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IN-VITRO CYTOTOTOXIC STUDIES OF *CAESALPINIA BONDUCELLA* F. USING AQUEOUS EXTRACT OF SEED POWDER AND CALLUS

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ABSTRACT: In the traditional system of Indian medicine *Caesalpinia bonducella* Flem. is widely used for its medicinal properties. All parts are valuable as it contains various secondary metabolites. Seeds contain 20-30% of oil. On the basis of phyto-chemical analysis of the seed it is known to contain alkaloids, flavonoids, glycosides, saponins and furano di terpenes. The plant possesses antidiabetic, antioxidant, antitumor, antiproliferative and anti inflammatory activities. "Cytotoxicity" is the ability to kill the cells. In the present study experiments were carried out to check for the cytotoxic nature of seeds and callus using *Daphnia* cultures. The method used is simple, inexpensive and even a low toxin amounts are sufficient to perform the test. Cytotoxic nature was tested using aqueous extracts of seed powder as well as callus. From the experiments it was observed that extract from seed powder as well as callus expressed cytotoxic activities, however, seed powder extract was more effective compared to callus extract.

INTRODUCTION: Plants are a valuable source of natural products for maintaining human health, with more intensive studies for natural therapies. In developed countries, about 80% individuals rely on traditional medicines which are derived from plants, for their primary health care needs ¹. There is a need to investigate such plants sources to better understand their pharmacological properties with safety and efficacy. Plant phytochemicals have been reported to prevent a number of diseases, including cancer, cardiovascular disease, infection and inflammation ².

Caesalpinia bonducella from family Leguminosae and sub family Caesalpiniae is a prickly shrub widely distributed all over the world especially in India, Sri Lanka and Andaman and Nicobar Islands. In India it is specially found in tropical regions. The seed of the plant, which is extremely bitter in taste, commonly known as natphal to the rural people of Tripura.

C. bonducella is used in traditional medicine like Ayurveda, Siddha, Unani and Homoeopathy ^{3, 4}. All parts of the plant have medicinal properties. Seeds are used to treat colic, convulsions, leprosy and palsy. The oil from seed helps to soften the skin and removes the acne. Singh and Raghav ⁵ reported that, *Caesalpinia bonducella*. Contain all major phytochemicals such as amino acids, fatty acids, hydrocarbons, isoflavones, phenolics, phytosterols and steroidal saponin. The presence of antioxidative activity of *Caesalpinia bonducella* seeds has been reported ^{6, 7}. The evaluation of acute

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and sub-acute toxicity of methanolic extract of *Caesalpinia bonducella* in albino mice has been reported⁸. The animal model selected for the present study is *Daphnia* which is one of the several small aquatic crustaceans commonly called “water fleas” because of their jerky swimming movements. The lifespan of a *Daphnia* does not exceed one year and depends on temperature, food availability and habitat. Cytotoxicity has been explained as those bioactive compounds which are toxic/fatal to living cells⁹.

Some toxic chemicals, toxic venom of different animals and immune cell are the main cause of cytotoxicity. Many cytotoxic compounds from natural products have been investigated for the discovery of the novel compound as an anti cancer drugs². With this background present study was carried out to test for the cytotoxic properties of *Caesalpinia bonducella* seed powder and callus extracts against *Daphnia*.

MATERIALS AND METHODS:

Collection of plant material: The dried fruits of *Caesalpinia bonducella* were collected in the month of November from the farmyard, located at Parle village, Satara district. Fruits were peeled and the seeds were sundried and stored in plastic bags.

In-vitro callus induction: Seeds were soaked in water for 6 h, testa was removed and the embryos were washed with liquid detergent for 15 min. Seed embryos were treated with 1% Bacillocide and 1% Bavistin, for 10 min each. Alternate washing was provided with sterilized distilled water. Finally the seed embryos were treated with 0.1% HgCl₂ for 5 min with continuous shaking. The embryos were washed with the sterilized distilled water, thrice to get rid of traces of HgCl₂. The embryos were cut into small pieces and were placed to the culture tubes containing Murashige and Skoog's¹⁰ media

supplemented with 3mgL⁻¹ 2, 4-Dichloro phenoxy acetic acid. The callus thus obtained was sub cultures repeatedly to get more biomass.

Preparation of Extracts: The dried seeds were ground to form a fine powder. The extracts were prepared using cold infusion method. Five g of powder was soaked in 50 ml of distilled water for 48 h. Same method was applied to get the extract from the callus. Five gram of callus was homogenized in mortar and pestle using distilled water, volume was made to 50 ml.

Preparation of *Daphnia* Culture: The dried eggs of *Daphnia* were hatched in glass beaker (500 ml) containing fresh water with constant aeration and illumination. After 24 h the newly hatched *Daphnia* were collected using pipettes. Ten nauplii were transferred to different vials having varying concentrations of extracts. The nauplii were counted macroscopically in the stem of pipette against a light background. The vials were maintained under illumination at room temperature 25°C to 28°C. Survivors were counted with the aid of 3x magnifying glass at specific time intervals. .

RESULTS:

From **Table 1**, it is evident that *Caesalpinia bonducella* seed powder extract has cytotoxic effect on *Daphnia*. Even the lowest concentration is found to be toxic to *Daphnia* as after 8 h 50% of the nauplii were found to be immotile. As the concentration of extract increased, cytotoxicity was also found to be increasing, with the highest concentration of 100% there are 50% of the nauplii affected just in 3 h. Form **Table 2**, it is clear that callus extract does not show the same severity as that of seed powder extract. Lower concentrations of extract do not affect the motility of *Daphnia* even up to 8 hr, however, highest concentration (100%) does affect the motility of the organisms.

