

IN VITRO ANTIMICROBIAL EFFICACY OF *DATURA INNOXIA* LEAF EXTRACT AGAINST GRAM POSITIVE BACTERIA

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ABSTRACT

Datura innoxia leaves were examined for potential antibacterial activity by preparing their crude aqueous and Ethanol extracts against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). The results of Disc diffusion method indicated that the pattern of inhibition depends upon the plants part, solvent used for extraction and the organism tested. Extracts prepared from leaf were shown to have better efficacy. Ethanol extracts provided potent antibacterial activity as compared to aqueous extracts. Ethanolic extract showed the antibacterial activity against gram positive bacteria. Gram-positive bacteria *Bacillus subtilis* were found most sensitive as compared to *Staphylococcus aureus*. This study concluded that *Datura innoxia*

leaf may present a potentially active antibacterial agent in near future.

KEYWORDS: *Datura innoxia*, Antibacterial activity, Drug resistance, Sterile Disc, Minimum inhibitory concentration.

INTRODUCTION

Antibiotics are one of most important weapons in fighting bacterial infections and have greatly benefited the health related quality of human life since their introduction. About 61% of new drugs developed between 1981 and 2002 were based on natural products and they are successful especially in infectious diseases.

Plants are major source of herbal medicines and the presence of secondary metabolites in plants implicated them for many therapeutic activities. It is estimated that there are 250,000 to 500,000 species of plants on Earth. Plants are used medicinally in different countries and

area source of many potent and powerful drugs (Semara *et al.*, 2006 & Suffredini *et al.*, 2006). A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources. In recent years, several researchers have reported that the alkaloids, phenolics, triterpenoids, glycosides, tannins, etc. are the major bioactive molecules from plant origins which have enormous potential to inhibit microbial pathogens (Hutchings *et al.*, 1996 & Karamanoli, K 2002). The most important bioactive constituents of these plants are alkaloids, tannins, flavonoids and phenolic compounds (Kumar *et al.*, 2007). A number of plants have been reported as anticancer, antiviral, antibacterial and antiamoebal agent (Ahn, 1994, Silva *et al.*, 1996, Iwu, 1999, Iwu *et al.*, 1999, & Scheck *et al.*, 2006).

One of the surveys conducted by the WHO reports that more than 80% of the world's population still depends upon the traditional medicines for various diseases (Priya *et al.*, 2002 & Steenkamp *et al.*, 2004). These plants have been reported as anticancer, antiviral, antibacterial and antiamoebal agents (Ahn, 1994, Silva *et al.*, 1996, Iwu, 1999, Iwu *et al.*, 1999 & Scheck *et al.*, 2006). Past two decades, Antibacterial properties of various plants and plant parts like root, stem, leafs, seeds and flowers have been well documented for some of the medicinal plants (Nandagopal *et al.*, 2007, Parekh & Chanda, 2007, Akinpelum & Onakoya 2006). Some plants have shown the ability to overcome resistance in such organisms which led the researchers' to isolate active principles and investigate mechanisms. A number of studies have been conducted for the selection of the crude plant extracts in a therapeutic treatment of bacterial infections (Ikram and Inamul, 1984, Izzo *et al.*, 1995, Bhattacharjee *et al.*, 2006). World Health Organization (WHO) has also suggested that the medicinal plants would be the best source for obtaining a variety of drugs (Basso *et al.*, 2005).

The present investigation was carried out with the major objective of antimicrobial screening of leafs of *Datura innoxia*, against gram positive Bacteria (*Bacillus subtilis* & *Staphylococcus aureus*).

MATERIALS & METHOD

Collection of Raw materials

Fresh plants of *Datura innoxia*, were collected in April 2012. These plants identification were confirmed by the Botanical taxonomist, in Gwalior. Then these plants were washed thoroughly 2- 3 times with running tap water and then sterile water and kept at room temperature in Departmental laboratory for 10 days for drying. After drying, all parts were to

be separated from plants. The dried parts of plants were pulverized into powdered form with the help of pestle and mortar. The powders of all plants are preserved in the boxes for use in making extraction.

Test Microorganism

The bacterial strains selected for present study were collected from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTech), Chandigarh, India. Gram positive Bacteria, *Bacillus subtilis* and *Staphylococcus aureus* were screened for present investigation. These bacterial cultures were maintained in nutrient agar slants at 37°C. Each of the microorganisms was reactivated prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth and incubated overnight at 37°C.

Chemical & reagents

The chemicals and media used for this study were from Hi Media, Qualigens and Merck and the glasswares from Borosil.

