

**Brine Shrimp Lethality Activity of land and fresh water Medicinal Plants****<sup>1</sup>Mehdi Yousefia, <sup>1</sup>Ensie Olyaei Juybari, <sup>1</sup>Mohamad Kazem Yahyapor, <sup>2</sup>Abbas Ali Dehpour, <sup>1</sup>Mahmood Hoseinzade**<sup>1</sup>Department of Fishery, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran<sup>2</sup>Department of biology, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran

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**ABSTRACT**

Brine shrimp larvae have been used as a bioassay for a variety of toxic substances. The method has also been applied to plant extracts in order to facilitate the isolation of biologically active compounds. In this study several species of medicinal plants and aquatic plant were collected and aqueous extracts of these plants were screened for their cytotoxicity. Out of the 6 plants tested, *Nymphaea alba* exhibited potent brine shrimp lethality with LC<sub>50</sub> 180 mg/ml. *Rumex acetosa*, *Allium rotundom*, *Sedum rubotictum* have also showed significant cytotoxicity with LC<sub>50</sub> of 600, 680, 570 mg/ml respectively. The present study supports that brine shrimp bioassay is reliable method for assessment of bioactivity of freshwater plants for their use in medicine.

**Key words:** *Nymphaea alba*, cytotoxicity, BSLT, LC<sub>50</sub>**Introduction**

Brine shrimp lethality assay (BSLT) is an important tool for preliminary assessment of cytotoxicity of herbal drugs. This method is used for the determination of LC<sub>50</sub> values of herbal extracts. It appears that BSLT is predictive of cytotoxicity and pesticidal activity<sup>1</sup>, since its introduction [1].

The research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and also to find different source of natural drug. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Nearly all medicinal preparations were derived from land plants, almost in the simple form of raw plant materials (dried under sunshine) or in the refined form of crude extracts. Several hundreds of plants have been known with medicinal applications in Iran, among them some have been subjected to the isolation of the active ingredients, however still several thousands have not been discovered for their medicinal compounds. Iran is one of the richest countries in the world as regards genetic resources of medicinal plants that some of them are export (such as Hana, Zaferan, Golab), to the world. In continuation of our efforts to verify the efficacy of traditional medicine, we have collected several medicinal plants from wetland locations in north of the country and three most abundant aquatic water to increase our information in this respect. We used the brine shrimp assay to determine the toxicity level of

this plant. The assay is considered a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, pesticides and cytotoxicity testing of dental materials [2-6].

*Allium rotundom*, *Rumex acetosa* and *Sedum rubotictum* are all herb distributed in North of Iran. The aerial and onion parts of plant are well investigated for chemical information. These plants was reported in various pharmacological activities like Antiinflammatory activity, Antibacterial activity, Anticancer activity, Diuretic activity etc [7-9]. In spite of frequent study of land herbal plant very few investigation are reported about the water plant. Therefore at present study we had an analysis 3 fresh water plant that is the most abundant in fresh water fish pond and water reservoir to understand more information about the habitat of the fish and their situation in the water. Every year million of fish fingerling are produce either for restocking or introduce for aquaculture [10]. The interaction between environment and fish culture are also very important [11] and very few study are reported in this respect. This data is necessary to understand the biology and ecology of fish and in other hand to compare the medicine properties of land plant and water plant as well.

In order to study the toxicity of these medicinal plants we performed brine shrimp lethality bioassay which based on the ability to kill *Artemia nupli* in laboratory condition.

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## Material and Methods

### Plant materials Extraction:

For the present study the plant materials, stem bark and leaf, were collected from the reliable source of Zagamarz region for medical plant of *Rumex acetosa*, *Allium rotundom*, *Sedum rubotictum* and irrigation reservoirs for aquatic plant of *Nymphaea alba*, *Chara algae*, *Sparganium crectum*, in North of Iran, and the identity of the drug was established by morphological study at the Department of Botany, Islamic Azad University of Qaemshahr, Iran (IAUQI). The whole six plants were shade dried at 37°C to 40°C and coarsely powdered through mesh 20.

