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ANTIBACTERIAL SCREENING OF *DATURA INOXIA* AGAINST MULTI-DRUG RESISTANCE STRAINS

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ABSTRACT

The development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has necessitated the search for new antibiotic from natural sources. Keeping this in view, we have studied the antibacterial effect of *Datura Inoxia* leaf by preparing their hexane, ethanol and aqueous extracts against multi-drug resistance strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The results of agar well diffusion assay indicated that the ethanol extract were most susceptible against *Staphylococcus aureus*, while aqueous extract were found to be ineffective against both the strains. This study has shown that the *Datura Inoxia* can act as promising plant for developing new antibiotics (herbal antibiotics) to Overcome the bacterial resistance problems worldwide.

KEYWORDS: *Datura Inoxia*, multi-drug resistance, agar well diffusion assay, herbal antibiotic.

INTRODUCTION

Multi Drug Resistance (MDR) is a condition enabling disease causing microorganism (bacteria, virus, fungi or parasites) to resist distinct antimicrobials. It is a worldwide problem in clinical medicine affecting our global health system. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the two well known MDR strains now a days. *Staphylococcus aureus* is a gram positive bacterial strain frequently found on our skin microflora and in the upper respiratory tract. [1] The strain is not always pathogenic, but it may sometimes be the root cause of skin infections, respiratory disease and food poisoning. Some *Staphylococcus* bacteria such as MRSA (methicillin resistance *Staphylococcus aureus*) are resistance to

certain antibiotics, making infections harder to treat. [2] *Pseudomonas aeruginosa* is a gram-negative bacteria, usually found in environments such as soil, water, humans, animals, plants, sewage and hospitals. [3] In addition, *Pseudomonas aeruginosa* is an opportunistic human pathogen that causes chronic infections in patient with cystic fibrosis. [4] Due to their intrinsic resistance, the strain exhibit resistant to many antibiotics and chemotherapeutic agents, making it very difficult to eliminate. [3] Plants have been used for centuries to treat variety of human diseases as they contain components of therapeutic values. [5] There are many medicinal plants which possess antibacterial activity. [6] *Datura Inoxia* (Family: Solanaeceae) is one of the good source of antibacterial medicinal plant which has been widely used as phytomedicine to cure various diseases. [7, 8] The species was first described by English botanist Philip Miller in 1768 and commonly called as thorn-apple, moonflower, sacred *Datura* etc. Present study was investigated to identify the antibacterial effects of *Datura Inoxia* leaf extracts against multi-drug resistance strains (*Staphylococcus aureus* and *Pseudomonas aeruginosa*).

MATERIALS & METHODS

Sample Collection and Preparation of Plant Extracts

Datura Inoxia was collected from nearby area of Amity University Campus, Lucknow. The plant leaves were washed thoroughly with tap water followed by distilled water to remove the dust particles and allowed to air dry on room temperature. The plant leaves were grinded to powder with the help of liquid Nitrogen and stored at -20°C till further use. For preparing extract, 5 gm of dry leaf powder was mixed with 50 ml hexane (Merck, India), ethanol and distilled water. The mixture was macerated in mortar and pestle and kept for 48 hr, to ensure maximum metabolite extraction. The final concentration was maintained as 100mg/ml by dissolving the dried plant extracts in 99.9% dimethyl sulfoxide (DMSO).

