In vitro evaluation of *Datura innoxia* (thorn-apple) for potential antibacterial activity

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Abstract Various parts of *Datura innoxia* were examined for potential antibacterial activity by preparing their crude aqueous and organic extracts against Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*) and Gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus*). The results of agar well diffusion assay indicated that the pattern of inhibition depends largely upon the plant part, solvent used for extraction and the organism tested. Extracts prepared from leaves were shown to have better efficacy than stem and root extracts. Organic extracts provided potent antibacterial activity as compared to aqueous extracts. Among all the extracts, methanolic extract was found most active against almost all the bacterial species tested. Gram-positive bacteria were found most sensitive as compared to Gram-negative bacteria. *Staphylococcus aureus* was significantly inhibited by almost all the extracts even at very low MIC followed by other Gram-positives. For *Escherichia coli* (a Gram-negative bacterium), the end point was not reached for ethyl acetate extract while it was very high for other extracts. The study promises an interesting future for designing a potentially active antibacterial agent from *Datura innoxia*.

Keywords *Datura innoxia* · Antibacterial activity · Drug resistance · Agar well diffusion assay · Minimum inhibitory concentration

Introduction

Antibiotics are an essential part for combating harmful bacterial infections *in vivo*. During the last decade, infectious diseases have played a significant role in the death of millions around the world, especially in developing countries like India. Because of the mutagenic nature of bacterial DNA, the rapid multiplication of bacterial cells, and the constant transformation of bacterial cells due to plasmid exchange and uptake, pathogenic bacteria continue to develop antimicrobial resistance, thus rendering certain antibiotics useless. An increased number of pathogens have also developed resistance [1, 2] to multiple antibiotics (Multiple Drug Resistance), threatening to develop complete immunity against all antimicrobial agents and therefore be untreatable. Thus, the search for novel antimicrobial agents is of the utmost importance.

Plants have been used for centuries as remedy for human diseases because they contain components of therapeutic values [3]. They are natural sources of antimicrobial agents primarily because of the large biodiversity of such organisms and the relatively large quantity of metabolites that can be extracted from them. The acceptance of these traditional medicines as an alternative form of health care has led researchers to investigate the antimicrobial activity of medicinal plants [4].

A lot of work has been done which aim at knowing the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections as possible alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant [5, 6].

India is one of the richest countries in the world with regard to diversity of medicinal plants [7]. Ayurveda, a

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form of Indian traditional medicine, mentions several plant species and outlines numerous medicinal uses for each. Extracts of these plants have served as medicine for thousands of years. Modern science has identified several secondary metabolites of various plant species that contain antimicrobial properties. *Datura innoxia* (thorn-apple), an Ayurvedic plant which was selected to evaluate antibacterial properties in the present study, is a poisonous species of the family *Solanaceae* native to North America. *Datura* plant has been found to contain a number of extremely toxic alkaloids; namely atropine, hyoscyne, hyoscyamine, and scopolamine [8]. Despite the plant's high toxicity, at appropriate doses of its extracts have been found to have medicinal effects recognized in traditional medicines around the world. In the present study, crude extracts of various parts *viz.*, leaves, stem, and root of *Datura* plant were prepared in different solvents and screened for their potent antibacterial activities. The study was further extended to determine the minimum inhibitory concentration(s) of active crude extracts against the susceptible bacterial species.

Materials and methods

Plant Collection

Datura plant was collected in late July and early August of 2006 from semi-arid, unshaded land of Ghaziabad (NCR), India. Fresh plant materials were washed under running tap water followed by sterilized distilled water, air-dried and then powdered with the help of sterilized pestle and mortar. The powders were further subjected for different extraction protocols as given below:

Aqueous extraction

10 g of air-dried powder of respective plant part *viz.*, leaves, stem or root was boiled in 400 ml distilled water till one fourth of the extract initially taken was left behind after evaporation. The solution was then filtered using muslin cloth. Filtrate was centrifuged at 5000 rpm for 15 min. The supernatant was again filtered using Whatman Filter No. 1 under strict aseptic conditions and the filtrate was collected in fresh sterilized bottles and stored at 4°C until further use.

Organic solvent extraction

10 g of air-dried powder was thoroughly mixed with 100 ml organic solvent (*viz.*, Ethanol, Methanol, Hexane or Ethyl acetate). The mixture was placed at room temperature for 24 h on shaker with 150 rpm. Solution was filtered through

muslin cloth and then re-filtered by passing through Whatman Filter No. 1. The filtrate thus obtained was concentrated by complete evaporation of solvent at room temperature to yield the pure extract. Stock solutions of crude extracts for each type of organic solvent were prepared by mixing well the appropriate amount of dried extracts with dimethyl sulphoxide (DMSO) to obtain a final concentration of 100 mg/ml. Each solution was stored at 4°C after collecting in sterilized bottles until further use.

