

Exploration of Antitumor Properties of Pollen Grains of Plant Species belonging to Fabaceae Family

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Abstract

Various bioactive compounds possessing the properties as antioxidant, antibacterial, antifungal, antimutagenic and anticancerous have been characterized and isolated from different parts of plants. Some studies in recent years have reported the occurrence of such bioactive compounds in pollen grains as well. Considering this, the present study was planned to examine antitumor activities of ethanolic extracts of pollen grains of four plant species of family fabaceae viz., *Bauhinia variegata*, *Cassia biflora*, *Cassia glauca* and *Cassia siamea* following potato disc tumor assay. It was seen that *Cassia glauca* has induced minimum number (5.93 tumors per disc) of tumors, followed by *Bauhinia variegata* (6.13 tumors per disc), *Cassia siamea* (6.80 tumors per disc) and *Cassia biflora* (6.93 tumors per disc) during potato disc assay whereas positive control i.e. only *Agrobacterium tumefaciens* culture has shown 19.73 tumors. Hence, the present study clearly indicates the antitumor potential of pollen grains of the studied plants. This study is the first report to show the antitumor activities of pollen grains of *Bauhinia variegata*, *Cassia biflora*, *Cassia glauca* and *Cassia siamea* plant species.

Keywords: Anticancer, *Cassia glauca*, potato disc tumor assay, tumors.

INTRODUCTION

Cancer is one of the most life-threatening diseases in both developed and developing countries. It is characterized by the deregulated proliferation of abnormal cells that invade and disrupt surrounding tissues^[1]. Different chemicals present in the environment can cause genetic mutations and are responsible for cancer^[2,3]. Due to biotic and abiotic stress, reactive oxygen species (ROS) are continuously formed in cells which include free radicals, as the superoxide anions and the hydroxyl ion and non-radical species, as H₂O₂ and singlet oxygen. All these factors cause damage to essential biomolecules such as carbohydrates, proteins, amino acids, lipids, nucleic acids, thus contributing to ageing and installation of chronic-degenerative diseases, such as cancer, cardiovascular diseases, arthritis, gastric ulcer etc.^[3, 4]. Since the exposure to different pollutants in environment is unavoidable, it becomes necessary to investigate the substances of natural origin for their anticancer potential. The different parts of plants viz., stems, leaves, seed, flowers, stamens etc. have been used as traditional medicine since the ancient time^[5,6,7]. Flowers parts of various plant species viz., *Crocus sativus*^[7], *Nymphaea nouchali*^[8], *Peltophorum pterocarpum*^[9], *Hibiscus sabdariffa*^[10], *Rhododendron arboreum*^[11] have been evaluated for their bioactivities. Pollen grains of some plant species have been explored for their antimutagenic activities^[2,12]. Pollens have also gained attention because of their bioactive properties such as antioxidant, antibacterial, antifungicidal and antimutagenicity^[13-18]. The bioactivity of the pollens was reported to be due to presence of compounds such as proteins, carbohydrates, amino acids, lipids, vitamins carotenoids, steroids, terpenes, flavonoids and polyphenols^[16,19-20].

Potato disc tumor assay is a widely used plant bioassay for evaluation of antitumor potential of various plants extracts and is based on the principle of infection of gram negative bacterium i.e. *Agrobacterium tumefaciens* on potato discs.

Many scientists have used this assay for antitumor activity of various plants extracts^[21-25]. Considering the applicability of potato disc tumor assay, this bioassay was selected to explore the antitumor potential of pollen grains of four plant species viz., *Cassia glauca*, *Cassia biflora*, *Cassia siamea* and *Bauhinia variegata* growing Guru Nanak Dev University campus.

MATERIALS AND METHODS

Collection of samples

The fresh flowers (about to anthesis) of *Cassia glauca*, *Cassia biflora*, *Cassia siamea* and *Bauhinia variegata* were collected from the Guru Nanak Dev University campus, Amritsar, Punjab (India). Identification of the plants was done using the botanical description from herbarium sheets with accession number 6474 dated 07/07/2009 (*Cassia glauca*), 6433 dated 15/07/2009 (*Bauhinia variegata*), 6455 dated 15/07/2009 (*Cassia siamea*) and 6487 dated 13/07/2009 (*Cassia biflora*), which were previously submitted in the herbarium of Department of Botanical and Environmental Sciences, GNDU, Amritsar.

Collection of pollen grains sample

The pollen grains were collected in pre weighted Petri plates by isolating anthers from flowers. The anthers were teased with the help of sharp forceps and were tapped in Petri plates in order to collect the pollen grains. The weight of Petri plates with pollens was noted again. 1 g of pollen grains was obtained from approximately 100-150 flowers which were further used to prepare the pollen extracts.

