Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences
4
ISSN- 0975-1491 Vol 5, Issue 2, 2013

Research Article

ANTIMICROBIAL SCREENING OF SEQUENTIAL EXTRACTS OF *DATURA STRAMONIUM L.*

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Received: 29 Jan 2013, Revised and Accepted: 12 Mar 2013

ABSTRACT

The growing phenomenon of antibiotic resistance, particularly to pathogenic microorganisms, in contemporary medicine, has led the concern of scientists for finding novel antimicrobial agents. The present study was aimed to evaluate the *in vivo* and *in vitro* antimicrobial properties of different plant parts (alcoholic extracts), whole plants (extracted sequentially with different organic solvents) of *Datura stramonium.* For this, antimicrobial properties were tested against bacteria *Escherichia coli, Staphylococcus aureus*, *Pseudomonas aeruginosa* and the fungal strains *Aspergillus flavus*, *Aspergillus niger, Fusarium culmorum* and *Rhizopus stolonifer.* All the solvent extracts showed significant activity against all the tested microorganisms. Methanolic extract are most active against all micro-organisms whereas all the extracts showed significant activity against *P. aeruginosa.* All the solvent extracts were also evaluated for their Minimum Inhibitory Concentration (MIC). All the solvent extracts showed lowest MIC against *A.niger.* Owning to the results, it can be concluded that the extracts of the *D.stramonium* can be used to design different antimicrobial agents.

Keywords: *Datura stramonium*, Antibacterial, Antifungal, Minimum Inhibitory Concentration

INTRODUCTION

Plants are rich in a wide variety of secondary metabolite such as tannins, terpenoids, alkaloids and flavonoids etc, which have been found to exhibit antimicrobial properties [1]. The modern allopathic system of medicine is known to produce serious side-effects and moreover the microorganisms have developed resistance against many antibiotics due to the indiscriminate use of antibacterial drugs. This creates problems in the treatment of infectious diseases. 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 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. The acceptance of traditional medicines as an alternative form of health care has led researchers to investigate the antimicrobial activity of medicinal plants [2, 3].

Datura stramonium Linn (Solanaceae) grows as a wasteland weed [4, 5] mostly in the tropical and temperate areas of the world. It is commonly known as Dhatura, Ummatta, Kanak, Shivpriya, Dhustura, Jimsonweed, Jamestown weed, Thorn apple, Devil`s Trumpet. In India, *D. stramonium* is considered as valuable medicine and regarded as antispasmotic, intoxicant, emetic, digestive, acrid, astringent, germicidal, anodyne antipyretic, antiseptic, antiphlogistic, antiproliferative narcotic, sedative, tonic, febrifuge, antidiarrhoeal, antihelminthic, alexiteric and useful in the treatment of Leucoderma, skin disorders, ulcers, bronchitis, jaundice, hysteria insanity, heart disease, and for fever and piles [6,7, 8,9]. Protection against severe organophosphate toxicity could be offered by *Datura stramonium* seed extracts [10]. It is beneficial in chronic coughs, hard and dry with violet paroxyam and scanty expectoration [11]. Leaves are useful in the treatment of ear ache [12].

Datura species have been screened for their antifungal activity against several species by number of workers [13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29]. Although very small number of work have been done on the antibacterial activity of this medicinal plant, it need further study for verification of its activity against disease causing microorganisms.

The present paper deals with the evaluation of antimicrobial potential of the *Datura stramonium* Linn.

MATERIALS AND METHODS

Collection

The plant *D.stramonium* was collected from Shyam nagar extension, Sodala, Jaipur. It was washed with tap water, dried at room temperature and different plant parts ground to fine powder separately.

Identification

The species specimen was authenticated and submitted in herbarium, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India and got the voucher specimen No. RUBL15656.

Source of test organism

The bacterial strains *Escherichia coli* (MTCC 1652), *Staphylococcus aureus* MTCC(3160), *Pseudomonas aeruginosa* (MTCC 847) were procured from the microbial type culture collection (Institute of Microbial Technology, Chandigarh, India) and the fungal strains *Aspergillus flavus (*MTCC 2456), *Aspergillus niger (*MTCC 282), *Fusarium culmorum* (MTCC 349) and *Rhizopus stolonifer (*MTCC 2591) were collected from Plant Pathology laboratory, department of botany, University of Rajasthan, Jaipur, Rajasthan, India.