TABLE 1: CYTOTOXIC EFFECT OF CAESALPINIA BONDUCELLA SEED POWDER EXTRACT ON DAPHNIA

Sr. No.	Concentration of aqueous extract (in %)	Effect on motility of nauplii after following hours (in %)			
		3h	5h	8h	24h
1.	0	100% M	100% M	100% M	50% IM
2.	20	100% M	100% M	50% IM	100% IM
3.	40	10% IM	50% M, 50% IM	100% IM	100% IM
4.	60	30% IM	50% M, 50% IM	100% IM	100% IM
5.	80	40% IM	30% M, 70% IM	100% IM	100% IM
6.	100	50% IM	20% M, 80% IM	100% IM	100% IM

(IM: Immotile, M: Motile)

TABLE 2: CYTOTOXIC EFFECT OF *CAESALPINIA BONDUCELLA* CALLUS EXTRACT ON *DAPHNIA*

Sr. No.	Concentration of aqueous extract (in %)	Effect on motility of nauplii after following hours (in %)			
		3h	5h	8h	24h
1.	0	100% M	100% M	100% M	50% M, 50% IM
2.	20	100% M	100% M	100% M	50% M, 50% IM
3.	40	100% M	100% M	100% M	40% M, 60% IM
4.	60	100% M	100% M	100% M	20% M, 80% IM
5.	80	100% M	100% M	50% IM	10% M, 90% IM
6.	100	100% M	100% M	50% IM	10% M, 90% IM

(IM: Immotile, M: Motile)

DISCUSSIONS: *Caesalpinia bonducella* seed powder extract is found to be cytotoxic on *Daphnia*, as after 24 h 100% of the nauplii were immotile whereas callus extract showed 90% immotility in them. *Daphnia* assay is a primary assay conducted to detect cytotoxic property of plant extract. Khalighi-Sigaroodi *et al.* on the basis of their experiments reported the cytotoxic nature of leguminous plants¹¹. *Caesalpinia gilliesii* expressed potent lethality activity against brine shrimp. Prema *et al.*¹² reported the potent cytotoxic activity of the combined extracts of *Cissus quadrangularis* and *Aegle marmelos* against the Colon cancer- HT-29 cell lines. The phytochemical constituents such as flavanoids and terpenoids are the major components which are responsible for the potential cytotoxic activity.

CONCLUSION: On the basis of above findings it is clear that seed powder extract affects the motility of *Daphnia* cultures adversely. Callus extract also shows adverse effect, but only when used at higher concentrations.

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REFERENCES:

1. FAO. *Trade in Medicinal Plants*, Economic and Social Department, Food and Agriculture Organization of the United Nations, Rome, Italy 2012.
2. Palasap A, Limpaboon T, Boonsiri P *et al.*: Cytotoxic Effects of Phytophenolics from *Caesalpinia mimosoides* Lamk on Cervical Carcinoma Cell Lines through an Apoptotic Pathway. *Asian Pacific Journal of Cancer Prevention* 2014, 15 (1): 449-454
3. Kirtikar K and Basu B. *Indian Medicinal Plants*. 2nd edition, Dehradun 1988.
4. Suryawanshi H and Patel M. Traditional uses, medicinal and phytopharmacological properties of *Caesalpinia crista* Linn- An overview. *International Journal of Research in Pharmacy and Chemistry* 2011, 1(4):1179-1183.
5. Singh V and Raghav P. Review on pharmacological properties of *Caesalpinia bonduc* L. *International Journal of Medicinal and Aromatic plants* 2012; 2(3): 514-530.
6. Sachan N, Verma S, Sachan A *et al.* An investigation to antioxidant activity of *Caesalpinia bonducella* seeds. *Annals of Pharmacy and Pharmaceutical Sciences* 2010; 1(2): 88-91.
7. Shukla S, Mehta A, John J *et al.* Antioxidant activity and total phenolic content of ethanolic extract of *Caesalpinia bonducella* seeds. *Food and Chemical Toxicology* 2009; 47(8):1848-51.
8. Preeja P, Suresh P, Doshi G *et al.* Pharmacognostical standardization and toxicity profile of the methanolic leaf extract of *Caesalpinia bonducella* (L) Fleming. *Deccan Journal of Natural Products* 2011; 2(2): 1-15.
9. Khan M, Asad A, Zaman G *et al.* Antioxidant and Cytotoxic Activities of Crude Methanolic Extract of *Medicago polymorpha*. *IOSR Journal of Pharmacy* 2013; 3(8):32-37.
10. Murashige T and Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Plant Physiology* 1962; 15: 473-497.
11. Khalighi-Sigaroodi F, Ahvazi M, Hadjiakhoondi A *et al.* Cytotoxicity and Antioxidant Activity of 23 Plant Species of Leguminosae Family. *Iranian Journal of Pharmaceutical Research*. 2012; 11 (1): 295-302.
12. Prema R, Dhana Sekar D, Sekhar K *et al.* *In vitro* cytotoxicity study on combined plants extracts *Cissus quadrangularis* and *Aegle marmelos*. *European Journal of Experimental Biology* 2012; 2(4):882-888

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