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Alkaloid rich fraction of *Datura alba* Rumph. ex Nees leaves possesses antitumor and antimitotic activity

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ABSTRACT

The antimitotic and antitumor activities of total alkaloids extracted from leaves of *Datura alba* Nees. were investigated using in vitro methods. The plant leaves were collected from local ground of Lahore College for Women University. The antitumor bioassay was performed using potato discs applied with plant alkaloid extract and activity was checked against *Agrobacterium tumefaciens*. The results demonstrated that the alkaloid rich fraction significantly repressed the tumor growth on potato discs at a concentration of 1000 ppm, giving 84.78% tumor inhibition after 21 days of incubation. The antimitotic bioassay was implemented using *Allium cepa* root tip bioassay. The findings suggested that the alkaloidal fraction also showed significant antimitotic effect on root tip cells of *A. cepa*. At 1000 ppm concentration, the alkaloidal extract revealed best antimitotic activity giving reduced (30 ± 0.577%) mitotic index after 72 h of treatment. The outcome of the present experiment, therefore, indicates that *Datura* alkaloids could be regarded as a potential source for the development of anti-tumor and antimitotic agents. The study could be extended further for drug development against cancers in humans.

1. Introduction

Human beings have been utilizing medicinal plants since primordial times as the fundamental source of medicines. The ethnomedicinal data related to folk medicines led to further exploration of medicinal plants as potential source of medicines resulting in the isolation of various natural products that have emerged as important pharmaceutics (Sharma and Mujundar, 2003). The most important characteristic of natural products contributing to their significance in drug discovery is their structural diversity (Veeresham, 2012). Secondary metabolites are biological entities that are not involved in the usual growth and development processes of the plants but play a major role in their defense system. These secondary metabolites including terpenes, phenolics and alkaloids are classified according to their biosynthetic origin. During the course of evolution diverse classes of these compounds have been associated with particular plant species and comprise the biologically active compounds in numerous therapeutically active medicinal and aromatic plants and useful foods (Roze et al., 2011).

Alkaloids are a large and structurally diverse group of compounds which have a heterocyclic nitrogenous ring system and a basic (alkaline) character e.g., atropine and nicotine. Alkaloid producing plants are commonly found in families like Fabaceae, Liliaceae, Solanaceae and Amaryllidaceae. Alkaloids are well known for their special pharmacological properties and are found in all plant parts like stems, roots, leaves, seeds, fruits and flowers. The literature reveals that alkaloids have diuretic, antitumor, antifungal, cardiotonic, antispermatogenetic, antiandrogenic, immunomodulatory, antipyretic and several effects on the central nervous system (CNS) (Patel et al., 2013).

Cancer is a major public health problem in both developed and developing countries. The instability of cell growth and death can cause tumors to be formed (Rashed, 2014). It is an irregular development of body cells. Such growths can be malignant (cancerous) or benign (noncancerous). After cardiovascular disease, it is the world's second killer (Kathiriya et al., 2010). Tumors are believed to be triggered by the mutual action between genomic liability and environmental poisons. Medicinal plants have been established as a common substitute for synthetic drugs for tumor inhibition and treatment in various countries around the world. Natural phytochemicals obtained from medicinal plants have contributed significantly towards the treatment of several human diseases including cancer (Mehta et al., 2010). Over 3000 plants worldwide have been reported to possess anticancer properties (Dai and Mumper, 2010). The potato disc bioassay is a commonly used method to check the antitumor potential of plants and plant derived compounds. The assay is established on the basis of infection caused by *Agrobacterium tumefaciens* on potato discs (Islam et al., 2009). The basis for this bioassay is that the mechanism of tumor induction by *A. tumefaciens* is similar for both animals and plants (Becker, 1975).

Abbreviations: CNS, central nervous system; DMSO, dimethylsulfoxide; IC_{50}, inhibitory concentration with 50% inhibition; LB, Luria–Bertani; ppm, parts per million.

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Antimitotic agents can modify or inhibit the process of cell division and thus are helpful in life threatening diseases like cancer (Gaikwad et al., 2011). The antimitotic activity can be tested using meristematic cells of *Allium cepa* root that are commonly used in testing of drugs for antimitotic activity. The growing tips of plant roots generally undergo repeated cell divisions and the rate of mitosis is higher in such regions compared to that of the other tissues. These cells can be used for initial screening of drugs with antitumor activity due to the presence of uniform meristematic cells, huge chromosomes and merely 16 chromosomes (Andrade et al., 2008; Thenmozhi et al., 2011).