Culture of test microbes

For the cultivation of bacteria, Nutrient Broth Medium (NBM) was prepared using 8% Nutrient Broth (Difco) in distilled water and agar-agar and sterilized at 15 lbs psi for 25-30 mins. Agar test plates were prepared by pouring ~15 ml of NBM into the petri dishes (10 mm) under aseptic conditions. A peptone saline solution was prepared (by mixing 3.56 g KH_2PO_4 + 7.23 g NaH_2PO_4 + 4.30 g, NaCl + 1 g peptone in 1000 ml of distilled water, followed by autoclaving) and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37°C for 24 hrs. However, for the cultivation of fungi, Potato Dextrose Agar medium was prepared by mixing 100 ml potato infusion + 20 g agar + 20 g glucose, followed by autoclaving) and the test fungi were incubated at 27°C for 48 hrs and the cultures were maintained on same medium by regular subculturing. To prepare the test plates, in both bacteria and fungi, 10 to 15 ml of the respective medium was poured into the petridishes and used for screening. For assessing the bactericidal efficacy, a fresh suspension bacteria was prepared in saline solution from a freshly grown agar slant, while for fungicidal efficacy, a uniform spread of the test fungi was made using sterile swab.

Preparation of test extracts

Powdered 50 g of *D. stramonium* leaves was soxhlet extracted with petroleum ether (60-80°C), ethanol, methanol and water successively. Similarly, 20 g of different plant parts (fruit, root, stem, leaf and callus) were homogenised separately with methanol only [30] and left overnight at the room temperature. Later, each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, the extracts were pooled individually and dried in vacuo and stored at 4°C in a refrigerator, until screened for antibacterial activity.

Bactericidal and Fungicidal Assay

For both, bactericidal and fungicidal assays *in vitro* Disc diffusion method was adopted [31] because of reproducibility and precision. The different test organism were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs of whatman no. 1 paper (6 mm in diameter), which were containing 4 mg (or 0.4 ml) of the test extracts or control (0.4 g/ml) of the respective solvent or gentamycin (10 mg/ml) or mycostatin (100 units/ml) as reference separately. Such treated discs were air-dried at room temperature, to remove any residual solvent which might interfere with the determination, sterilized and inoculated. These plates were initially placed at low temperature for 1 hrs, so as to allow the maximum diffusion of the compounds from the test discs into the plate and later, incubated at 37°C for 24 hrs in case of bacteria and 48 hrs at 27°C for fungi, after which the zones of inhibition could be easily observed. Five replicates of each test extract were examined and the mean values were then referred.

The inhibition zone (IZ) in each case were recorded and the activity index (AI) was calculated as compared with those of their standard reference drugs (AI = inhibition zone of test sample/inhibition zone of standard).

Determination of MIC

Test for antibacterial activity

The antibacterial assay was carried out by microdilution method [32, 33, 34] in order to determine the antibacterial activity of compounds tested against the human pathogenic bacteria. The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^7 CFU/ml. The inoculum was prepared daily and stored at +4°C until use. Dilutions of the inoculum were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum. All experiments were performed in duplicate and repeated three times.

Microdilution test

The minimum inhibitory concentrations (MICs) were determined using 96-well microtitre plates. The bacterial suspension was adjusted with sterile saline to a concentration of 1.0 $X10^7$ cfu/ml. Compounds to be investigated were dissolved in broth LB medium (100 µl) with bacterial inoculums (1.0 X 10^5 cfu per well) to achieve the wanted concentrations (1 mg/ml). The microplates were incubated for 24 h at 48°C. The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The optical density of each well was measured at a wavelength of 655 nm by Microplate reader (Perlong, ENM8602) and compared with a blank and the positive control. Gentamycin was used as a positive control (1 mg/ml DMSO). All experiments were performed in duplicate and repeated three times.

