

# Brine shrimp lethality assay of *Sesbania grandiflora* ethanolic extract – *in vitro* study

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## ABSTRACT

To evaluate the lethality assay of *Sesbania grandiflora* using *in vitro* parameter. Brine shrimp lethality bioassay is a standard method to investigate the cytotoxicity of herbal extract. *S. grandiflora* commonly known as Agathi in Tamil that appears to have historical usage as an antibacterial and anti-inflammatory agent. A total of 20 nauplii were added into three replicates of each concentration of the plant extract. After 24 h, the surviving brine shrimps were counted, and percentage of mortality was assessed. This study reveals that *S. grandiflora* leaf possesses significant cytotoxic activity when tested *in vitro*. From this study, it can be concluded that ethanolic extract of *S. grandiflora* possessed marked cytotoxic effect.

**Keywords:** Antibacterial, Brine shrimp, cytotoxicity, *Sesbania grandiflora*

## Introduction

*Sesbania grandiflora* is commonly known as Agathi keerai in Tamil. It is a popularly used herbal extract. Various literatures prove that there are about 60 global species belonging to genus *Sesbania* which are commonly found to be grown in Africa, Australia, and Asia. *S. grandiflora* is commonly found in tropical regions. The vernacular name of *S. grandiflora* is also known as agusta, bagphal, and agate (Bengali).<sup>[1,2]</sup> *S. grandiflora* is a small, erect, fast growing perennial (4-5 m in just 6 months), and sparsely branched tree that reaches 10-15 m in height and diameter up to 12 cm.<sup>[3]</sup> It is traditionally used for anti-inflammation,<sup>[4]</sup> antimicrobial activities,<sup>[5,6]</sup> anticancer,<sup>[7]</sup> antioxidative activities,<sup>[8,9]</sup> anti-ulcer activity,<sup>[10,11]</sup> and immunomodulatory activity,<sup>[12]</sup> and associated diseases such as hepatic diseases,<sup>[13,14]</sup> respiratory diseases,<sup>[15]</sup> and renal diseases.<sup>[16]</sup> The juice of the bark or root is traditionally used for the treatment of cancer in India.<sup>[17]</sup> Brine shrimp lethality bioassay is a simple, high throughput cytotoxicity test of bioactive chemicals. It is based on killing the ability of test compounds on a simple zoological organism-brine shrimp (*Artemia salina*).<sup>[18]</sup> Brine shrimp lethality bioassay is widely used in the evaluation of toxicity of heavy metals, pesticides, medicines especially natural plant extracts, etc.<sup>[19,20]</sup> Hence with this

regard, we have chosen *S. grandiflora* leaf against the brine shrimp to evaluate the lethality assay.

## Materials and Methods

### Plant material

*S. grandiflora* leaf was obtained as a gift sample from Green Chem Lab, Bengaluru, India.

### Brine shrimp lethality assay

The eggs of brine shrimp were procured from Philadelphia, USA. In a small water tank containing seawater, the eggs were incubated for 48 h for hatching. Required light was provided with Philips 40Watts lamp for 12 h cycle. After 24 h, the larvae were used for the experiments. The nauplii of brine shrimp were challenged in different test tubes containing 10 mL of sea water and 20 larvae. To this, extracts of leaves at different concentrations (10-50 mg/mL) were added. After 24 h, the viability of larvae was observed, and mortality was recorded. Nauplii were considered dead when they were immobile and stayed at the bottom of the test tubes.<sup>[21-23]</sup> The percent mortality of brine shrimp was calculated with the formula given below.

[% mortality = Number of brine shrimp dead/Number of brine shrimp introduced × 100]

## Results and Discussion

This study was conducted to evaluate *in vitro* brine shrimp lethality assay of an ethanolic extract of *S. grandiflora* and compare the cytotoxicity results with beneficial effects of the plant.

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The results obtained from different concentrations of the extract toward brine shrimp lethality assay are given in Table 1. It shows that extract is highly toxic to brine shrimp with an increase in concentration.

It was observed that at a concentration of 10 mg/ml 100% of mortality is seen in brine shrimp in 24 h. At 20 mg/ml concentration, 100% of brine shrimp was killed in 24 h and 30 mg/ml concentration, 100% of the brine shrimp was killed in 24 h. At a concentration of 40 mg/ml and 50 mg/ml, 100% mortality of brine shrimp was noticed in 24 h, and these concentration prove the larvicidal efficacy of the *S. grandiflora* ethanolic leaf extract. The results were represented in Table 1 and Figures 1-6.

