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Antifungal Activities of Methanolic Extracts of *Datura inoxia*

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Abstract
This study was conducted to investigate antifungal activities of methanol extracts from the leaves, seeds, stems and roots of *Datura inoxia*. Growth inhibition was determined against five fungal species *Aspergillus flavus, Aspergillus niger*, *Alternaria solani, Fusarium solani* and *Helianthus sporium.* Methanol extracts showed more activity against fungi, giving percentage inhibition ranging from 18.27% to 85.35% particularly to *Fusarium solani* and *Helianthus sporium.* It was concluded that medicinal plants can contribute hugely to the traditional medicines through providing ingredients for drug or having played central roles in the drug discoveries.
Keywords: Drugs, *Datura inoxia*, antifungal, antimicrobial.

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INTRODUCTION

In modern and traditional medicines, medicinal plants constitute as an effective source. Plant acts as a biosynthetic laboratory, for its chemical compounds and most are derivatives of a few biochemical motifs. These include alkaloids, phenolics, turpenoids and glycosides. The use of plants as medicine was documented in the Babylonian circa 1770 BC in the code of Hammurabi and in ancient Egypt circa 1550 BC. The ancient Egyptians believed that medicinal plants to have utility even in the after life of their pharaohs (Anna, 1993). The use of medicinal plants has been recorded in old civilizations (Baqar, 2001). The manufacturers of allopathic and herbal medicines should conduct organized research on medicinal plants and thus save foreign exchange spend on their imports (Shinwari and Malik, 1989).

Pakistan has unique biodiversity on earth comprising of various climatic zones with variety of plant species. *Datura* plant is member of *Solanaceae* family, which comprises about 3,000 species (Bohs and Olmstead, 1997). Plant pathogenic fungi are causing extensive damage to crops resulting in yield losses (Al-Askar, 2012; Alwathnani and Perveen, 2012). Antifungal compounds obtained from the higher plants are known to be as important factors for controlling some plant diseases (Tapwal *et al*., 2011). In recent times, several scientists in the world express their attention in the application of plant product as bio- pesticide (Singh and Srivastava, 2013). The aim of current study was to investigate antifungal activity of *Datura inoxia* extracts.

MATERIALS AND METHODS

Plant material

The fresh matured plant of *Datura inoxia* was collected during the month of June from a natural population of university of Peshawar, KP Pakistan. The plant species were described by using standard morphological characteristic features according to flora of Pakistan.

Test microorganisms

The following five pathogenic fungal strains were used:

Aspergillus niger, Aspergillus flavus, Alternaria solani, Helianthus sporium and *Fusarium solani* were obtained from stock culture of Microbiological Research Laboratory, Centre of Biotechnology and Microbiology, University of Peshawar.

Fungal species were subcultured on Sabouraud Dextrose Agar plates from slanted stock culture stored at 4°C. For growth and maintenance of fungal culture SDA (Sabouraud Dextrose Agar) was used.

Preparation of extracts

Fresh leaves, stem, roots, and seeds of *D. inoxia* were collected, washed under running tap water, dried out and used for extraction. These plant parts were kept in dark room for drying at 37 ^{0}C for 15 to 20 days. Portions of the air dried leaves, stems, roots and seeds were extracted separated in methanol for the extraction of active compounds. The specimens were kept on shaker at room temperature for 15 days. The extracts were filtered and soluble extracts were collected in isolated flask. The solvents were dried by rotatory evaporator at 50 ºC for 1 hour. Finally 5 gram crude extract of leaves and seeds were obtained separately. Similarly 8 gm extracts from stem and roots were isolated separately (Hussain *et al.,* 2016). The extracts were kept in small sterilized plastic bottles at (-20°C) until use (Hoque *et al.,* 2008).

Antifungal activity test

SDA media were used for antifungal testing. 25 ml sterilized media were poured into sterilized plates. The plates were incubated for 24 hrs to check the sterility. The tested fungal species were inoculated on these plates to refresh the stock culture. SDA plates were used for the antifungal sensitivity test. Sterilized cork borer having 4mm diameter was used to make holes on SDA plates. Solutions of 2 mg of each extract was dissolved in 1 ml DMSO and poured in eppendorf tubes. Agar well diffusion method (Perez *et al.,* 1990; Alsohiby *et al.,* 2016) was employed. 10 µl solutions were pipette out and poured in wells to check the antifungal activities of each parts of *Datura inoxia* separately. The SDA plates were incubated at 25 ºC for seven days. DMSO was used as negative control and 2 mg of Griseofulvin dissolved in standard sterile distilled water was considered as positive control.

