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Assessment of Cytotoxic Activity of *Agave cantula* Using Brine Shrimp (*Artemia salina*) Lethality Bioassay

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ABSTRACT

The essence of the present study is to focus on the cytotoxicity of the aqueous and alcoholic extracts of the Indian medicinal plant *Agave cantula* belonging to the family Agavaceae. Brine shrimp lethality bioassay method was established for the present study and the cytotoxicity was reported in terms of lethality concentration (LC_{50}). The shrimps were hatched and active shrimps were collected and used for the assay, 10 active shrimps were added to the 0.5 mL diluted test solution and the surviving (larvae) shrimps were counted after 24 h and lethality concentration LC_{50} was assessed. In the present study, aqueous and alcoholic extracts of *Agave cantula* exhibited potent brine shrimp lethality LC_{50} as 15 and 12.5 mg, respectively. The present study supports that brine shrimp bioassay is simple, reliable and convenient method for assessment of bioactivity of medicinal plants and extends the support for further research.

Key words: *Agave cantula,* brine shrimp lethality, cytotoxicity, lethality concentration-50, *Artemia nauplii*

INTRODUCTION

Plants are put to medicinal use all over the world since time immemorial. The importance of medicinal plants and traditional health systems in solving the healthcare problems of the world is gaining increased attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing tremendously. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc. Recent estimates suggest that several thousands of plants have been known with medicinal applications in various cultures (Farnsworth and Soejarto, 1991).

In modern era, the medicinal plants have been gradually replaced by synthetic drugs. But of late, it is being realized that several diseases were found to develop drug resistance to synthetic drugs and also responsible for many of adverse effects. At present majority of the people are relying for their primary health care on traditional medicine. The present study concentrates on the cytotoxic activity of the folklore claimed plant *Agave cantula* using brine shrimp lethality bioassay which is based on the ability to kill laboratory cultured brine shrimp (*Artemia nauplii*).

Artemia salina the brine shrimp is an invertebrate component of the fauna of saline aquatic and marine ecosystem. It plays an important role in the energy flow of the food chain (Lewan *et al*., 1992). And it can be used in a laboratory bioassay in order to determine the toxicity

by the estimation of the medium lethality concentration LC_{50} (Meyer *et al.*, 1982). which have been reported for a series of toxins and plant extracts (Lagadic and Caquet, 1998).

For the past 30 years, the *Artemia nauplii* have been used to detect general toxicity (Persoone and Wells, 1987) in teratology screens (Sleet and Brendel, 1983, 1985; Acey and Tomlison, 1988; Kerster and Schaffer, 1983) and in ecotoxicology (Sorgeloos *et al.*, 1978; Persoone and Wells, 1987). From a pharmacological point of view, a good relationship has been found with the brine shrimp lethality test to detect antitumoral compounds in terrestrial plant extracts (Solís *et al.*, 1993; Meyer *et al.*, 1982; Mackeen *et al.*, 2000).

Agave cantula is a perennial, Stout, Scapigerous herb common in waste lands, along road sides and often planted as fencing plant (Chetty *et al.*, 2008). Various parts of the plant were reported to be used as laxative, emmenagogue, scurvy, syphilis, scrutula, swelling, retention of urine, anticancer, cytotoxic, diuretic, aphrodisiac, antisyphilitic (Farooq, 2005) in folklore claims.

Agave cantula was reported to have chemical constituents like flavone glycoside, sterol glycosides, neotigogenin, hecogenin and the plant also contains tigogenin (Khare, 2004).

Reports in tribal medicine suggest the use of leaf extract of *Agave cantula* for its cytotoxic activity, our present study aims at bringing the cytotoxic activity of the traditional Indian medicinal plant *Agave cantula* into lime light. A preliminary study was carried out for the cytotoxic activity of the plant extract. Later these results provoked us to find the lethality concentrations (LC_{50}) of the extracts using brine shrimp lethality bioassay method.

MATERIALS AND METHODS

Plant collection and extraction: The plants were collected from the Regional Forest Research Centre (RFRC), Rajahmundry and was authentified by RFRC in 2009 September.

The leaves were collected, garbled and dried under sunlight. The dried leaves were powdered coarsely. The powdered leaves of *Agave cantula* were weighed upto 100 g and packed in a bag and the alcoholic extract was obtained using soxhlet extractor and ethanol was used as solvent (Kokate, 1991). The extraction was preceded for a certain period until the leaves get completely extracted. The extract was concentrated, collected and stored in refrigerator. For aqueous, the powdered leaves were macerated with distilled water using chloroform as a preservative. The extract was concentrated, collected and stored in refrigerator. The aqueous and alcoholic extracts obtained from the above methods were used for the cytotoxicity study.

