IN-VITRO EVALUATION OF DATURA SPECIES FOR POTENTIAL ANTIMICROBIAL ACTIVITY

Gachande B D and E M Khillare

Department of Botany, N. E. S. Science College, Nanded.
gachandebhagwat@gmail.com

ABSTRACT

Various parts of four *Datura* plant species *viz.D.ferox, D.inoxia, D.metal* and *D.stramonium* were examined for their potential antimicrobial activity in aqueous and ethanolic extracts against pathogenic bacteria such as *Bacillus subtilis*-2699, *Escherichia coli*-2803, *Staphylococcus aureus*-2602, *Proteus vulgaris*-2027, *Salmonella typhi*-2501; and pathogenic fungi such as *Aspergillus flavus*- 525, *Aspergillus niger* (local culture), *Candida albicans*-3100 and *Rhizopus stolonifer* (local culture).The results of agar well diffusion assay indicate that the pattern of inhibition depends largely upon plant part used and organism tested. The ethanolic extracts were showed good inhibitory activity than the aqueous extracts. Extract prepared from leaves were shown to have better efficacy than stem, and root.

Key words: *Datura* sp., aqueous and ethanolic extracts antimicrobial activity.

INTRODUCTION

Datura also known as thorn apple, prickly burr, jimson weed, moon flower, devil's weed, devil's cucumber and devil's trumpet. It is a member of the family Solanaceae (Nightshade family). All *Datura* plants contain tropane alkaloids such as scopolamine, hyoscyamine, and atropine, primarily in their seeds and flowers (Preissel and Preissel, 2002). Because of the presence of these substances, *Datura* has been used for centuries in some cultures as a poison and as a hallucinogen (Preissel and Preissel, 2002; Adams and Garcia, 2005). It may be mixed with other psychoactive agents, such as the San Pedro cactus in its traditional use in South America (Bussmann and Sharon, 2006). There can be a 5:1 toxin variation across plants, and a given plant's toxicity depends on its age, where it is growing, and the local weather conditions. There are also several reports in the medical literature of deaths from *Datura stramonium* and *Datura ferox* intoxication (Michalodimitrakis and Koutselinis, 1984; Boumba *et al.,* 2004; Steenkamp *et al.,* 2004).

Use of plants as a source of medicine has been inherited is an important component of the health care system. There are about 45,000 plant species in India. The official documented plants with medicinal potential are about 3,000 but traditional practitioners use more than 6,000 plants. India is the largest producer of medicinal herbs and is appropriately called the Botanical garden of the world (Ahmedulla & Nayar, 1999). Approximately 20% of the plants found in the world have been submitted to pharmacological or biological test (Suffredini *et al.,* 2004). The systemic screening of antimicrobial plant extracts represents a continuous effort to find new compounds with the potential to act against multiresistant fungi & bacteria. In the present study, *in-vitro* antibacterial and antifungal activity of plant extracts from *Datura* sp. was studied.

MATERIALS AND METHODS

Collection and maintenance of micro-organisms:

Pathogenic bacteria such as *Bacillus subtilis*-2699, *Escherichia coli*-2803, *Staphylococcus aureus*-2602, *Salmonella typhi*-2501 and *Proteus vulgaris*-2027; pathogenic fungi such as *Aspergillus flavus*-525, *Candida albicans*-3100 obtained from National Collection of Industrial Microorganisms (NCIM), Pune and *Aspergillusniger*(local culture), *Rhizopusstolonifer*(local culture) were used as test organisms. Bacterial strains were maintained in nutrient broth and sub-cultured on nutrient agar slants.

Fungal pathogens i.e. *Aspergillus flavus*, *A, niger*and *Rhizopus stolonifer* were maintained on Potato dextrose agar (PDA) slants and *Candida albicans* on MGYP medium (Yeast culture). Nutrient broth, nutrient agar and Potato dextrose agar (Hi-media, Mumbai) and other chemicals were obtained from qualigens (Mumbai) of analytical grade.

Collection of plant materials:

Fresh *Datura* plants belong to four species viz. *D. ferox* L. Amoen, *D. metel* L., *D. inoxia*Mill.Gard and *D. stramonium* L. were collected from different localities of Marathwada region. The leaves of *Datura* sp. were washed thoroughly with tap water and ones with distilled water and dried in cool, dark place. After complete drying the leaves were crushed to powder form. The powdered samples were hermetically sealed in separate polythene bags until the time of extraction.