All of the samples were collected in June 2011. The plant materials were dried under shade and grinded to a coarse powder. Powdered plant materials (each 25 g) were individually extracted with water / hydroalcohol / alcohol (200 ml) and then filtered. Filtrates were concentrated dried under vacuum and subjected for activity studies. The extract was used in the concentration of 20, 40, 80, 160  $\mu$

### Hatching the brine shrimp:

Brine shrimp eggs were hatched in artificial sea water prepared from commercial sea salt 40 g/l. The compartments plastic chamber are used, the eggs were sprinkled into the compartment which was darkening. After 48 hours incubation at room temperature (25-29°C), nauplii were collected by pipette from the lighted side of the chamber.

### Bioassay:

The bioactivity of the extracts were monitored by the method previously described by Meyer *et al.* [1]. The sample were dissolved in DMSO (up to 2% of final dosage) and diluted with sea water. Serial dilutions were made in the wells of 96-well microplates in triplicate in 500  $\mu$ l sea water. Control wells with DMSO were included in each experiment. A suspension of nauplii containing 10-15 organisms (100  $\mu$ l) was added to each well. The plates were covered and incubated at room temperature (25-29°C) for 5 and 24 hours. Plates were then examined under the binocular stereomicroscope and the numbers of dead (non-motile) nauplii in each well were counted. One hundred microliters of methanol were then added to each well to immobilize the nauplii and after 15 minutes the total numbers of brine shrimp in each well were counted. Analysis of the data was performed by probit analysis on a Finney computer program to determine the lethal concentration to half of the test organisms (LC<sub>50</sub>).

### Statistical analysis:

The percentage lethality was calculated from the mean survival larvae of extracts treated tubes and control. LC<sub>50</sub> values were obtained by best-fit line method. The LC<sub>50</sub> values of the plant extracts were obtained by a plot of percentage of the shrimp nauplii killed against the concentrations of the extracts and the best-fit line was obtained from the data by means of regression analysis.

### Results:

The lethality activity and LC<sub>50</sub> values of the brine shrimp obtained for extracts of fresh water and terrestrial plants are given in Table 1 and 2.

**Table 1:** The lethality of extracted aquatic plant at a dosage of 10, 100, 200, 500 and 1000 mg/l at 5h and 24 h test with artemia nupli.

Dosage of extraction		10mg/ml		100mg/ml		200mg/ml		500mg/ml		1000mg/ml		LC <sub>50</sub>
Duration		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
<i>Chara algae</i>	5 h	0.0	0.0	2.5	2.4	3.3	2.4	6.7	2.4	13.3	2.4	3750
<i>Nymphaea alba</i>	5 h	1.7	2.9	5	0	8.3	2.9	10	0	20	5	2500
<i>Sparganium crectum</i>	5 h	0.0	0.0	5	4	6.7	2.4	8.4	2.4	13.4	2.4	4670
<i>Chara algae</i>	24h	3.3	2.9	6.7	2.9	8.3	2.9	18.3	5.8	26.7	5.8	2400
<i>Nymphaea alba</i>	24h	28.3	7.6	35	13.2	45	5	75	8.7	100	0.0	180
<i>Sparganium crectum</i>	24h	6.7	2.9	8.3	5.8	8.3	2.9	18.3	7.6	23.3	5.7	3670

The Lethality plant extracts of *Sparganium crectum*, *Rumex acetosa* and *Chara algae* at 5 hours treatment showed the minimum effect on artemia nupli and was significantly different with those of the others (P<0.05). Alcoholic extract of *Nymphaea alba* showed most prominent activity with LC<sub>50</sub> of 180 mg/ml among fresh water weeds and terrestrial plant (Table 1 & 2). *Chara algae* and *Sparganium crectum* at 24h treatment exhibited minimum brine shrimp lethality with LC<sub>50</sub> values higher than 2400

and 3000 mg/ml respectively.

