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Research article

In-vitro antibacterial activity of sequential crude extracts from *Datura stramonium* seeds

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Abstract

The growing phenomenon of antibiotic resistance, particularly to pathogenic microorganisms, in current medicine, has directed the concern of scientists for finding novel antimicrobial agents from plant origin with negligible side effect. The present study was aimed to phytochemical investigation and antimicrobial activity of seed extract of *Datura stramonium* in sequentially with different organic solvents. For this, antimicrobial properties were tested against bacteria Escherichia coli, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Bacillus subtilis* by cup plate method. Among the tested bacterial *Klebsiella pneumoniae* was the most inhibited majorly with the chloroform extract. *Daturastramonium* chloroform seed extract produced maximum zone of inhibition 26 mm against *Klebsiella pneumoniae* and 12 mm against *Bacillus subtilis* and 13 mm against Escherichia coli. *Datura Stramonium* methanol seed extract produced maximum zone of inhibition 27 mm against *Pseudomonas aeruginosa* and 15 mm against *Bacillus subtilis*, 14 mm against *Staphylococcus aureus* and 19 mm against Escherichia coli. *Datura stramonium* petroleum ether seed extract produced 16 mm zone of inhibition against Escherichia coli. *Datura stramonium* aqueous seed extract exhibits 24 mm zone of inhibition against *Bacillus subtilis*. All the experienced solvent extracts showed potential antimicrobial activity Index against various tested microorganisms. Owning to the results, it can be concluded that the extracts of the *Datura stramonium* can be used to design different herbal antimicrobial agents

Introduction

Plants are rich in a wide diversity of secondary metabolites which have been found to exhibit antimicrobial, antioxidant, anti-infectious and antitumour activities [1]. The affordability, reliability, availability and low toxicity of bio-medicals in therapeutic use has made them widespread for implementation in medical health care sector. An increased number of pathogens have also developed resistance to multiple antibiotics (Multiple Drug Resistance), Because of the mutagenic nature of bacterial DNA, the rapid multiplication of bacterial cells, constant transformation of bacterial cells and therefore be untreatable. Thus countless studies have been directed on medicinal plants for antimicrobial activities and efficacy.

Datura plant known as thorn apple, prickly burr, jimson weed, moonflower, devil's weed, devil's cucumber and devil's trumpet. It is a member of the family Solanaceae. *Datura* plants contain tropane alkaloids like scopolamine, hyoscyamine, and atropine,primarily

in their seeds and flowers [2] .Because of the presence of these fundamental biomedicals, D. *stramonium* is considered as treasured medicine and useful in the treatment of Leucoderma, skin disorders, ulcers, bronchitis, jaundice, hysteria insanity, heart disease, and for fever and piles [3-6].

Although very small number of work have been done on the antibacterial activity of

D. *stramonium* seed, it need further study for verification of its activity against disease causing microorganisms. In this study, we have carried out the preliminary study of phytochemical investigation and antibacterial activities of the seeds of *Datura* metel.

Figure 1. Medicinal use of D. stramonium

Materials and methods

Collection of plant:

Datura stramonium was collected from medicinal garden of Ashokrao Mane College of Pharmacy Pethvadgaon. The plant specimen was authenticated by Vijaysinh Yadav College of Arts & Science, Peth Vadgaon.

Extraction:

The solvents used for extraction of D. *stramonium* seeds were Chloroform 99.0-99.4%, methanol 99.8%, Petroleum ether and sterile water. The 100 grams of the seed powder was soaked in 300 ml of each solvent for 7 days then the soaked samples were filtered out using Whatman No.1 filter paper, then the filtrate was evaporated in Rotary Evaporator at 40ºC. The crude extract was stored into refrigerator which is further used for analysis of phytochemical and antibacterial activities.

Phytochemicals Analysis:

The Phytochemical analysis of seed extract was carried out for the presence of flavanoids, glycosides, alkaloids, steroids, oil, Protein, carbohydrates, tannins, saponins, Phenols, using standard qualitative method as described by standard phytochemical procedures. (Evans WC and Evans, 2003).

Figure 2. *D. stramonium* seed

Standardization of microorganism:

A loop full of test organism was inoculated in 5ml of sterile Nutrient Broth and incubated for 18 -24 hours. 18 hours culture of the organism was dispensed into 20ml of sterile Nutrient Broth and incubated for 3-5 hours to standardize the culture to 106 Cfu/ml by McFarland turbidity standards. A loop full of the standardized cultures was used for the antimicrobial activity. List of bacteria used for antibacterial activity is shown in table 1.

Screening of extract for antimicrobial activity by Cup plate method:

Sterile nutrient agar plates were prepared and seeded with standardized bacterial inoculums. Sterile cork-borer (diameter 4 mm) was used to bore cup on each plate (contains 5 cup /bores). Sterile Micro-pipette was used to transfer different concentrations of each plant extract on each labeled well, while the fourth well was for streptomycin as standard positive control for bacteria. The plates were left for 15 minutes to allow for maximum diffusion into the medium then incubated at 37°C for 24 hours. Diameter zones of inhibition around each well were measured and recorded, along with tested samples; positives

antibiotic control. Antimicrobial activity was expressed as the average diameter of the inhibition zones of three replicates. The inhibition zone (IZ) in each case was recorded and the activity index (AI) was calculated as compared with those of their standard reference drugs.