RESULTS AND DISCUSSION

Methanol extracts from stem, leaves, roots, and seeds *Datura inoxia* demonstrated antifungal activity. Among the fungal species, *Fusarum solani* was more sensitive as compared to others. The lowest growth was shown by *Fusarium solani* (35 mm) and highest growth by *Aspergillus niger* (75 mm) in SDA slants against leaf extracts (Table 1). The % age inhibitions of leaves extracts were calculated to be more against *F. solani* up to 79 % and less inhibition up to 30 % against *A. niger* (Table 2). The lowest growth was shown by *F. solani* (55 mm) and highest growth by *A. niger* (85 mm) in SDA slants against stem extracts (Table 1). The % age inhibitions of stem extracts were calculated to be more against *F. solani* up to 57.31 % and less inhibition up to 18.29 % against *A. niger* (Table 2). The lowest growth was shown by *F. solani* (30 mm) and highest growth by *A. niger* (80 mm) in SDA slants against root extracts (Table 1). The % age inhibitions of root extracts were calculated to be more against *F. solani* up to 85.36 % and less inhibition up to 24.39 % against *A. niger* (Table 2). The lowest growth was shown by *F. solani* (38 mm) and highest growth by *A. niger* (72 mm) in SDA slants against seed extracts (Table 1). The % age inhibitions of seed extracts were calculated to be more against *F. solani* up to 75.60 % and less inhibition up to 34.14 % against *A. niger* (Table 2). Our results of antifungal activity of methanol extracts of *Datura inoxia* are supported by previous studies (Shama *et al.,* 2014).

The methanol extracts of leaves, stem, roots and seeds against fungal species showed inhibition ranging from 85.36 to 18.29 %, in which *Fusarium solani* were more affected. *A. niger* showed more resistance. DMSO was used as negative control and Griseofulvin was used as a positive control (Table 2). The lowest growth was shown by *F. solani* (20 mm) and highest growth by *A. niger* (40 mm) in SDA slants against Griseofulvin (Table 1). The higher activity of antifungal drug than plant extracts is supported by the findings of Shama et al. (2014). These findings supported the use of plant parts for the treatment of wounds (Okigbo and Omodamiro, 2006).

Plants continue to contribute a myriad of natural products which discover wide range of applications in medicine and other products. In recent years the incidence of multiple resistances in human pathogenic microorganisms has been increasing day by day, mostly because of the indiscriminate use of antimicrobial drugs. The adverse side effects of certain antibiotic and the occurrence of formerly uncommon infections are a severe problem in medical field (Shito, 2001). That's why scientists have their keen interest to find out the new antimicrobial substances from different medicinal plants. The screening of plants for antimicrobial activity has demonstrated that higher plants are a probable source of novel antibiotic protypes (Grimes *et al*., 1996).

The inherited use of plants as a foundation of medicine is a crucial part of the health care system. Almost 20% of the plants present in the world have been evaluated for pharmacological or biological tests (Suffredini *et al*., 2004).

Al-Hakami et al. (2016) documented antimicrobial activity of Cinnamon barks (Aqueous and Ethanolic Extracts) against *Candida albicans*. Hussain et al. (2016) investigated the antibacterial activities methanolic crude plant extracts of leaves, stem, root and seeds of *Datura inoxia*.

Table 1. Antifungal activities of fungal isolates against extracts.

Compound	Growth on SDA (milimeter)				
	Aspergillus niger	***Aspergillus flavis***	***Alternaria solani***	***Heliantus spori***	***Fusarium solani***
Leaf Extracts	75mm	63mm	58mm	55mm	35mm
Stem Extracts	85mm	65mm	80mm	72mm	55mm
Root Extracts	80mm	70mm	68mm	45mm	30mm
Seed Extracts	72mm	65mm	68mm	65mm	38mm
DMSO	90mm	80mm	85mm	80mm	80mm
Antifungal	40mm	45mm	40mm	30mm	20mm

Table 2. Calculated % inhibition zones.

Compound	Inhibition zones (% age)				
	Aspergillus niger	***Aspergillus flavis***	***Alternaria solani***	***Heliantus spori***	***Fusarium solani***
Leaf Extracts	30.48	45.12	51.21	54.87	79.26
Stem Extracts	18.29	42.68	24.39	34.14	57.31
Root Extracts	24.39	36.58	39.02	67.07	85.36
Seed Extracts	34.14	42.68	39.02	42.68	75.60

CONCLUSION

The methanol extracts of *Datura inoxia* showed activities against tested fungal species. It was concluded that medicinal plants have a huge contribution to the traditional and western medicines by granting ingredients for drugs or having played major roles in the drug discoveries.

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CONFLICT OF INTEREST

The authors declare that this article content has no conflict of interest.

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