Antimicrobial Activity Assay [9, 10, 11]

The agar gel diffusion method was carried out on Muller Hinton Agar plates to assess antimicrobial activity assay. The media were prepared as per the supplier's instructions and sterilized by autoclaving at 121^{o}C for 15 min. Lyophilized cultures of *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 were obtained from Hi Media Lab., India and maintained by subculturing on blood and macconkey's agar plates. The pure isolates of bacteria were diluted with 0.9% normal saline solution before applying onto the plates to meet the 0.5 McFarland turbidity standards. The plates were then inoculated with the bacterial suspensions using

sterile swab sticks dipped in it. The swab sticks must be pressed firmly against the wall of the tube to avoid taking too much colonies and was swabbed onto the surface of the agar three times in the different directions by rotating the plate each time to ensure that the bacteria distribute evenly on the agar. Next, on these plates, wells of 6 mm size were dug with the help of a sterile cork borer. Each plate had three wells at equal distance. For each pathogen as well as for plant sample, separate plates were prepared. In each plate, 100 µl of the hexane, ethanol and aqueous extracts were loaded. These plates were then incubated for 24 hr at 37°C. After incubation period, the antibacterial activity was expressed in terms of zone of inhibition (diameter in mm). Each experiment was repeated three times and mean of all values was taken. Commercial antibiotic discs for the strains- Gentamycin and Azithromycin (HiMedia, India; for *S. aureus*) and Gentamycin and Ceftazidime (HiMedia, India; for *P. aeruginosa*) were used as positive control while DMSO were used as negative control to compare the antibacterial activity of the extracts.

RESULTS AND DISCUSSION

Antibacterial susceptibility studies of hexane, ethanol and aqueous extracts of *Datura Inoxia* leaf against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, are summarized in Table 1. The aqueous and hexane extracts of *Datura Inoxia* leaf did not inhibit the growth of the isolates of *Pseudomonas aeruginosa*, as there was no zone of inhibition, signifying that strains of *Pseudomonas aeruginosa* tested are resistant to it despite good agar diffusion, but its ethanol (17 mm) extract showed moderate antibacterial activity and represented as ++ sign. *Pseudomonas aeruginosa* isolates were susceptible to standard antibiotic discs tested (Ceftazidime- 20 mm and Gentamycin- 32 mm) with appreciable zone of inhibition. Other workers have also demonstrated the antibacterial effect of ethanolic extract of *Datura* species against *Pseudomonas aeruginosa*. [12, 13]

The hexane and ethanol extracts of the plant showed moderate to strong antibacterial activity against *Staphylococcus aureus* with zone of inhibition measured as hexane extract (16 mm) and ethanol extract (25 mm), represented as ++ and +++ sign, while aqueous extract were found resistant to the strain. The isolates of *Staphylococcus aureus* showed mild to moderate antibacterial activity against Azithromycin (15 mm) and Gentamycin (20 mm). Negative control (DMSO) was found ineffective against the tested strains. Similar results were also observed by Gachande and khillare (2013), [14] they also showed the antibacterial activity in ethanolic extract of *Datura Inoxia* leaf, but no activity in its aqueous extract against *S.*

aureus. It is clear from the present study that organic solvents are better in extracting compounds for antimicrobial activities compared to water based method. [15]

Table 1: Antibacterial Activity of *Datura Inoxia* Leaf Extracts Against Pseudomonas Aeruginosa and Staphylococcus Aureus.

Test organisms	Zone of Inhibition (mm)						
	Standard antibiotic discs			Extracts (mg/ml)			DMSO (99.9%)
	CAZ (30µg)	AZM (15µg)	G (10µg)	Hexane	Ethanol	Aqueous	
Pseudomonas aeruginosa	20	-	32	-	17	-	-
Staphylococcus aureus	-	15	20	16	25	-	-

Avg. ZOI :< 15mm (+ or mild); <25mm (++ or moderate); >25mm (+++ or strong)

CAZ- ceftazidime, AZM- azithromycin, G- gentamicin

DMSO- dimethyl sulfoxide

CONCLUSION

From the present study we conclude that ethanolic extract of *Datura Inoxia* leaf were came out to be more effective against the strains tested, which is a positive sign to develop new antibiotic for solving multi-drug resistance problems. The future prospects of present research work will include purification of extract and its characterization by several methods like high performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), gas chromatography-mass spectroscopy (GC-MS) and electrospray ionization tandem-mass spectroscopy (ESI-MS).

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