Datura alba Rumphius ex Nees belonging to the family Solanaceae is known for its medicinal uses. All parts of the plant have medicinal value, but only the leaves and seeds are formally used. It is extensively used for the treatment of asthma, healing of burn wounds, muscle spasm, whooping cough, hemorrhoids and skin ulcers etc. (Uddin et al., 2012). In the whole plant of *Datura,* many different alkaloids are found, which increase slowly but surely with increase in age of the plant. Major alkaloid components of *D. alba* comprise of large number of tropane alkaloids containing hyoscyamine, a number of withanolides, littorine, hyoscine, valtropine, acetoxytropine, fastusine and several tigloyl-esters of tropine and pseudotropine. Daturanolone, triterpene, β-sitosterol and daturadiol are mainly present in the fruit wall while the seeds predominantly contain tropane alkaloids along with daturanolone and fastusic acid. The nor-tropane alkaloids, calystegines, have also been found in a number of *Datura* species and are known to possess glycosidase inhibitory activity (Ghani, 2003). The ether extract of *D. stramonium* exhibited antitumor effects through inhibition of mitosis process (Ahmad et al., 2009). In a study by Nazeema Banu et al. (2014) the methanol extracts of leaves and stem of *D. metel* were evaluated for anticancer activity against MCF-7 cell lines. The study confirmed that the leaf extract had significant anticancer potential against MCF-7 cell lines than the stem extract. Several withanolides were isolated from the methanol extract of flowers of *D. metel* that were screened for inhibitory activity against various cancer cell lines and were found to be active with IC_{50} values ranging from 0.05 to 3.5 μM (Pan et al., 2007). On the basis of all these reports the total alkaloids from leaves of *D. alba* were extracted and were tested for antitumor and antimitotic activity.

2. Materials and methods

2.1. Collection and preparation of plant material

Leaves of mature *D. alba* were collected from Lahore College for Women University. The plant was identified using Flora of Pakistan and other available resources and a voucher specimen was deposited in Prem Madan Herbarium (Voucher no. LCWU-18-233). The leaves were carefully separated from the plant, washed and surface sterilized. The leaf material was dried and crushed into powdered form to be used for further alkaloid extraction.

2.2. Extraction of total alkaloids

Approximately 70 g of powdered leaf material was soaked in 200 mL of methanol for 5 days with constant stirring. The methanol extract was filtered and the process was repeated again in order to ensure that no extractables remained in the residues. The methanol was recovered by rotary evaporator at 40 °C. The residues from the methanol extracts were air dried and stored for further use. The filtrate was acidified with 0.1 M H_2SO_4 and extracted with chloroform. The organic phase-I contained neutral and acidic materials which was stored while the aqueous phase-I was tested for alkaloids with Dragendorff's reagent. This aqueous phase-I was basified with 20% Na_2CO_3 to pH 9/10 and again extracted with chloroform. The aqueous phase-II obtained contained the water soluble material whereas organic phase-II was washed with H_2O and dried with Na_2SO_4. The chloroform was evaporated in a rotary at 40 °C. The latter solvent was completely dried up and the residue left was weighed to calculate the amount of crude alkaloids. The residue was tested with Dragendorff's reagent to confirm the presence of alkaloids.

2.3. Antitumor activity

Antitumor activity of alkaloid extract of *D. alba* was determined by using the potato disc method as stated by Ferrigni et al. (1982).

2.3.1. Preparation of bacterial culture

Culture of *A. tumefaciens* pathogenic strain (LBA4404) was grown on LB (Luria–Bertani) agar medium. LB medium was prepared by dissolving 1.25 g of LB in 50 mL of distilled water and pH was set at 7.0. It was autoclaved in 100 mL flask. Four to five isolated colonies from culture plate of *A. tumefaciens* were transferred to LB broth and incubated for 48 h at 30 °C. In a test tube, 10 ml of phosphate buffer with pH 7.2 was taken and about six to seven loops of bacterial suspensions were added in it.

2.3.2. Sample preparation

Plant material was prepared by dissolving 10 mg of plant alkaloidal extract in 1 mL of DMSO (10 mg/mL or 10,000 ppm) to make stock solution. From this stock solution additional dilutions (1000 ppm, 100 ppm, and 10 ppm) were prepared.

2.3.3. Preparation of inoculum

To prepare final concentration of 1000, 100 and 10 ppm, 1.5 mL of inoculum was prepared from initial stocks by adding 0.15 mL of each of the stock solution in three autoclaved test tubes. Finally 0.75 mL of double distilled (autoclaved) water and 0.60 mL culture of bacteria were added in each test tube. 0.15 mL of DMSO replacing alkaloid solution served as negative control while 0.15 mL of DMSO and 1.35 mL of double distilled (autoclaved) water served as blank. In order to avoid contamination, each solution was prepared in a Laminar flow hood and all precautionary measures were considered.

2.3.4. Preparation of agar plates

To prepare the plane agar medium, 15 g/L of plane agar was dissolved in distilled water and sterilized in an autoclave. Three plates were used for each concentration (1000 ppm, 100 ppm, 10 ppm) and three plates for three controls. In each petri plate, about 20 mL of autoclaved agar solution was transferred and allowed to solidify.

2.3.5. Preparation of potato discs

Red skinned potatoes were surface sterilized by dipping them in 0.1% $HgCl_2$ solution in a beaker for 10 min. The potato was then rinsed thrice with autoclaved, distilled water and dried. A sterilized borer was used to make cylinders of potato. The potato cylinders were splashed in distilled water in a petri plate. Thick discs (5 mm) of potato cylinders were made in petri plates. Potato discs were sterilized with autoclaved water and transferred on solidified agar plates (10 discs for each plate). On the surface of all discs, 50 μL of inoculum was poured. Within 10–20 min, the inoculum was diffused. The plates were made air-tight by wrapping them with parafilm. These petri-plates were put into an incubator at 28 °C for 21 days.

2.3.6. Staining of potato discs

Lugol's solution was prepared in distilled water by adding 10% KI and 5% Iodine in it. The potato discs were coated with lugol's solution and allowed to diffuse for 15 min. The discs were examined under light microscope. The unstained portions of discs were the tumors. No. of