### Conclusion

Its well-known fact that various cell line studies are there to prove the cytotoxicity efficacy of the herbal extract. In those cell lines,

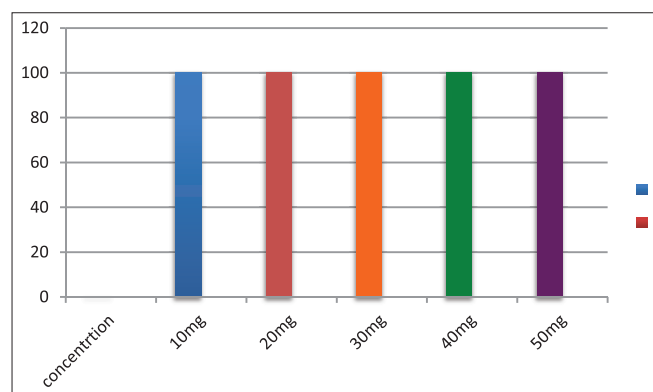


Figure 1: Brine shrimp lethality assay of *Sesbania grandiflora* leaf extract

the maintenance is cumbersome and requires specialized media and protocol. Few cell lines are not economical also. Brine shrimp lethality assay has been emerged to prove the cytotoxicity efficacy of the herbal extract which in the recent years are gaining popularity. The procedure is cost effective. In conclusion, *S. grandiflora* leaf extract revealed that it possess significant cytotoxicity activity against brine shrimp.

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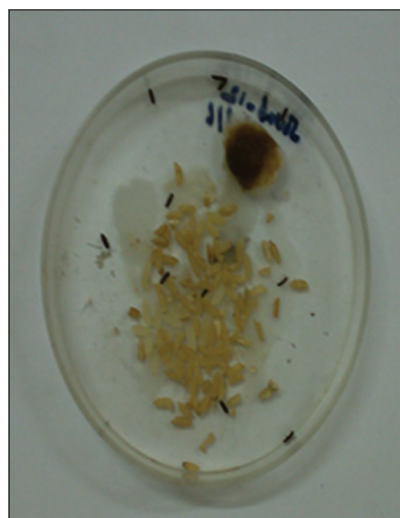


Figure 3: 20 mg/ml concentration

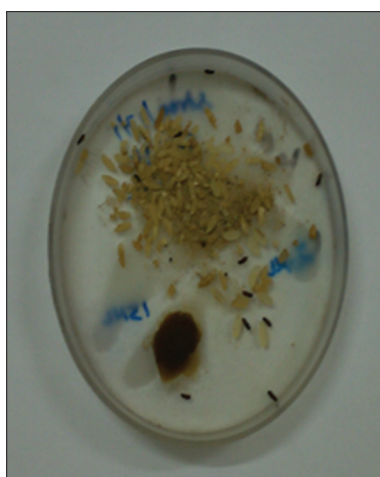


Figure 2: 10 mg/ml concentration



Figure 4: 30 mg/ml concentration

Table 1: Brine shrimp lethality assay of *C. fimbriata* extract

Sample	Incubation period	Concentration (mg/ml)				
		10 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml
<i>S. grandiflora</i> extract	24 h	100%	100%	100%	100%	100%

*C. fimbriata*: *Caralluma fimbriata*, *S. grandiflora*: *Sesbania grandiflora*

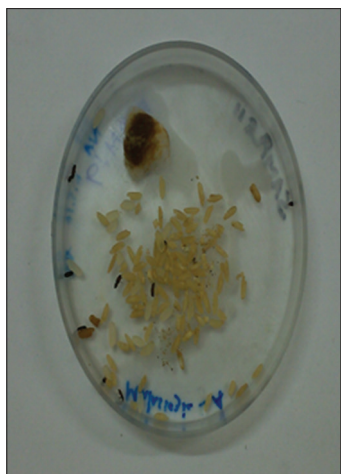


Figure 5: 40 mg/ml concentration

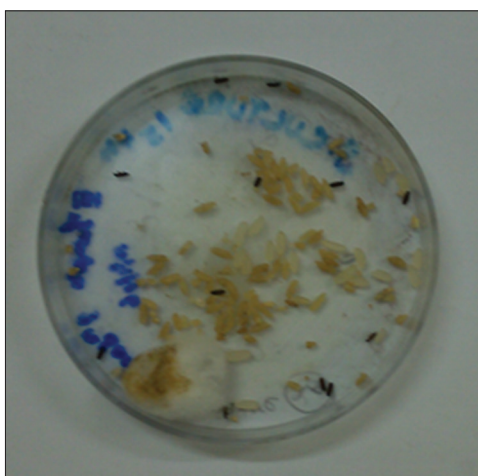


Figure 6: 50 mg/ml concentration

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