Extract Preparation

Aquoes Extract

Weigh the 10gm of shade, dried plant material were macerated with 100 ml sterile distilled water in a warring blender for 10 minutes. The macerated were first filtered through double layered muslin cloth. Then this extract was centrifuged at 4000g for 30 minutes. Supernatant was filtered through what man no.1 filter paper and sterilized at 120°C for 30 minutes. The extract was preserved aseptically in a brown bottle at 5°C until further use.

Ethanol Extract

Weigh the sample (10gm) in conical flasks with cotton plug which containing 100 ml of 95% ethanol solvent. Flask was kept in shaking incubator for overnight shaking at 35° Celsius at 110 rpm. After overnight shaking, filtration was done with the help of what man no. 1 filter paper into another clean conical flask. After it, filtrate was subjected to centrifugation at 7000 rpm at 18°C for 15 minutes. The supernatant of the leafs were collected into a separate clean air tight bottles and were stored in a refrigerator at 4°C for further antimicrobial testing.

Antibacterial Assay

In-vitro bacterial test was carried out by Disc Diffusion method. Tetracycline antibiotic discs were used as positive controls while corresponding extraction solution was used as negative

control. The discs of tetracycline were prepared by impregnating disc with 5mg/ml concentration and then left for air drying. Then disc of the plants samples were made by impregnating the discs with extraction of each sample and left for air drying. The discs for negative control were prepared by impregnating the discs with respective solvents used to prepare extracts. After then bacterial cultures were taken by loop and were spread on Muller hinton agar plates with the help of spreader. Then discs of the samples, disc of positive control and discs of negative control were placed on seeded agar plates. The inoculated plates were incubated at 37°C for 24 hours and antibacterial activities were calculated and evaluated by measuring the zone of inhibition against the tested bacteria.

RESULTS & DISCUSSIONS

Sample extracts of the plant parts were used and tested against two Gram's positive Bacteria namely, *Staphylococcus aureus*, & *Bacillus subtilis*. These extracts were: Aqueous and Ethanol extracts of leaf of *Datura innoxia*. Ethanol extract of *Datura innoxia* plants leaf shows good antimicrobial activity against *Staphylococcus aureus*, & *Bacillus subtilis*. The zone of inhibition is shown in Figure 1, II & Table I & II.

Table 1: Effect of Ethanolic and Aqueous Extracts of Sample of *Datura innoxia* Plants on *Bacillus subtilis*.

Name of Plants	Zone of Inhibition of Ethanol Extracts (In mm)	Zone of Inhibition of Aqueous Extracts (In mm)
Tetracycline (Positive Control)	35	35
Negative Control	-	-
<i>Datura Innoxia</i> Leaf	15	-

Table 2: Effect of Ethanolic and Aqueous Extracts of Sample of *Datura innoxia* Plants on *Staphylococcus aureus*.

Name of Plants	Zone of Inhibition of Ethanol Extracts (In mm)	Zone of Inhibition of Aqueous Extracts (In mm)
Tetracycline (Positive Control)	35	35
Negative Control	-	-
<i>Datura Innoxia</i> Leaf	13	-



Figure: 1. Zone of inhibition of Ethanol extract of Leaves of *Datura Innoxia* against *Bacillus subtilis*



Figure: 2. Zone of inhibition of Ethanol extract of Leaves of *Datura Innoxia* against *Staphylococcus aureus*

These studies reveals that the use of ethanol solvent in the preparation of plant extracts provides more consistent antibacterial activity while aqueous extract of *Datura innoxia* showed no significant activity. The extract nature and mode of action of the active constituents is quite obscure at this stage. Further work may however reveal whether these components act as intracellular bacterial enzyme inhibitor or impair the cell wall synthesizing system of the cell, or any other biological reaction destruction which causes inhibition of bacterial growth. This observation clearly indicates that the polarity of antibacterial compounds make them more readily extracted by ethanol solvent.

CONCLUSION

The present study demonstrates the antibacterial potential of crude extracts of *Datura innoxia* leafs. The results indicate that Ethanol extracts of this plant have significant antibacterial potential against the bacterial strains of clinical significance and observe the traditional medicinal value of the plant. The study concludes that the plants are a reserve of biologically

active substances. The extracts obtained by the plant can potentially be used in the treatment of infectious diseases caused by microorganisms that are showing emergence of resistance to currently available antibiotics. These studies also support the folkloric usage of the studied plants and suggest with antibacterial agents in new drugs for the therapy of infectious diseases caused by pathogens.

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