*Rumex acetosa*, *Allium rotundom* and *Sedum rubotictum* as terrestrial plant have also shown moderate brine shrimp lethality and the LC<sub>50</sub> values were found to be lower than 680 and noted in Table 1 and 2. The degree of lethality was found to be directly proportional to the concentration of the extract. Maximum mortalities took place at a concentration of 1000  $\mu$ g/ml whereas least mortalities were at 10  $\mu$ g/ml concentration.

**Table 2:** The lethality activity of extracted terrestrial plant at a dosage of 10, 100, 200, 500 and 1000 mg/ml at 5h and 24 h test with *artemia nupli*

Dosage of extraction		10mg/ml		100mg/ml		200mg/ml		500mg/ml		1000mg/ml		LC <sub>50</sub>
Duration		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
<i>Allium rotundum</i>	5 h	.0	0.0	2	2.9	5	0.0	12	2.9	38	5.7	1240
<i>Sedum rubotictum</i>	5 h	1.7	2.4	5	0.0	5	0.0	8.3	2.4	16.7	6.2	3000
<i>Rumex acetosa</i>	5 h	0.0	0.0	3.3	2.9	3.3	2.9	6.7	2.9	13.3	2.9	3750
<i>Allium rotundum</i>	24h	2	2.9	7	2.9	15	5	33	5.7	83	10.4	680
<i>Sedum rubotictum</i>	24h	6.7	2.9	13.3	5.8	26.7	7.6	33.3	5.8	53.3	2.9	570
<i>Rumex acetosa</i>	24h	21.7	7.6	41.7	2.9	43.3	2.9	48.3	10.4	56.7	5.8	600

### Discussion:

The brine shrimp lethality assay is reliable and simple bioassay discovery for testing plant extracts bioactivity use as indicator for cytotoxic and anti-tumor activity of plant [12]. In the present study the brine shrimp lethality of extracts of three terrestrial and three aquatic plants was determined using the procedure of Meyer *et al.* [1].

Among six terrestrial and aquatic plants, the most active extract was *Nymphaea alba* (Nymphaeaceae) methanol fraction of leaves. This fraction has a potential to be a candidate for more investigation of cytotoxic compounds.

*Nymphaea alba* belongs to Nymphaeaceae family, is a herbaceous aquatic plant, grows up to 50 cm in height are mainly in rice field reservoir, whose leaves float or submerge in water. Many bioactive and pharmacologically important compounds have been obtained from *Nymphaea* spp and used in medicine and pharmacy [13].

Economically the use of Nuphar is in medicine [14]. Contemporary analyses have illustrated potential pharmacological value in Nuphar [15]. Alkaloids of several species display atropine-like and papaverinelike activity [16], insecticidal effects [17], or anti-tumor activity in some animal cancers [15]. With the above in mind, the leaves of *Nymphaea alba* were tested for antibacterial activity against some bacteria isolated from wounds, since it is being used in traditional medicine. However, neither the cytotoxic activity of Nymphaeaceae growing spontaneously in rice field reservoir in Iran has been published to the best of our knowledge. Crude extracts *Nymphaea alba* resulting in LC<sub>50</sub> values of less than 180 µg/ml for 24 hours were considered significantly active and had the potential for further investigation. The result of the phytochemical analysis of the Ethanolic extracts of *Nymphaea* lotus leaves showed the presence of bioactive compounds such as tannins, flavonoids, alkaloids, anthraquinones, saponins, cardiac glycosides and phenolics. The results also showed that *S. aureus*, *S. pyogenes* and *E. coli* isolated were highly susceptible to *Nymphaea lotus* [18] of Nymphaeaceae.

Although several potent antibacterial compounds have been isolated from the leaves of Nymphaeaceae, its leaf have been rarely tested for cytotoxic investigation. This study found that Nymphaeaceae exhibited higher cytotoxic potency

than the other plants we assumed as medicine plants. Therefore, further isolation of the highly active fractions of the leaf, rizome, flowers and the seed of *Nymphaea alba* may lead to the discovery of new cytotoxic compounds. Besides cytotoxic activity, these fractions should also be evaluated for the pesticide activity.

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