Bacterial strains

The bacterial strains selected for present study were collected from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTech), Chandigarh, India. A total of five bacteria namely *Escherichia coli* and *Salmonella typhi* (both are Gram-negative bacteria) and *Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus* (all Gram-positive) 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.

Antibacterial assay

In vitro antibacterial activities of all aqueous and organic extracts of different parts of *Datura* plant were determined by standard agar well diffusion assay [9]. Petri dishes (100 mm) containing 18 ml of Mueller Hinton Agar (MHA) seeded with 100 µl inoculum of bacterial strain (inoculum size was adjusted so as to deliver a final inoculum of approximately 10⁸ CFU/ml). Media was allowed to solidify and then individual Petri dishes were marked for the bacteria inoculated. Wells of 6 mm diameter were cut into solidified agar media with the help of sterilized cup-borer. 100 µl of each extract was poured in the respective well and the plates were incubated at 37°C for overnight. DMSO and sterilized distilled water were used as negative control while tetracycline antibiotic (one unit strength) was used as positive control. The experiment was performed in triplicate under strict aseptic conditions and the antibacterial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced by the respective extract at the end of incubation period.

Determination of minimum inhibitory concentration

Extracts producing an inhibition zone ≥ 15 mm in diameter were screened to determine minimum inhibitory concen-

trations (MICs) by standard two-fold microbroth dilution methodology given by NCCLS [10]. A stock solution of each active extract was serially diluted in 96-wells microtiter plate with Mueller Hinton broth to obtain a concentration ranging from 0.50 µg/ml to 1024 µg/ml. A standardized inoculum for each bacterial strain was prepared so as to give inoculum size of approximately 5×10^5 CFU/ml in each well. Microtiter plates were then kept at 37°C for an overnight incubation. Following incubation, the MIC was calculated as the lowest concentration of the extract inhibiting the visible growth of bacterial strain. All the chemical ingredients used in present study were of analytical grade, and were purchased from Hi Media, India.

Results and discussion

Predictions of antibacterial activity in herbal compounds extracted from plant parts depend largely upon the type of solvent used for extraction. Table 1 shows the antibacterial activity in terms of zone of inhibition (in mm diameter) of dry powder extracts in aqueous and organic solvents from different parts (leaves, stem and root) of *Datura innoxia*.

Data indicated that extracts prepared from leaves exhibited better antibacterial activities than those prepared from stem and root. Leaf extracts were shown to have the zone of inhibition ranging from 9.11 mm to 26.76 mm. These

Table 1 *In vitro* antibacterial activity of aqueous and organic extracts of *Datura innoxia*

Extraction Type		Zone of Inhibition* (in mm diameter)				
		<i>E. coli</i>	<i>S. typhi</i>	<i>B. cereus</i>	<i>B. subtilis</i>	<i>S. aureus</i>
Leaf Extracts	Ethanol	12.64	–	17.56	15.33	23.83
	Methanol	17.11	15.22	19.43	18.55	26.76
	Ethyl Acetate	14.32	–	14.19	12.23	18.12
	Aqueous	–	–	10.75	09.11	13.23
Stem Extracts	Ethanol	–	–	11.32	08.10	12.26
	Methanol	10.21	–	12.52	10.12	13.19
	Ethyl Acetate	–	–	09.32	08.87	11.66
	Aqueous	–	–	–	–	12.98
Root Extracts	Ethanol	–	–	12.73	–	12.98
	Methanol	10.43	–	–	–	12.11
	Ethyl Acetate	–	–	–	–	10.22
	Aqueous	–	–	–	–	–
Positive Control	Tetracycline	35.62	33.29	26.71	22.97	30.46
Negative Control	Distilled Water	–	–	–	–	–
	DMSO	–	–	–	–	–

*Zone of inhibition (in mm diameter) including the diameter of well (6 mm) in agar well diffusion assay. Assay was performed in triplicate and results are the mean of three values. In each well, the sample size was 100 µL. Tetracycline: One unit strength.

Microorganisms: *E. coli* - *Escherichia coli*; *S. typhi* - *Salmonella typhi*; *B. cereus* - *Bacillus cereus*; *B. subtilis* - *Bacillus subtilis*; *S. aureus* - *Staphylococcus aureus*

Table 2 Minimum inhibitory concentration (MIC) of active extracts of *Datura innoxia*

Test Agent		Minimum Inhibitory Concentration* (µg/ml)			
		<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>B. cereus</i>
Leaf Extracts	Ethanol	>1024	256	512	512
	Methanol	512	<128	256	256
	Ethyl acetate	ND**	256	>1024	512

*Minimum inhibitory concentrations (µg/ml) of active extracts screened from agar-well diffusion assay against sensitive bacterial species by microbroth dilution assay standardized by NCCLS.