Test for antifungal activity

The micromycetes were maintained on Potato dextrose agar and the cultures stored at 4°C and sub-cultured once a month. In order to investigate the antifungal activity of the extracts, a modified micro dilution technique was used [35]. The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0 – 10^7 in a final volume of 100 µl per well. The inoculums were stored at 4°C for further use. Dilutions of the inoculum were cultured on solid potato dextrose agar to verify the absence of contamination and to check the validity of the inoculum. Minimum inhibitory concentration (MIC) determinations were performed by a serial dilution technique using 96-well microtiter plates. The compounds investigated were dissolved in respective solvents (1 mg/ml) and added in potato dextrose broth medium with inoculum. The microplates were incubated for 72h at 28°C, respectively. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs.

RESULTS AND DISCUSSION

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [36]. The results of antimicrobial screening of sequential extracts of leaves and methanolic extracts of all plant parts are shown in Table 1.

Table 1: Bactericidal and fungicidal efficacy of sequential and methanolic extracts of *D. stramonium*

Microorganisms		Sequential extracts (Leaf)				Methanolic extracts				
		EtOH	MeOH	PE	Aqueous	Fruit	Stem	Root	Leaf	Callus
Bacteria										
	AI	0.918	0.875	0.68	0.779	0.744	0.829	0.631	0.875	0.696
E. coli	IZ	15.4+0.82	19.8+0.63	13.4+0.56	12.6+0.52	17.8+0.71	13.4+0.89	15.1+0.19	19.8+0.63	17.1 +0.07
MTCC 1652	AI	0.67	0.861	0.582	0.547	0.774	0.582	0.656	0.861	0.743
P. aeruginosa	IZ	22.4+0.86	22.2+0.59	18.9+0.11	20.7+0.52	11.9+0.43	13.3+0.56	12.8+0.62	22.2+0.59	21.3 +0.32
MTCC 647	AI	1.01	0.965	0.87	0.9	0.517	0.578	0.556	0.965	0.968
Fungi										
R. stolonifer	IZ	20.1+0.62	21.5+0.41	10.3+0.63	12.6+0.34	11.4+0.05	17.3+0.35	18.2+0.54	21.5+0.41	19.01+0.21
MTCC 2591	AI	0.957	1.02	0.49	0.6	0.542	0.823	0.87	1.02	0.901
A. niger	IZ	10.8+0.30	11.2+0.41	9.4 + 0.06	9.8 +0.42	8.4+0.65	9.1+0.23	7.3+0.63	11.2+0.41	12.1+0.02
MTCC 282	AI	0.843	0.875	0.734	0.765	0.656	0.71	0.57	0.875	0.945
F. culmorum	IZ	15.0+0.43	17.3+0.35	10.4+0.21	8.5 +0.35	9.5+0.80	10.3+0.45	14.5+0.25	17.3+0.35	18.9+0.07
MTCC 349	AI	0.84	0.969	0.584	0.476	0.532	0.577	0.812	0.831	1.059
A. flavus	IZ	11.3+0.22	12.1+0.72	07.5+0.06	9.2+0.91	10.4+0.56	8.5 +0.35	10.8+0.30	12.1+0.72	12.8+0.10
MTCC 2456	AI	0.83	0.889	0.551	0.676	0.764	0.625	0.794	0.889	0.941

IZ = Inhibition zone (in mm) including the diameter of disc (6 mm)

Activity index = $\frac{Inhibition\ area\ of\ the\ test\ sample}{Inhibition\ area\ of\ the\ Standard}$

Abbreviation: EtOH = Ethanol; MeOH = Methanol; PE = Petroleum ether; AQ = Aqueous; Standards : zentamycin = 100 unit per disc; Gentamycin = 10 mg/disc; mycostatin (100 units/ml)

Results are mean value SD from at least three experiment,

$S.E.(\sigma_x) = \frac{\sigma}{\sqrt{n}}$; σ=Standard deviation; n = no. of set

All the solvent extracts of *D.stramonium* inhibited the growth of all the seven micro-organisms tested. In the sequential extract of leaf, ethanolic extract showed greater bactericidal activity against *P. aeruginosa* and fungicidal activity against *R. stolonifer.* In plant parts and callus cultures maximum bactericidal activity was exhibited by leaf against *P. aeruginosa* and fungicidal activity against *R. stolonifer*. In the present investigation, all the extracts showed nearly similar considerable antimicrobial activity, indicating the suitability of these solvents for dissolving most of the bioactive compounds of plants responsible for the activity. Methanol extract is highly active against all the tested micro-organisms compared to other solvent extracts. Callus showed higher activity as compared to leaves against *A. flavus, A. niger* and *F. culmorum.*

