

## INVITRO CYTOTOXICITY ASSAY OF ACACIA CATECHU ETHANOLIC SEED EXTRACT USING BRINE SHRIMP

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### ABSTRACT

Context: *Acacia catechu* is a tree which possesses lots of therapeutic value.

Objective: To evaluate the lethality assay of *Acacia catechu* seed using Invitro parameter.

Background: *Acacia catechu* wild is an indigenous plant having tremendous medicinal properties and well reviewed in literature. Ethanolic extract of *Acacia catechu* seed has already been reported to possess selected pharmacological activities like anti diabetic, but there are no reports on cytotoxicity assay of this extract.

Methods: Twenty nauplii were added into three replicates of each concentration of the plant extract. After 24,48 hours, the surviving brine shrimps were counted and percentage of mortality was assessed.

Results: The present study reveals that *Acacia catechu* possess significant cytotoxic activity when tested invitro.

Conclusion: From the present study it can be concluded that ethanolic seed extract of *Acacia catechu* possessed marked cytotoxic effect The effect was plausibly due to the presence of phytochemical contents present in it.

Clinical significance: Novel cytotoxic, pesticidal compounds can be isolated from plant based sources through the investigation of cytotoxic activity against brine shrimps.

### INTRODUCTION

*Acacia catechu* belongs to the family *fabaceae* also known as black cutch and karungali in Tamil. In Kerala people drink it in boiling water to prevent digestive problems. Various part of the plant leaves, bark, heartwood possess diverse pharmacological actions for management of various disorders <sup>1-5</sup>The pharmacological activities in various parts of the plant has been extensively studied. Phytochemical constituents like Catechin, Epicatechin, Cyanidol, Quercetin,

Epigallocatechin gallate, Rutin, Isorhamnetin, Taxifolin is found to be present in *A.catechu*.<sup>6-</sup>

<sup>10</sup> The various part of the extract reports the antipyretic, anti-inflammatory, antidiarrheal, hypoglycemic, hepatoprotective, antioxidant and antimicrobial activities including anti caries and anti plaque activity<sup>11-15</sup>

### MATERIALS AND METHODS

#### Plant material

*Acacia catechu* seeds were collected from

Hosur, Tamil Nadu and were authenticated by Green Chem lab, Bengaluru ,India.

#### PREPARATION OF ETHANOLIC EXTRACT

Seeds were shade dried for a week. Dried seeds 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* seeds 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 extract were stored at 4 °C until used.

#### BRINE SHRIMP LETHALITY ASSAY

The eggs of Brine shrimp 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 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 (1, 2 3, 4 and 5 mg/mL) were added. After 48 hours, the viability of larvae was observed and mortality was recorded.<sup>16,20</sup> 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

The present study was conducted to evaluate invitro brine shrimp lethality assay of ethonolic extract of *Acacia catechu* seed and correlate cytotoxicity results with known pharmacological actions of the plant. The results obtained from different concentrations of brine shrimp lethality assay are given in Table1. It shows that *Acacia catechu* extract is highly toxic to brine shrimp with increase in concentration. It was observed that at a concentration of 100 µl / ml 50% of mortality is seen in brine shrimp in 24 and 48 hours. At 200 µl /ml concentration 55% of brine shrimp were killed in 24 hours and 75% were killed in 48 hours, at 300 µl / ml concentration 100% of the brine shrimp were killed and at 24 & 48 hours. At a concentration of 400 µl /ml 100% mortality of brine shrimp was noticed in 24 hours and at 48 hours even, at 500 µl / ml concentration about 100% of brine shrimp were killed and these concentration proves the larvicidal efficacy of the *Acacia catechu* seed ethanolic extract. The results were tabulated in table 1 and depicted in fig 1.

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

Sample	Incubation Period	Concentration(mg/ml)				
		100 µl /ml	2 00 µl /ml	300 µl /ml	400 µl /ml	500 µl /ml
<i>Acacia catechu</i> seed extract	24 h	50	55	100	100	100
	48 h	50	75	100	100	100

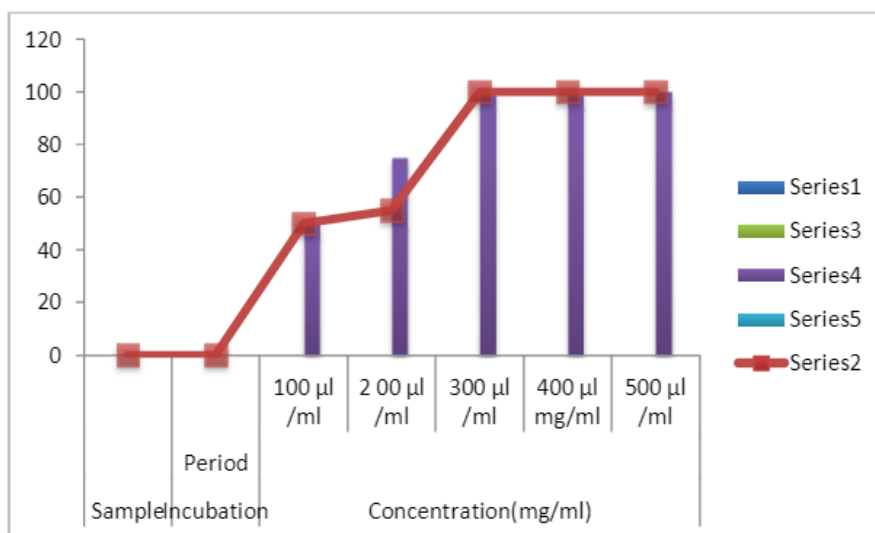


Fig 1 :Brine shrimp lethality assay of *Acacia catechu* seed

## CONCLUSION

Seed of *Acacia catechu* extract possess lots of medicinal value. Brine shrimp lethality assay is an economical and cost effective method for investigating the cytotoxic activity. Hence from the results it can be concluded that that *Acacia catechu* seed is a promising candidate against brine shrimp and possess cytotoxic activity.

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## CONFLICT OF INTEREST

Nil

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