, the lichen *P. perlata* was extracted with solvents of varying polarity such as hexane, ethyl acetate, acetone, and methanol and tested for hatch inhibition of cysts of *Artemia salina* and cytotoxic activities against *A. salina* naupilii.

MATERIALS AND METHODS

Preparation of Extracts

The lichen *P. perlata* (Huds.) Ach. was purchased commercially and then ground by using an electric blender. The powdered material was extracted by soaking 25 g powder at room temperature in Erlenmeyer flasks containing 250 ml of solvents such as hexane, ethyl acetate, acetone, and methanol for 72 h. The extraction process was carried out in triplicates. After 72 h, the extract was filtered through Whatman No. 1 filter paper and the solvent was evaporated under vacuum in a rotary evaporator and the dried extracts were stored at 4°C until further assay.

Brine Shrimp Lethality Bioassay

Brine shrimp lethality bioassay is considered a useful tool for preliminary assessment of toxicity. The method is attractive, because it is very simple, inexpensive and sensitive (Krishnaraj *et al.*, 2005). 10 nauplii were drawn through a glass capillary and placed in test tubes containing 10 ml of artificial seawater solution and 0.5 ml of the diluted plant extract (5.0, 10, 25, 50, 100 ppm) was added to it and maintained at room temperature for 24 h under constant aeration and light source. The test was also carried out on control (artificial sea water). The *Artemia* mortality in both treated and control was recorded after 24 h and the percentage of mortality calculated.

Mortality at treatment

$$\% \text{ Mortality} = \frac{100 - \text{mortality at control}}{100 - \text{mortality at control}} \times 100$$

Statistical Analysis

The lethal concentrations, LC₅₀ and LC₉₀ at which concentrations (ppm) 50% and 90% larvae showed mortality, 95% confidence limit of upper and lower confidence levels were calculated by Probit analysis (SPSS, version 11.5).

RESULTS AND DISCUSSION

The brine shrimp mortality of *P. perlata* is given in Tables 1-4. The highest brine shrimp mortality of 100% was recorded with hexane and methanol extracts at 100 and 200 ppm, respectively. The ethyl acetate extract at 200 ppm showed 92.6% brine shrimp mortality, whereas acetone showed 82.0% mortality and 213.29 ppm.

In our study, a dose-dependent result was recorded. The hatch inhibition of *A. salina* cysts was increased with increasing concentration of plant extracts. A similar observation was made by Sabai *et al.* (2001) and Otang *et al.* (2013).

The highest cytotoxic potential among all the plant extracts tested was explored from the hexane extract of *P. perlata* which showed 100% brine shrimp mortality at 100 ppm.

Table 1: LC of hexane extract of *P. perlata* against brine shrimp (*A. salina*)

Concentration (ppm)	Brine shrimp mortality (%)	LC ₅₀ (ppm)	LC ₉₀ (ppm)	95% fiducial limit (ppm)		Chi-square
				LCL	UCL	
12.5	22.40	26.28	66.63	16.53-38.62	43.94-191.37	12.379*
25	36.80					
50	78.60					
100	100.00					
200	100.00					
Control	1.0					

*Significant at $P < 0.05$. *P. perlata*: *Parmelia perlata*, *A. salina*: *Artemia salina*, LCL: Lower confidence limit, UCL: Upper confidence limit, LC: Lethal concentration

Table 2: LC of ethyl acetate extract of *P. perlata* against brine shrimp (*A. salina*)

Concentration (ppm)	Brine shrimp mortality (%)	LC ₅₀ (ppm)	LC ₉₀ (ppm)	95% fiducial limit (ppm)		Chi-square
				LCL	UCL	
12.5	16.5	45.02	211.10	38.67-52.27	163.60-296.76	3.056
25	32.4					
50	48.0					
100	72.5					
200	92.6					
Control	0.0					

P. perlata: *Parmelia perlata*, *A. salina*: *Artemia salina*, LCL: Lower confidence limit, UCL: Upper confidence limit, LC: Lethal concentration

Table 3: LC of acetone extract of *P. perlata* against brine shrimp (*A. salina*)

Concentration (ppm)	Brine shrimp mortality (%)	LC ₅₀ (ppm)	LC ₉₀ (ppm)	95% fiducial limit (ppm)		Chi-square
				LCL	UCL	
12.5	13.6	62.80	384.19	53.01-75.25	271.97-624.14	0.992
25.0	25.9					
50.0	42.8					
100.0	59.6					
200.0	82.0					
Control	0.0					

P. perlata: *Parmelia perlata*, *A. salina*: *Artemia salina*, LCL: Lower confidence limit, UCL: Upper confidence limit, LC: Lethal concentration

Table 4: LC of methanol extract of *Parmelia perlata* against brine shrimp (*A. salina*)

Concentration (ppm)	Brine shrimp mortality (%)	LC ₅₀ (ppm)	LC ₉₀ (ppm)	95% fiducial limit (ppm)		Chi-square
				LCL	UCL	
12.5	8.00	97.30	493.50	60.89-217.06	219.20-5370.65	9.775*
25.0	16.60					
50.0	21.80					
100.0	44.60					
200.0	79.40					
Control	0.0					

*Significant at $P < 0.05$. *P. perlata*: *Parmelia perlata*, *A. salina*: *Artemia salina*, LCL: Lower confidence limit, UCL: Upper confidence limit, LC: Lethal concentration

Krishnaraj *et al.* (2005) found that the hydroalcoholic extract of the lichen showed an LC₅₀ 730 ppm which is comparatively very higher than the results obtained with all the extracts of *P. perlata* in this study.

The methanolic and ethanolic extract of *P. perlata* showed its effectiveness in inhibiting drug resistant *Helicobacter pylori* isolates (Gehlot *et al.*, 2016). The results of methanol extract of *Piper betle* showed 100% mortality of cysts only at 500 ppm with an LC₅₀ value of 85.50 ppm (Mae *et al.*, 2014). These concentrations are much higher than our results. Sharma *et al.* (2014) identified dibenzofuran, 2-acetyl-9b-carbmethoxy-7,9-dihydroxy-8-methyl-1,3(2H,9bH)-dibenzofurandione, and 2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bH)-dibenzofurandione also known as (+) - usnic acid, as heterocyclic natural compounds from lichen *P. perlata*. The ethyl acetate, acetone, and methanol extract of *P. perlata* were found to possess inhibitory effect against *Staphylococcus aureus* (Vidyalakshmi and Kruthika, 2012).

Further investigations on *P. perlata* to find out the cytotoxic potential compound through a bioassay guided separation and also to find out the mechanism of action is suggested by this study.

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