Preparation of pollen extracts

Pollen extracts were prepared using the protocol given by Carpes et al.^[26] with certain modifications. 1 g of pollen grains was milled and homogenized with the help of needle end in Petri plate. 7.5 ml of 70 % ethanol was added to the pollen mixture and kept at 70°C for 1 h with agitation of 1 min after every 10 min interval. The supernatant was separated and solid residue was re-extracted with 70 % ethanol. The process is repeated 3-4 times and the

supernatants were pooled in a 25 ml conical flask. Collected supernatant was considered as 100 % extract. These extracts were stored at 5°C till further analysis. Different concentrations viz., 25 %, 50 %, 75 % and 100 % of pollen extracts were made using double distilled water.

Estimation of antitumor potential of pollen extracts

Potato disc assay was used to evaluate the antitumor properties of pollen grains using standard protocol of Coker et al.^[27] with modifications. *Agrobacterium tumefaciens* strain MTCC (Microbial Type Culture Collection and Gene Bank) No. 431 was purchased from IMTECH, Chandigarh. After the procurement, *Agrobacterium tumefaciens* culture was grown in nutrient medium (peptone, beef extract, yeast extract and NaCl) by keeping in incubator shaker at conditions of temperature (25 ± 2 °C), shaking (225 rpm) and time (18 h). Russet potatoes were purchased from local market and were washed thoroughly under running water for 2-3 min. The potatoes were peeled off with sterile knife and discs of 0.5 cm × 1 cm (height × diameter) were cut using sterile cork borer. The discs were disinfested using 10 % bleach solution. 5 sterile potato discs were placed in agar plates (Petri dish) and immersed gently up to 2/3 rd of its height. 400 µl of pollen extract and 400 µl of freshly prepared culture of *Agrobacterium tumefaciens* were mixed in a vial. 50 µl of the mixture was put on each disc. Petri plates were then covered with lids and sealed with parafilm. After sealing, the Petri plates were placed in B.O.D. incubator for 12 days to induce tumors. 50 µl of solution (25 µl of *Agrobacterium* culture + 25 µl sterile distilled water) was used as positive control whereas sterile double distilled water and ethanol were used as negative controls. After 12 days of incubation period, potato discs were stained with Lugol's solution (5 % potassium iodide + 5 % Iodine) and scored for number of induced tumors using stereomicroscope at 25X magnification.

RESULTS AND DISCUSSION

The present study revealed the antitumor potential of pollen grains of plant species (Figure 1). Among the four species studied, minimum number (5.93 tumors per disc) of tumors was observed by pollen extract of *Cassia glauca*, followed by *Bauhinia Variegata* (6.13 tumors per disc), *Cassia siamea* (6.80 tumors per disc) and *Cassia biflora* (6.93 tumors per disc). The positive control i.e. only *Agrobacterium tumefaciens* culture has showed 19.73 tumors. From the present study, it was clearly indicated that the pollen extracts of *Cassia glauca* has shown maximum tumor reducing potential. known as Crown gall which is a neoplastic disease induced by Ti plasmid of *Agrobacterium tumefaciens*. *Agrobacterium tumefaciens* shows similar pathogenic effects as *Helicobacter phyrihenselae*, a tumor causing bacteria in human^[28-29]. Many scientists have reported the inhibitory effects of extracts of different medicinal plants on growth of tumors^[6,21,23-25]. Kuete et al.^[30] listed the genus *Treulia* for antitumor activities using crown gall tumor assay. The leaves extracts of *T. Africana*, *T. acuminata* and *T. obovoidea* have shown tumor-reducing activity as 89.67%, 92.16% and 96.67%, respectively while the twigs of *T. acuminata* had tumor-reducing activity as 87.18%. Fatima et al.^[21] evaluated the biological activities of *Rumex dentatus* L. using methanol and hexane extracts for antitumor potential using potato disc tumor assay. The authors prepared leaf, stem and root extracts using methanol and hexane by simple maceration. They observed the inhibitory effects of the extracts of *R. dentatus* in terms of tumor induction on the potato discs produced by *Agrobacterium* strains At10 and At6.

