Octa Journal of Biosciences
International peer-reviewed journal
Octa. J. Biosci. Vol. 3(1):7-8

ISSN 2321 – 3663
Jun. 2015

Octa Journal of Biosciences

journal homepage: www.sciencebeingjournal.com

In Vitro Cytotoxic Studies of *Pongamia pinnata* Pierre Using Aqueous Extracts of Seed Powder and Callus

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ARTICLE INFO

Received 29 April 2015
Revised 20 May 2015
Accepted 30 May 2015
Available online 30 June 2015

Keywords: *Pongamia pinnata*, callus, cytotoxicity, *Daphnia*

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ABSTRACT

Plants have been widely used as curative agents for different ailments since time immemorial. *Pongamia pinnata* Pierre is one of such versatile plant. The seeds of the plant contain about 40% oil known as pongamol. It is a having a value in folklore medicine in the treatment of skin diseases, rheumatism etc. All parts of the plant are used in the treatment of tumors, piles, skin diseases, wounds and ulcers. In the present study, experiments were carried out to test the cytotoxic nature of the plant. Plant extracts were prepared using seed powder and callus. Cytotoxicity was tested on *Daphnia* cultures. Results of the present study showed better responses when seed powder extracts were used. Hence, seed powder can be a good source for the cytotoxic compounds.

INTRODUCTION

For thousands of years mankind is using plant source to alleviate or cure illnesses. Plants are the source of novel chemical compounds, which are having potential to be used as medicines and other applications. Plants contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which are deposited in their specific parts such as leaves, flowers, bark, seeds, fruits, root, etc.(Gupta et al, 2012). Palasap et al. (2014) reported that, plant phytochemicals have been used to prevent a number of diseases, including cancer, cardiovascular disease, infection and inflammation.

Sagwan et al., (2010) mentioned Pongamia is a genus having one species only *Pongamia pinnata* P. also known as Pongamia glabra (Vent); *Derris indica* (Lamk.) which belongs to family Leguminosae and sub-family Papilionaceae. It is commonly known as Karanja originated in tropical and temperate Asian countries (Kagithoju et al, 2011). The whole plant is medicinally important. Seeds contain 27 to 40% of bitter fatty oil and traces of essential oil. Seeds shows the presence of secondary metabolites like karanjin, pongamol and glabrin in them (Sreelakshmi and Reddy, 2012). Sagwan et al. (2011) on the basis of their studies with the root, stem, leaves, seed and callus of *Pongamia pinnata* suggested that there is a correlation between the phenolic content and antioxidant activity of the plant. In the traditional medicinal system, such as Ayurveda and Unani, the *Pongamia pinnata* plant is used for anti-inflammatory, anti-plasmodial, anti-nonciceptive, anti-hyperglycamic, anti-lipidperoxidative, anti-diarrhoeal, anti-ulcer, anti-hyperammonic and antioxidant activity (Chopade et al., 2009). Dahanukar et al. (2000) reported that seed oil is used in various treatments such as scabies, herpes, leucoderma, rheumatism and skin diseases.

Mirzaei and Mirzaei (2013) mentioned that the over consumption of medicinal plants can lead to excessive accumulation of herbs in the body which may cause toxicity. In considering the adverse effects, it is necessary to study the toxicity of the plants though they found to be valuable to humans. The toxicological studies are performed generally on animals which are costly and also animals are harmed in all the process. To avoid this, an alternative laboratory studies are performed (Lagarto et al., 2001). For the present cytotoxicity study, the animal model selected was *Daphnia* which is one of the several small aquatic crustaceans commonly called "water fleas" because of their jerky swimming movements. The most prominent external features of *Daphnia* are a single large compound eye and two pairs of slightly branched antennae. Cytotoxicity has been explained as those bioactive compounds which are toxic/ fatal to living cells (Khan et al., 2013). Many cytotoxic compounds from natural products have been investigated for the discovery of the novel compound as an anti cancer drugs (Palasap et al., 2014). On the basis of this background present study was carried out to test for the cytotoxic properties of *Pongamia pinnata* seed powder and callus extracts against *Daphnia*.

