



## Brine Shrimp Lethality Assay of *Acacia catechu* Bark Ethanolic Extract

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### ABSTRACT

Brine shrimp lethality bioassay is a standard method to investigate cytotoxicity of herbal extracts. This method is cost effective hence easily employed *in vitro* to check the larvicidal efficacy of the plant extract. *Acacia catechu* commonly known as karungali in Tamil is a deciduous tree possesses immense pharmacological actions. It has lots of nutritive and medicinal value. Hence, the aim of the present study is to evaluate the larvicidal activity of *Acacia catechu* bark extract.

**Keywords:** Brine shrimp, Lethality, *Acacia catechu*, Bark.

### INTRODUCTION

**A** *Acacia catechu* (AC) (Family: *Fabaceae*, subfamily: *Mimosoideae*) known as Black cutch.<sup>1</sup> AC is medium sized thorny deciduous tree mainly found in India and also found in deciduous forests around the world.<sup>5,9,10</sup> It grows up to 13 meters in height. The foliage is softly textured; light green and oval-shaped. The branches are thin and spike like due to tiny thorns grown around the exterior. The sap wood of AC is large and yellowish white and heart wood is small and red in colour.<sup>2</sup> The leaves, bark and heartwood have many nutritional and medicinal uses.

The extract of *Acacia catechu* extract have been reported to have various pharmacological effects like immuno modulatory, anti-pyretic, hypoglycaemic, hepatoprotective activity.<sup>4</sup> In stomatitis, halitosis, dental caries and cavities. The bark is anthelmintic, antipyretic, antiinflammatory and antileprotic. Bark and cutch are antidiarrhoeal, astringent and stomachic. Catechu or Cutch tree bark is useful in melancholia, conjunctivitis and haemoptysis.<sup>3,7,8</sup>

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of extracts of medicinal plants of India. This type of experiments were done to prove the larvicidal activity.<sup>11</sup>

### MATERIALS AND METHODS

#### Plant material

*Acacia catechu* Willd. bark (AAE/9007) were collected from Hosur, Tamilnadu and was authenticated by Dr.H.B Singh, Raw materials herbarium & museum, NISCAIR, New Delhi. The voucher specimen is preserved for further use in Green Chem lab, Bangalore.

#### Ethanolic extraction

Barks were shade dried for a week. Dried barks were milled to fine powder. Powder was passed through 100 mesh sieve and stored in a sealed polythene bag. 2.5kg of powdered *Acacia catechu* bark were extracted with 10 liters of Ethanol, at 65°C temperature, for 1 hour, in a 20 liter round bottom flask with Graham condenser attached. Condenser was cooled circulating with chilled water. After 1 hour of extraction, round bottom flask was cooled to room temp and the extract were filtered and collected. The Marc was extracted repeatedly with 10 liters of Ethanol, twice. The extracts were filtered and collected. The combined extracts was evaporated to dryness under reduced pressure in a Buchi Rotary Evaporator (Switzerland) at 65°C, to obtain 150g of powder extract. The w/w yield of the prepared extract was 6%. The extracts were stored at 4 °C until used.

#### Brine Shrimp Lethality Assay<sup>12</sup>

The eggs of *Artemia salina* were procured from Philadelphia, USA. In a small water tank containing sea water, the eggs were incubated for 48 hours for hatching. Required light was provided with Philips 40 Watts lamp for 12 hours cycle. After 48 hours, the larvae were used for the experiments. The nauplii of *Artemia salina* were challenged in different test tubes containing 10 mL of sea water and 20 larvae. To this, extracts of leaves at different concentrations (1, 2 3, 4 and 5 mg/mL) were added. After 48 hours, 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. The percent mortality of brine shrimp was calculated with the formula given below.

[% mortality = No.of brine shrimp dead /No.of brine shrimp introduced x 100 ]



## RESULTS AND DISCUSSION

*Artemia salina* Nauplii was used as a reference organism to study the larvicidal activity for various reasons such as availability of the cyst throughout the year, suitable test species to evaluate the relative toxicity for a broad range of chemical compounds and mainly the early larval stage of *Artemia* can survive several days without food, which prove them to be suitable for acute toxicity tests.<sup>13,14</sup>

*Acacia catechu* bark studied for its bioactivity against the Nauplii larvae of *Artemia Salina* the bark extract alone showed significant level of lethality. The ethanolic extract of bark showed 25 % lethality against the larvae at the concentration of 5mg/ml. at 1mg /ml and 2mg/ml concentration the bark extract of *Acacia catechu* did not show any mortality. When the concentration is increased mortality rate is significantly observed. *Acacia catechu* bark extract showed low level of larvicidal toxicity. The mortality rate recorded for different concentrations of *Acacia catechu* bark is presented in Table 1.

**Table 1:** Brine shrimp lethality assay of *Acacia catechu* bark extract

Sample	Incubation period	Concentration (mg/ml)				
		1	2	3	4	5
<i>Acacia catechu</i> bark against <i>Artemia salina</i>	24 h	0	0	10	15	20
	48 h	0	0	10	25	25

## CONCLUSION

*Acacia catechu* is an evergreen tree possessing good medicinal value. It exhibits lots of pharmacological actions and used in treatment of various disease conditions. In conclusion, the present study stated that *Acacia catechu* ethanolic bark extract showed low level of toxicity against brine shrimp.

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