**ND-Not Detected.

Microorganisms: *E. coli* - *Escherichia coli*; *S. aureus* - *Staphylococcus aureus*; *B. subtilis* - *Bacillus subtilis*; *B. cereus* - *Bacillus cereus*.

extracts were found more inhibitory for Gram-positive bacteria as compared to Gram-negatives. Among all the bacterial strains tested, *Staphylococcus aureus* was found most susceptible with maximum inhibition by methanolic extract producing zone of inhibition >25 mm. Stem and root extracts were found almost ineffective in inhibition of bacterial strains producing zone of inhibition ≤ 13.19 mm.

Staphylococcus aureus (a Gram-positive bacterium) was found susceptible to various plant extracts evaluated in different studies such as methanolic extract of *Psidium guajava* [11], *Verbascum* sp. [12, 13], *Solanum tomentosum* [14], *Curcuma longa* (turmeric) [15, 16]. In another study, *Psidium guajava* (Goiaba) leaf extract found to have significant antimicrobial activity against *Staphylococcus aureus* [17].

Extracts prepared in organic solvents consistently displayed better antibacterial activity than that of aqueous extracts. Furthermore, extracts prepared in methanol was observed most inhibitory (diameter of zone of inhibition ranging from 15.22 mm to 26.76 mm) followed by those prepared in ethanol and ethyl acetate, respectively. Aqueous extracts of leaves were found inhibitory only against Gram-positive bacteria with a very little diameter of zone of inhibition (<14mm). Tetracycline (a positive control) showed inhibition diameters ranging from ~23 mm to 35 mm against all test microorganisms. Control experiments using sterile distilled water and DMSO (negative control) showed no inhibition of any bacteria.

Active extracts thus obtained (leaf extracts prepared in organic solvents) were further subjected for determination of minimum inhibitory concentration (MIC) by two-fold microbroth dilution method against susceptible bacterial species viz., Gram-positive *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* and Gram-negative *Escherichia coli*. Table 2 indicated that methanolic extracts was found significant inhibitor than other extracts. MIC of this extract was <128 $\mu\text{g/ml}$ against *Staphylococcus aureus* while it was 256 $\mu\text{g/ml}$ against other two Gram-positive bacteria and 512 $\mu\text{g/ml}$ against Gram-negative *Escherichia coli*. The next potent inhibitor was ethanol extract with MIC ranging between 256 $\mu\text{g/ml}$ and 512 $\mu\text{g/ml}$ for Gram-positive bacteria. The concentrations of ethanolic extracts tested were not enough to inhibit *Escherichia coli* (MIC >1024 $\mu\text{g/ml}$). Ethyl acetate extract were found suitable for inhibition of *Staphylococcus aureus* with MIC 256 $\mu\text{g/ml}$ and 512 $\mu\text{g/ml}$ for *Bacillus cereus*. MIC was not observed for this extract against *Escherichia coli*. Furthermore, Gram-positive bacterial species were found most sensitive as compared to Gram-negatives.

Our results are supported by one of the work in which the antibacterial activity of the methanol extracts of the

aerial parts of the *Datura innoxia* and *Datura stramonium* was investigated by the paper disk diffusion method using 25 μl of 80% methanol solutions having 2.5, 1.25 and 0.725 mg/ml concentrations. The extracts showed marked activity against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) in dose-dependent manner. No activity was found against *Escherichia coli* and *Pseudomonas aeruginosa* [18] in contrast to our study where ethanol and methanol extracts showed the activity against *Escherichia coli* but comparatively at higher concentrations than that of Gram-positive bacteria.

The present study reveals that the use of organic solvents in the preparation of plant extracts provides more consistent antibacterial activity as compared to aqueous extracts. This observation clearly indicates that the polarity of antibacterial compounds make them more readily extracted by organic solvents, and using organic solvents does not negatively affect their bioactivity against bacterial species. The data also showed that some antimicrobial substances could only be extracted by organic solvents, suggesting that organic solvents are clearly better solvents of antimicrobial agents.

Furthermore, Gram-positive bacteria were found more susceptible than Gram-negative bacteria. This is probably due to the differences in chemical composition and structure of cell wall of both types of microorganisms.

All antimicrobial activities occurred in a concentration-dependent manner as suggested by MIC determination, however, the efficacy of extracts are lesser than to that of standard antibiotic, tetracycline.

In the present study, we evaluated the antibacterial potential of various crude extracts of different parts of *Datura innoxia*. The results indicated that extracts of this plant have significant antibacterial potential against the bacterial strains of clinical significance and confirms the traditional medicinal value of the plant. 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. Furthermore, active plant extracts can be subjected to various pharmacological evaluations by several methods such as NMR (nuclear magnetic resonance), Mass Spectrometry etc. for the isolation of the therapeutic antimicrobials.

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