Preparation of aqueous extracts:

Aqueous extraction was carried out by decoction process (Davis, 1956). This was carried out by boiling theleaf powder of *Datura*species in hot water, one part of dried powder and 5 parts of sterilized water were taken in boiling water flasks and boiled till one fourth of the extracts left behind after evaporation. After boiling the extracts were filtered through double layered muslin cloth. The filtrate was centrifuged at 5000 rpm for 15 min; the supernatant was again filtered using Whatman No. 1 filter paper under aseptic conditions. The filtrate was collected in fresh sterilized bottles and stored at 4° C until further use.

Preparation of solvent extracts:

Ethanolic extracts were prepared by soxhelet extraction. In this process 10 gm of dried powder from different plant parts of *Datura*sp. After completion of process the concentrated active constituents from plant materials were kept in sterilized glass bottles and stored at 4°C until further use. The traces of solvents and water were removed by keeping the bottles at 50°C in oven for 1 hr.

Determination of antibacterial activity:

Antibacterial activity of *Datura*plant extracts (aqueous and Ethanolic) was carried out by well diffusion method (Parez *et al.,* 1990). Pre-prepared nutrient agar plates were inoculated with test bacteria by spreading one loopful of bacterial inoculums (24 hr broth culture) on surface of the media. Five mm diameter wells were punched in the agar. 0.5 ml of aqueous extracts was added into the well. Well containing sterile distilled water act as negative control for aqueous extracts. Ethanolicextracts (10mg) were mixed with 1 ml of Dimethyl sulfoxide (DMSO) and 0.5 ml of this solution was added into the well. Well containing DMSO alone act as negative control for ethanolic extracts and antibiotic streptomycin (1mg/1ml) act as positive control. The plates were incubated at 37±2C° in bacteriological incubator for 48 hr. The zone of inhibition was measured and recorded.

Determination of antifungal activity

Antifungal activity of *Datura*plant extracts (aqueous and ethanolic) was carried out by well diffusion method (Parez *et al.,* 1990). Pre-prepared PDA plates were inoculated with test fungi (*Aspergillus flavus*, *A. niger* and *Rhizopus stolonifer*) and MGYPA (yeast culture) plates were inoculated with test fungus *Candida albicans* by spreading 1 ml of standard spore suspension on surface of the media. Five mm diameter wells were punched in the agar. 0.5 ml of aqueous extracts was added into the well. Well containing sterile distilled water act as negative control for aqueous extracts. Solvent extracts (10mg) were mixed with 1 ml of Dimethyl sulfoxide (DMSO) and 0.5 ml of this solution was added into the well. Well containing DMSO alone act as negative control and Fluconazole (0.1ml) act as positive control. The plates were incubated at room temperature (28±2C°) for72 hr. The zone of inhibition was measured and recorded.

RESULTS AND DISCUSSION

Results presented in Table-1 revealed that, the aqueous extracts of different parts (roots, stem and leaves) only leaves extracts were inhibited the bacterial growth in vitro. The bacteria *Bacillus subtilis* and *Eshcherichia coli* were highly sensitive to the leaf extracts of *Datura ferox* as compared to other species. There is no antifungal activity was observed in case of aqueous extracts the leaf extracts *of D. ferox, D.metal* and *D.strsmonium* were effective against *B. subtilis* and *E.coli* where as leaf extract of *D.inoxia* was effective to *S.typhi*. Antimicrobial activity of ethanolic extracts of *Datura* species were depicted in Table-2. It is clear from the results that, the root extracts of *D. ferox* showed maximum zone of inhibition against *B. subtilis* (22mm) and *Staphalus aureus* (18 mm). Whereas the root extract of *D. metal* and

Table 1: Antimicrobial activity of aqueous extracts of *Datura* species

Name of organism	Zone of inhibition (mm)													
	D. ferox			*D. inoxia*			*D. metel*			*D. stramonium*			Control (water)	Positive Control
	RT	ST	LS	RT	ST	LS	RT	ST	LS	RT	ST	LS		
Bacillus subtilis	-	-	20	-	-	-	-	-	16	-	-	16	-	30
Escherichia coli	-	09	21	10	-	-	09	-	18	08	-	10	-	40
Proteus vulgaris	-	-	-	10	-	13	-	-	-	-	-	-	-	41
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-	-	-	12
Salmonella typhi	-	-	-	-	-	16	-	-	11	-	-	-	-	32
Aspergillus flavus	-	-	-	-	-	-	-	-	-	-	-	-	-	60
Aspergillus niger	-	-	-	-	-	-	-	-	-	-	-	-	-	20
Candida albicans	-	-	-	-	-	-	-	-	-	-	-	-	-	22
Rhizopus stolonifer	-	-	-	-	-	-	-	-	-	-	-	-	-	45