Formula for AI = inhibition zone of test sample/inhibition zone of standard

Result and Discussion

Medicinal plants represent a rich source of antimicrobial agents and source of many potent and powerful drugs [7].

Phytochemicals Analysis

Table No. 02 revealed the phytochemical properties of the various seed of Datura stramonium which showed the presence of glycoside (for aqueous extract), steroids (for aqueous extract), alkaloids(for aqueous, methanol extract), tannins (for aqueous extract), Oil (for chloroform extract), saponins (for aqueous extract), steroidal (for aqueous extract) and carbohydrate (for aqueous, methanol, chloroform, petroleum ether extract).

The results of antimicrobial screening of seed extracts are shown in Table No 3. Table No.3 and Figure No.3 revealed the activities of the sequential seed extracts against various selected gram positive and gram negative test microorganisms. The bacterium (Pseudomonas aeruginosa) was resistant to methanol, chloroform, water extract; Bacillus subtilis was resistant to Petroleum ether extract; Staphylococcus aureus was resistant to Petroleum ether, chloroform, water extract; Klebsiella pneumonia was resistant to methanol, Petroleum ether, water extract. Klebsiella pneumonia was sensitive to chloroform extract. Activity index (AI) was calculated as compared with those of their standard reference drugs mentioned in Table No.3.

Table 1. List of Bacteria used for antibacterial study

Name	Gram + / -	Shape of bacteria
Pseudomonas aeruginosa	Gram Negative	Rod Shaped
Staphylococcus aureus	Gram Positive	Cocci (Round), Appear as Cluster
Klebsiella pneumoniae	Gram Negative	Rod Shaped
Bacillus subtilis	Gram Positive	Rod Shaped
E. coli	Gram Negative	Rod Shaped

Table 2. Phytochemical analysis of aqueous extract of *Datura stramonium* leaves

Sr. No.	Phytochemical Constituents	Seed Extract			
		Water	Chloroform	Methanol	Petroleum Ether
1	Carbohydrates Molish Test Fehling Test Benedicts Test Starch	+ + + +	+ + + +	+ + + +	+ + + +
2	Tannins	+	-	-	-
3	Flavonoids	-	-	-	-
4	Proteins	-	-	-	-
5	oil	-	+	-	-
6	Steroidal	+	-	-	-
7	Glycosides	+	-	-	-
8	Saponins	+	-	-	-
9	Alkaloids	+	-	+	-

Table 3. Antibacterial study of various *D. stramonium* seed extracts

Name of extract	Antibacterial study of various D. *stramonium* seed extracts									
	Bacillus subtilis		***Pseudomonas aeruginosa***		***Staphylococcus aureus***		***Klebsiella pneumoniae***		***E. coli***	
	Zone of Inhibition Mean of bacteria (mm)	Activity index-	Zone of Inhibition Mean of bacteria (mm)	Activity index-	Zone of Inhibition Mean of bacteria (mm)	Activity index-	Zone of Inhibition Mean of bacteria (mm)	Activity index-	Zone of Inhibition Mean of bacteria (mm)	Activity index-
chloroform	12	0.4	-	-	-	-	27	0.9	13	0.4
methanol	15	0.5	26	0.7	14	0.7	-	-	19	0.7
pet. ether	-	-	-	-	-	-	-	-	16	0.5
water	24	0.8	-	-	-	-	-	-	-	-
standard	30	-	35	-	20	-	29	-	29	-

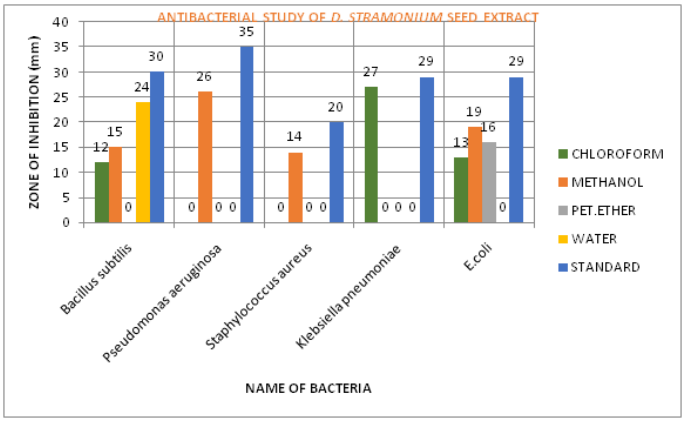

Figure 3. Antibacterial study of D. stramonium seed extract

Conclusion

In the present study, the preliminary antimicrobial activity of D. *stramonium* seeds with different solvent systems was investigated. Results obtained in the study showed varying degrees of antibacterial activities against microbes tested here which supports the traditional usage of plant in the treatment of various diseases. D. *stramonium* seeds can be a source of high pharmacological importance and great potential source of new drugs as an effective antimicrobial agent for medicinal purposes.

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