MIC in microbiology is the lowest concentration of antimicrobials that will inhibit the visible growth of microorganisms after overnight incubation. MIC is important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agent. Table 2 shows the results of MIC for methanolic extract of plant parts and sequential extract of the leaves. In sequential extracts methanolic extract was most effective against *P.aeruginosa* and methanolic extracts of callus was found to be most effective against *E.coli*. For fungi, the methanolic extract (sequential) of leaves and methanolic extract of callus were most effective against *A.niger*(minimal MIC). MIC values have been already reported by [37] (*P. aeruginosa*: 15% w/v; *K. pneumonia*: 25% w/v and *E. coli*: 20% w/v). For bactericidal activity *S. aureus,* and *E. coli* which causes frequent urinary tract infections are the most common organisms used by other workers [38] also, *P. aeruginosa* (Urine infection) and chosen as the test organisms because of their high resistance to the antibacterial agents. Thus any plant which could exhibit pronounced activity against either of these organisms might yield an important antibiotic [39]. Reports on the antibacterial and antifungal activity have been evaluated by number of workers on different species of *Datura*: *D. metel* [40,41] *D.innoxia* [42] . It was also reported that antifungal activity was due to a pyrrole derivative. Several bioactive molecules including antifungal from plants have been reported. Antifungal activity is due to the presence of withanolide and a novel compound 2-(3,4-dimethyl 2,5-dihydro-1H-pyrrol-2-yl)–1- methylethyl pentanoate [9].

CONCLUSION

In the present study, the antibacterial and antifungal activity of *D.stramonium* was investigated. Results obtained in the study indicates that it posess considerable antimicrobial activity that supports the traditional usage of plant in the treatment of various diseases. However advance studied are required to identify and characterize the chemical compounds (primary or secondary metabolites) responsible for the activity so that the plant can be used as a natural antimicrobial agent.

Table 2: Minimum Inhibitory Concentration (µg/µl) of methanolic and sequential extracts of *D. stramonium*

Micro-organisms		Leaf					Methanol				
		EtOAc	MeOH	PE	Aqoeous	Standard	Fruit	Stem	Root	Leaf	Callus
Bacteria											
S. aureus	MIC	24.34±0.20	25.31±0.22	29.7±0.22	27.45±0.3	22.5±0.23	28.26±0.24	30.8±0.29	25.31±0.23	29.34±0.30	29.11±0.26
E. coli	MIC	44.42±0.39	38.07±0.35	47.36±0.5	48.43±0.56	33.4±0.27	40.94±0.55	47.36±0.52	44.95±0.51	38.04±0.48	14.98±0.17
P. aeruginosa	MIC	39.99±0.36	20.80±0.2	22.71±0.21	22.11±0.21	20.10±0.20	29.8±0.27	28.58±0.28	31.03±0.44	20.8±0.23	20.86±0.20
Fungi											
R.stolonifer	MIC	23.25±0.2	44.15±0.43	33.67±0.35	31.22±0.32	22.3±0.21	34.74±0.32	26.24±0.27	25.19±0.22	43.32±0.44	24.5±0.22
A. niger	MIC	11.92±0.14	11.7±0.15	13.16±0.13	12.84±0.1	10.4±0.09	13.97±0.11	13.41±0.14	14.87±0.16	11.7±0.14	10.97±0.01
F.culmorum	MIC	35.72±0.35	31.75±0.31	43.61±0.35	46.93±0.45	30.8±0.29	45.21±0.60	43.82±0.53	36.59±0.32	36±0.39	60.06±0.68
A. flavus	MIC	23.75±0.21	20.68±0.2	29.41±0.31	24.84±0.25	20.3±0.21	25.09±0.26	27.91±0.25	24.46±0.29	22.55±0.20	21.49±0.24

MIC = Minimum inhibitory concentration

Abbreviation: EtOH = Ethanol; MeOH = Methanol; PE = Petroleum ether; AQ = Aqueous; Standards : zentamycin = 100 unit per disc; Gentamycin = 10 mg/disc

Results are mean value SD from at least three experiment

$S.E.(\sigma_x) = \frac{\sigma}{\sqrt{n}}$; σ = Standard deviation ; n = no. of set

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