MATERIALS AND METHODS

Collection of plant material

The dried fruits of *Pongamia pinnata* were collected in the month of April to May 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 4 to 6 hours, testa was removed and the embryos were washed with liquid detergent for 15 min. Seed embryos were treated with 1% Bacillocid and 1% Bavistin, for 10 minutes each. Alternate washing was provided with sterilized distilled water. Finally, the seed embryos were treated with 0.1% $HgCl_2$ for 7 minutes with continuous shaking. The embryos were washed with the sterilized distilled water, thrice to get rid of traces of $HgCl_2$. The embryos were cut into small pieces and were placed to the culture tubes containing Murashige and Skoog's (1962) 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 gram of powder was soaked in 50 ml of distilled water for 48 hours with intermittent shaking. Same method was applied to get the extract from the callus. Five gram of callus was homogenized in mortar and pestle using distilled water and volume was made to 50 ml. The supernatant thus obtained was used for the experiments.

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 hours 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 AND DISCUSSIONS

Table 1 shows the effect on *Daphnia* when suspended in aqueous extract of *Pongamia pinnata* seed powder. From the table it is evident that, as the concentration increased there was increase in immotile organisms. The concentrations in the range of 20% to 60% adversely affected the nauplii leading to 60-70% to be immotile. After 5 hours 80%

Table 1: Effect of varying concentrations of P. pinnata seed extract on the motility of *Daphnia nauplii* at different incubation periods

Sr. No.	Concentration of aqueous extract (in %)	Percentage of *Daphnia* immotile			
		3h	5h	8h	24h
1.	0	00	00	00	10
2.	20	00	60	70	90
3.	40	00	70	80	100
4.	60	00	70	80	100
5.	80	00	100	100	100
6.	100	00	100	100	100

Table 2: Effect of varying concentrations of *P. pinnata* callus extract on the motility of *Daphnia* nauplii at different incubation periods

Sr. No.	Concentration of aqueous extract (in %)	Percentage of *Daphnia* immotile			
		3h	5h	8h	24h
1.	0	00	00	30	30
2.	20	00	20	40	50
3.	40	00	10	40	70
4.	60	00	30	40	80
5.	80	00	30	50	85
6.	100	10	40	50	90

and 100% concentrations affected 100% nauplii. The effect of aqueous extract of *P. pinnata callus* of on *Daphnia* is depicted in table 2. From the table it is clear that 20% concentration resulted in paralyzing 40% of nauplii after 8 hours of incubation. As the concentration increased (40%) the effect was observed in 5 hours, with 10% of the nauplii becoming immotile. Higher concentration such as 100%, showed a significant activity with a short time period of 3 hours. After 24 hours, 80% and 100% concentration, 15% and 10% of the nauplii were found to be motile. On the basis of the results of present experiments, it can be concluded that, seed powder extract of *Pongamia pinnata* was found to be more potent in terms of toxicity towards *Daphnia* when compared with callus extract. The phytochemical constituents such as flavanoids and terpenoids are the major components which are responsible for the potential cytotoxic activity. Khalighi-Sigaroodi et al. (2012) on the basis of their experiments reported the cytotoxic nature of leguminous plants.

Krishnaraju et al. (2005) mentioned aqueous extracts of some medicinal plants were screened for their cytotoxicity using brine shrimp lethality test. Out of the 120 plants tested, Pistacia lentiscus exhibited potent brine shrimp lethality with LC50 2.5 µg. *Aristolochia indica* (Aristolochiaceae), *Boswellia serrata* (Burseraceae), *Ginkgo biloba* (Ginkgoaceae), *Garcinia cambogia* (Clusiaceae), and *Semecarpus anacardium* (Anacardiaceae) have also showed significant cytotoxicity with LC50 13, 18, 21, 22, and 29.5 µg respectively. They also found Pongamia pinnata having very minute cytotoxic activity with LC50 is 155 µg.