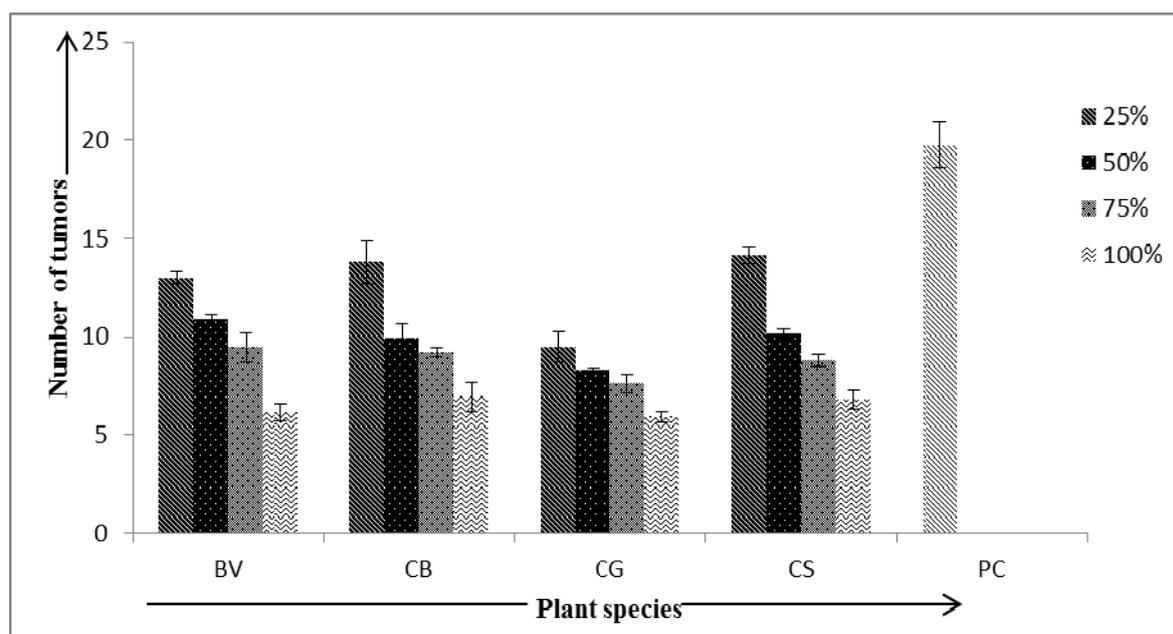


Figure 1. Effect of pollen grains extracts of plants species on reduction in number of tumors by following potato disc assay.

BV: *Bauhinia variegata*; CB: *Cassia biflora*; CG: *Cassia glauca*; CS; *Cassia siamea*

Agrobacterium tumefaciens cause a disease in plants Islam et al.^[24] examined the antitumor activity of leaf methanol extract of *Oldenlandia diffusa* (willd.) Roxb. Three strains of *A. tumefaciens* viz., AtTa0112, AtAc0114 and AtSl0105 were used for induction of tumors and methanol extract of *Oldenlandia diffusa* (willd.) Roxb was used for antitumor activity. At 1000 ppm, the percentage tumor inhibition was found to be 40.98, 41.93 and 41.89 % for AtTa0112, AtAc0114 and AtSl0105 strains, respectively. Galsky and Wilsey^[22] compared the activities of various plant materials against initiation of crown gall tumors for cytotoxicity as well as inhibition of Leukemia in the mice and observed strong correlation between the antileukemic activity of the samples and their ability to inhibit crown gall tumor formation on potato discs. Mahmood et al.^[31] reported 75 % inhibition of tumor by *Withania somifera* L. Dunal and *Datura innoxia* Mill. while 50 % inhibition by *Solanum surrattense* Burm. f.

Although different plants including leaves, stem, roots and bark have been explored for their antigenotoxicity, antimutagenicity and antitumor activities but very few reports are available on these bioactivities of pollen grains. Barzin et al.^[2] examined the antimutagenic response of pollen grains of *Phoenix dactylifera* using Ames assay. The authors observed that pollen grains of *Phoenix dactylifera* had 46 % antimutagenic response. In another report, Jatón et al.^[12] studied the inhibitory effects of secalosides (a glycosides compound isolated from pollen grains of *Secale cereale*) on S180 sarcoma. The compound showed very strong antitumor activity against S180 sarcoma.

CONCLUSION

Among the four species studied in the present study, *Cassia glauca* has shown maximum tumor reducing potential. It was seen that *Cassia glauca* has induced minimum number (5.93 tumors per disc) of tumors, followed by *Bauhinia variegata*, *Cassia siamea* and *Cassia biflora* during potato disc assay. The present study clearly indicates the antitumor potential of pollen grains of the studied plants. This study is the first report to show the antitumor activities of pollen grains of *Bauhinia variegata*, *Cassia biflora*, *Cassia glauca* and *Cassia siamea* plant species.

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