CYTOTOXIC BIOASSAY

Brine shrimp lethality bioassay: Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of extracts of *Agave cantula*. Brine shrimp lethality bioassay is easily mastered, costs little and it utilizes small amount of test material. This provides a front line screen that can be backed up by more specific and expensive bioassays, once the active compound has been isolated. It is evident that brine shrimp lethality bioassay is predictive of cytotoxicity and pesticidal activity (Krishnaraju *et al.*, 2005).

This *in vivo* lethality test has been successfully used as a preliminary study of cytotoxic and antitumour agents.

Brine shrimp eggs were collected from Laila Implex Research Centre, Vijayawada Andhra Pradesh, India, as gift sample for the research work.

Hatching of *Artemia salina* shrimps: Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a vessel filled with simulated sterile artificial sea water (brine solution) (Dissolve 38 g of sea salt, sodium chloride in 1000 mL of distilled water adjust the pH to 8.5 using 1N NaOH) under constant aeration for 48 h. The active shrimps were collected and used for the assay (Krishnaraju *et al*., 2005).

About 4.5 mL of brine solution was taken in to each test tube. Suitable dilutions of the test substance (extract) were made as per the concentrations. The 0.5 mL diluted test solution was added to the test tubes.

- Ten active shrimps were added in to each test tube by drawing them with glass capillary tube
- The solution should be mixed thoroughly with the help of a cyclo mixer
- The surviving (larvae) shrimps were counted after 24 h and lethality concentration LC_{50} was assessed

RESULTS AND DISCUSSION

After enumerating the number of shrimps surviving after 24 h the percentage inhibition was evaluated. The lethality concentration 50 (LC_{50}) of the standard, podophyllotoxin was found to be 0.8 μg; the datas are represented in the Table 1.

The results obtained for the bioassay with alcoholic extract of *Agave cantula* was tabulated in Table 2, the lethality concentration (LC_{50}) was found to be 12.5 mg. There was a gradual increase in the percentage inhibition with the increase in concentration of alcoholic extract.

Significant results were produced for the bioassay with aqueous extract of *Agave cantula* and from the Table 3, the lethality concentration 50 (LC_{50}) of the aqueous extract of *Agave cantula* was found to be 15 mg.

The brine shrimp lethality bio assay represents a rapid, inexpensive and simple bioassay for testing plant extract's bioactivity which in most cases correlates reasonably well with cytotoxic and

Table 1: Percentage inhibition by podophyllotoxin treated (Standard)

Concentration of extract/mL	No. of shrimps surviving after 24 h			Total No. of shrimps alive	Inhibition (%)
	T1	T2	T3		
Control	7	8	8	23	23.3
0.1 μg	6	7	8	21	30.0
0.5 μg	5	7	5	17	43.3
1.0 μg	3	3	2	8	73.3
2.5 μg	1	1	1	3	90.0
5.0 μg	1	0	1	2	93.3

Table 2: Percentage inhibition by alcoholic extract of *Agave cantula* treated

Concentration of extract/mL	No. of shrimps surviving after 24 h			Total No. of shrimps alive	Inhibition (%)
	T1	T2	T3		
2.5 mg	7	8	7	22	26.6
5 mg	6	7	7	20	33.3
10 mg	5	6	5	16	46.6
15 mg	5	4	5	14	53.3
20 mg	5	3	4	12	60.0
25 mg	2	3	2	7	76.6

Table 3: Percentage inhibition by Aqueous extract of *Agave cantula* treated

Concentration of extract/mL	No. of shrimps surviving after 24 h			Total No. of shrimps alive	Inhibition (%)
	T1	T2	T3		
2.5 mg	9	9	8	26	13.3
5 mg	8	7	8	23	23.3
10 mg	7	7	7	21	30.0
15 mg	5	6	4	15	50.0
20 mg	3	2	4	9	70.0
25 mg	2	1	1	4	86.6

anti tumour properties (McLaughlin *et al.*, 1993). The brine shrimp assay was proposed by Michael *et al.* (1956) and later developed by Vanhaecke *et al.* (1981) and Sleet and Brendel (1983). 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 (Harwig and Scott, 1971; McLaughlin *et al.*, 1991; Martinez *et al.*, 1999; Barahona and Sanchez-Fortun, 1999; Pelka *et al.*, 2000).

The brine shrimp assay is very useful tool for the isolation of bioactive compounds from plant extracts (Sam, 1993).

The significant lethality of brine shrimp due to extracts of *Agave cantula* is an indicative of the presence of potent cytotoxic components which warrants further investigation.

CONCLUSIONS

Although, the brine shrimp lethality bioassay is rather inadequate regarding the elucidation of the mechanism of action, it is very useful to assess the bioactivity of the plant extracts. In the course of our studies, the brine shrimp lethality assay actually has proven to be convenient system for monitoring biological activities of several plant species that are used in the Indian traditional medicine.

If this study is extended for assessment of cytotoxic activity after isolation of pure compounds some useful drugs may develop out of the research.

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