CONCLUSION

On the basis of present study, it is clear that aqueous extracts of seed powder as well as callus of *Pongamia pinnata* showed cytotoxic properties, though seed powder extract was found to be more effective.

REFERENCES

1. Chopade, V., Tankar, A., Pande, V., Tekade, A., Gowekar, N., Bhandari, S. and Khandake, S. (2009): Pongamia pinnata: Phytochemical constituents, traditional uses and pharmacological properties. *International Journal of Green Pharmacy*, 2:72-75.
2. Dahanukar, S., Kulkarni, R. and Rege, N. (2000): Pharmacology of medicinal plants and natural products. *Indian Journal of Pharmacology*, 32: 81-118
3. Gupta, A., Naraniwal, M. and Kothari, V. (2012): Modern extraction methods for preparation of bioactive plant extracts, *International Journal of Applied and Natural Sciences*, 1(1): 8-26.
4. Kagithoju, S., Godishala, V., Pabba, S., Kurra, H. and Swamy, N. (2011): Antibacterial activity of flower extract of Pongamia pinnata Linn- An elite medicinal plant. *International Journal of Pharmacy and Pharmaceutical Scinces*, 4(3):130-132.
5. Khalighi-Sigaroodi, F., Ahvazi, M. , Hadjiakhoondi, A. , Taghizadeha, M., Yazdania, D., Shahram Khalighi-Sigaroodi, S. and Bidel, S. (2012): Cytotoxicity and Antioxidant activity of 23 plant species of Leguminosae family. *Iranian Journal of Pharmaceutical Research*, 11(1): 295-302.
6. Khan, M., Asad, S., Zaman, G., Rehman, H., Rehman, S., Iqbal, A., Ullah, A., Ullah, I, S. and Ali, S. (2013): Antioxidant and Cytotoxic Activities of Crude Methanolic Extract of Medicago polymorpha. *IOSR Journal of Pharmacy*, 3(8):32-37.
7. Krishnaraju, A., Rao, T., Sundararaju, D., Vanisree, M., Tsay, H. and Subbaraju, G. (2005): Assessment of Bioactivity of Indian Medicinal Plants Using Brine Shrimp (Artemia salina) Lethality Assay. *International Journal of Applied Science and Engineering*, 3(2): 125-134.
8. Lagarto, P., Silva, Y., Guerra, S. and Iglesias, B. (2001): Comparative study of the assay of Artemia salina L. and the estimate of the medium lethal dose (LD50 value) in mice, to determine oral acute toxicity of plant extracts. *Phytomedicine*, 8(5), 395–400.
9. Mirzaei, M. and Mirzaei, A. (2013): Comparison of the Artemia salina and Artemia uramiana bioassays for toxicity of four Iranian medicinal plants. *International Research Journal of Biological Sciences*. 2(3): 49-54.
10. Murashige, T. and Skoog, F. (1962): A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Plant Physiology*; 15: 473-497.
11. Palasap, A., Limpaiboon, T. and Boonsiri, P. (2014): Cytotoxic Effects of Phytophenolics from Caesalpinia mimosoides Lamk on Cervical Carcinoma Cell Lines through an Apoptotic Pathway. Asian Pacific. *Journal of Cancer Prevention*, 15 (1): 449-454.
12. Sagwan, S., Rao, D. and Sharma, R. (2010): A review on Pongamia pinnata (L.) Pierre: A great versatile Leguminous plant. *Nature and Science*, 8(11): 130-139.
13. Sagwan, S., Rao, D. and Sharma, R. (2011): In-vitro and In-vivo antioxidant activity and total phenolic content of Pongamia pinnata (L.) Pierre: an important medicinal plant. *International journal of biotechnology*, 4(6): 568-574.
14. Sreelakshmi, L. and Reddy, J. (2012): Detection of Karanjin from callus cultures of Pongamia pinnata. *Journal of Pharmacognosy*, 3(2):67-70.