Note: RT=root, ST=stem, LS=leaf; Positive control for bacteria-Streptomycin, for fungi-Fluconazole

Table 2: Antimicrobial activity of ethanol extracts of *Datura* species

Name of organism	**Zone of inhibition (mm)**													
	D.ferox			***D.inoxia***			***D.metel***			***D.stramonium***			**Control (DMSO)**	**Positive Control**
	RT	**ST**	**LS**	**RT**	**ST**	**LS**	**RT**	**ST**	**LS**	**RT**	**ST**	**LS**		
Bacillus subtilis	22	24	**27**	13	18	20	9	19	27	15	15	31	-	30
Escherichia coli	11	12	**25**	15	10	15	7	15	20	9	11	18	-	40
Proteus vulgaris	-	-	-	-	-	**12**	-	-	11	-	-	-	-	41
Staphylococcus aureus	18	16	20	12	12	**27**	21	15	25	20	17	24	-	12
Salmonella typhi	-	-	7	10	20	18	15	18	**22**	9	11	10	-	32
Aspergillus flavus	-	-	10	-	-	10	-	-	9	-	-	8	-	60
Aspergillus niger	-	-	-	-	-	5	-	-	-	-	-	-	-	20
Candida albicans	-	-	8	-	-	-	-	-	-	8	-	10	-	22
Rhizopusstolonifer	-	-	-	-	-	-	-	-	-	-	-	-	-	45

Note: RT=root, ST=stem, LS=leaves; Positive control for bacteria-Streptomycin, for fungi-Fluconazole

D. stramonium showed maximum zone of inhibition against *Staphaureus* (21 & 20 mm). The stem extracts of *D. ferox, D. inoxia* and *D. metal* showed maximum zone of inhibition against *B.subtilis* and *S.typhi.*The diameter of inhibition zone ranges in between 18-24 mm.The extracts of leaves of all four *Datura* sp. were found to be inhibitory against *B. subtilis, E. coli* and *Staph. aureus.* The leaf extracts of *D.inoxia* and *D. metal* were found effective against *S. typhi* and the diameter of inhibition zone ranges in between 18-31 mm.

The similar results were also observed by Rajesh and Sharma (2002) they were studied the antimycotic properties of *Datura metal* against different species of *Aspergillus*; Eftekhar *et al.* (2005) studied the antimicrobial activity of *Datura innoxia* and *D. stramonium* against *Escherichia coli* and *Pseudomonas aeruginosa*; Kaushik and Goyal (2008) studied *in-vitro* antibacterial activity of various parts of *Datura innoxia* by preparing aqueous and organic extracts against gram-negative bacteria i.e. *Escherichia coli* and *Salmonella typhi*; gram-positive bacteria i.e. *Bacillus cereus*, *B. subtilis* and *Staphylococcus aureus*; Satish *et al*., (2007) tested the aqueous extract of 52 plants from different families for their antifungal potential against eight important species of *Aspergillus* Among fifty-two plants tested, aqueous extract of *Acacia nilotica*, *Achraszapota*, *Datura stramonium*, *Emblica officinalis*, *Eucalyptus globules*, *Lawsonia inermis*, *Mimuso pselengi*, *Peltophorum pterocarpum, Polyalthia longifolia*, *Prosopis juliflora*, *Punica granatum*and *Sygigium cumini* have recorded significant antifungal activity; Sharma *et al.* (2009) studied antibacterial and antifungal activities of some common plants and weeds; they found weed plant *Datura stramonium* showed pronounced antibacterial and antifungal activity against *Escherichia coli, Staphylococcus aureus*, *Bacillus subtilis, Klebsiella pneumonia, Aspergillus niger and Candida albicans.*

Siva Sakthi *et al.* (2011) studied antibacterial evaluation and phytochemical screening of *Datura metal* leaf extracts against bacterial pathogens *viz. Escherichia coli, Salmonella typhi, Shigella flexeri, Klebsiella pneumonia, Vibrio cholera* and *Pseudomonas aeruginosa* in different organic solvents and reported that ethanol and ethyl acetate extract showed maximum zone of inhibition. In case of antifungal activity of four *Datura* species, only the leaf extracts of *D. ferox and D.stramonium* showed little antifungal activity.Benito Johnson *et al.* (2011) also reported that the alcoholic extracts displayed higher antibacterial and anti fungal activity than aqueous extract against *Staphylococcus, E.coli* and *Aspergillus species flavus* and *C. albicans*.

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