

## Evaluation of Brine Shrimp Lethality Bioassay (BSLA) of extract and fractions of *Hertia intermedia*

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### Abstract:

*In this research study, the extract and fractions of Hertia intermedia were evaluated for toxicity. Brine shrimp lethality assay was the technique used for the determination of mortality of Artemia salina which is an important experimental organism for the evaluation of toxicity. Methanol extract, n-hexane fraction and butanol fraction of*

*Hertia intermedia* showed no cytotoxicity while aqueous fraction showed cytotoxicity at higher concentration. The % mortality at 1000 ug/ml showed by aqueous fraction was 53.3%. The standard drug Etoposide showed 46.6% mortality.

**Keywords:** Brine Shrimp Lethality Bioassay (BSLA), extract, fractions, *Hertia intermedia*

## INTRODUCTION

Medicinal plants are the best resources for remedies provided by the nature. The constituents present in these traditional plants are used as herbal drugs with least toxicity and side effects [1]. Ethnomedicinal plants are used for the treatment of different diseases in developing countries [2]. Tribe Senecioneae, family Compositae consists of important genus *Hertia* which comprises of 12 species, present in south west Asia and north and south of Africa [3]. *Othonnopsis intermedia* is the alternative name of *Hertia intermedia* which is found in Balochistan for instance in Chaman, Quetta, Khanozai. *Hertia intermedia* are tiny shrubs with flowers of yellow colour [4]. The medicinal plant, *Hertia intermedia* is traditionally used as a painkiller in Pakistan. The phytochemicals are known as hertidins [5]. The phytochemical which are investigated are terpenoids, tannins, flavonoids, coumarins, and steroids [6]. The native herbalists and experienced elders of the society are the traditional doctors from whom people seek treatment and advices [7].

## MATERIALS AND METHODS

### Collection and Identification of plant

The medicinal plant, *Hertia intermedia* was collected from Yaro, Pishin and identified by a taxonomist Prof. Dr. Rasool Bakhsh Tareen, Department of Botany, University of Balochistan, Quetta, Pakistan. Under the co-supervision of Prof. Dr. Muhammad Anwar Panezai, the extraction and fractionation of *Hertia intermedia* was

carried in the Institute of Biochemistry, University of Balochistan, Quetta.

### **Extraction and Fractionation**

The plant, *Hertia intermedia* was shade dried for the period of month, grinded into powdered and then 3kg was soaked in 10 litres of methanol for 7 days. Filtered and then methanol was vaporized with the help of rotary evaporator. The crude extract was 104gm which was further fractionated with n-hexane and aqueous to obtain n-hexane fraction 6.5 gm and aqueous fraction 35 gm. Aqueous fraction was further fractionated with butanol to produce butanol fraction 3 gm [8, 9].

### **Brine Shrimp Lethality Assay**

For the evaluation of toxicity, brine shrimp lethality assay was used. In a hatching tray which was shallow rectangular dish and filled with artificial seawater, brine shrimp eggs were added to hatch brine shrimp (*Artemia salina*). 38 gm per litre sea salt was used to prepare artificial seawater. After 48 hours, with the help of Pasteur pipette, active neuplii were collected. In this research study, 20mg extract and fractions were dissolved in 5ml of DMSO and from this 10ug, 100ug and 1000ug were transferred to sample vials. In each vials, 30 neuplii were added with the help of Pasteur pipette and them 5ml artificial seawater was added to each vial to adjust the final volume. With the help of magnifying glass, the number of survivor neuplii were counted [10].

## **RESULTS AND DISCUSSION**

For the testing of bioactivity of extracts and fractions of medicinal plants, brine shrimp lethality is used which is simple bioassay, inexpensive and rapid assay. In this assay, four extract and fractions of *Hertia intermedia* are used such as methanol extract, n-hexane fraction, aqueous fraction and butanol fraction. Methanol extract, n-hexane fraction and butanol fraction showed no cytotoxicity while aqueous fraction showed cytotoxicity at higher concentration. At 1000

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ug/ml, maximum mortality was shown. The toxicity of extract and fractions of *Hertia intermedia* represented in table 1, 2, 3 and 4.

**Table 1. Brine Shrimp Lethality Bioassay of Whole plant Methanol Extract of *Hertia intermedia***

| Dose (ug/ml) | No. of Shrimps | No. of Survivors | % Mortality | % Viability | STD. Drug | % Mortality |
|--------------|----------------|------------------|-------------|-------------|-----------|-------------|
| 10           | 30             | 30               | 0%          | 100%        | Etoposide | 46.6%       |
| 100          | 30             | 30               | 0%          | 100%        |           |             |
| 1000         | 30             | 24               | 20%         | 80%         |           |             |

**Table 2. Brine Shrimp Lethality Bioassay of Whole plant n-hexane fraction of *Hertia intermedia***

| Dose (ug/ml) | No. of Shrimps | No. of Survivors | % Mortality | % Viability | STD. Drug | % Mortality |
|--------------|----------------|------------------|-------------|-------------|-----------|-------------|
| 10           | 30             | 30               | 0%          | 100%        | Etoposide | 46.6%       |
| 100          | 30             | 29               | 3%          | 97%         |           |             |
| 1000         | 30             | 17               | 43.3%       | 56.7%       |           |             |

**Table 3. Brine Shrimp Lethality Bioassay of Whole plant aqueous fraction of *Hertia intermedia***

| Dose (ug/ml) | No. of Shrimps | No. of Survivors | % Mortality | % Viability | STD. Drug | % Mortality |
|--------------|----------------|------------------|-------------|-------------|-----------|-------------|
| 10           | 30             | 27               | 10%         | 90%         | Etoposide | 46.6%       |
| 100          | 30             | 26               | 13.3%       | 86.7%       |           |             |
| 1000         | 30             | 14               | 53.3%       | 46.7%       |           |             |

**Table 4. Brine Shrimp Lethality Bioassay of Whole plant butanol fraction of *Hertia intermedia***

| Dose (ug/ml) | No. of Shrimps | No. of Survivors | % Mortality | % Viability | STD. Drug | % Mortality |
|--------------|----------------|------------------|-------------|-------------|-----------|-------------|
| 10           | 30             | 30               | 0%          | 100%        | Etoposide | 46.6%       |
| 100          | 30             | 27               | 10%         | 90%         |           |             |
| 1000         | 30             | 24               | 20%         | 80%         |           |             |

## CONCLUSION

Brine shrimp lethality assay is a useful technique for evaluation of bioactivity of extract and fractions of medicinal plants. In this research study, we conclude that methanol extract, n-hexane fraction and butanol fraction of *Hertia intermedia* showed no cytotoxicity while aqueous fraction showed cytotoxicity at higher concentration.

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