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EVALUATION OF ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACTS OF DATURA STRAMONIUM ON SELECTED BACTERIA

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Abstract:
This study was carried out with an objective to investigate the antibacterial potentials of leaves of Datura stramonium. The aim of the study is to assess the antimicrobial activity and to determine the zone of inhibition of extracts on some bacterial and fungal strains. In the present study, the microbial activity of methanolic extracts of leaves of Datura stramonium. (an ethnomedicinal plant) was evaluated for potential antimicrobial activity against medically important bacterial and fungal strains. The antimicrobial activity was determined in the extracts using agar disc diffusion method. The antibacterial and antifungal activities of extracts (5, 25, 50, 100, 250 µg/ml) of Datura stramonium were tested against two Gram-positive—Staphylococcus aureus, Streptococcus pyogenes; two Gram-negative—Escherichia coli, Pseudomonas aeruginosa human pathogenic bacteria; and three fungal strains—Aspergillus niger, Aspergillus clavatus, Candida albicans. Zone of inhibition of extracts were compared with that of different standards like ampicillin, ciprofloxacin, norfloxacin, and chloramphenicol for antibacterial activity and nystatin and griseofulvin for antifungal activity. The results showed that the remarkable inhibition of the bacterial growth was shown against the tested organisms. The phytochemical analyses of the plants were carried out. The microbial activity of the Datura stramonium was due to the presence of various secondary metabolites. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

KEYWORDS: *Antibacterial activity and Datura stramonium.*

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1. INTRODUCTION OF ANTI- MICROBIAL ACTIVITY:

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization [1, 2]. They usually contain many biologically active ingredients and are used primarily for treating mild or chronic ailments. According to World Health Organization (WHO), about 80% of the world population relies chiefly on the plant based traditional medicine especially for their primary healthcare needs. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs [3, 4].

Infectious diseases are a major cause of morbidity and mortality worldwide. The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents, intravenous catheters, organ transplantation and ongoing epidemics of human immunodeficiency virus (HIV) infections [5, 6]. This situation provided the impetus to the search for new antimicrobial substances from various sources like medicinal plants. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects. Therefore, there is a need to search for new infection-fighting strategies to control microbial infections [7].

Plant medicines are used on a worldwide scale to prevent and treat infectious diseases[8]. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, Terpenoids and flavonoids having been found in vitro since they have antimicrobial properties and may serve as an alternative, effective, heap and safe antimicrobial for the treatment of microbial infections[9]. Plant based antimicrobial compounds have great therapeutic potential as they have lesser side effects as compared with synthetic drugs and also little chance of development of resistance. Therefore an attempt has been made to study the antibacterial activity of ten medicinally important plants viz. Artocarpus heterophyllus, Berberis aristata, Chromolaena odorata, Embelia ribes, Jasminum angustifolia, Mahonia leschenaultii, Pluchea lanceolata, Plumbago indica, Terminalia chebula, Vitex negundo.[10]

INTRODUCTION OF BACTERIA

Clinical isolated bacteria used in the study are *Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus.*

Escherichia coli

Classification

Escherichia coli is the most commonly encountered member of the family Enterobacteriaceae in the normal colonic flora and the most common cause of opportunistic infections. All members of the family Enterobacteriaceae are facultative, all ferment glucose and reduce nitrates to nitrites and all are oxidase negative.[11]

Table-1 Classification of *E. coli*

Domain	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Enterobacteriales
Family	Enterobacteriaceae
Genus	*Escherichia*
Species	*coli*

Figure 1.7:Gram stain of *E. coli.*

Morphology and identification

Escherichia coli is gram-negative, non-sporing bacilli with most strains being motile and generally possessing both sex pili and adhesive fimbriae. Because most strains rapidly ferment lactose, colonies grown on MacConkey media are smooth, glossy, and translucent and are rose-pink in colour. Some strains grown on on blood agar result in colonies being surrounded by zones of haemolysis. Colonies are smooth, circular, 1 – 1,5mm in diameter and yellow opaque if lactose fermenting (blue, if non-lactose fermenting) when grown on cystine-lactose-electrolyte deficient (CLED) mediu.[12]

Epidemiology

Strains of *Escherichia coli* predominate among the aerobic commensal bacteria present in the healthy gut.[1]

***Escherichia coli* Infections**

Escherichia coli was initially considered a non-harmful member of the colon flora, but is now associated with a wide range of diseases and infections including meningeal, gastrointestinal, urinary tract, wound and bacteremia infections in all age groups.[14]

Other infections caused by *Escherichia coli* include peritonitis, cholecystitis, septic wounds and bedsores. They may also infect the lower respiratory passages or cause bacteraemia and endotoxic shock especially in surgical or debilitated patients.[15]

Antimicrobial Susceptibility

Within the community, *Escherichia coli* strains are commonly susceptible to all agents active against the Enterobacteriaceae. However, because of the

frequent occurrence of R plasmids, strains acquired in hospitals may be resistant to any combination of potentially effective antimicrobics and therapy must therefore be guided by susceptibility testing.[16]

Staphylococcus aureus

Classification

Members of the genus Staphylococcus (staphylococci) are Gram-positive cocci that tend to be arranged in grape-like clusters.[17]

Table-2 Classification of *S. aureus*

Domain	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Staphylococcaceae
Genus	*Staphylococcus*
Species	*aureus*

6. METHODOLOGY:

Plant collection and identification

Datura stramonium is an aromatic much branched erect herb with 4 angled stems, bearded nodes leaves. It is a common weed of open lands. For the present study fresh plants were collected from locality and brought to laboratory in air tight polythene bags for further processing.

Preparation of leaf extract

The extraction of the *Datura stramonium* leaves was carried out using known standard procedures. The plant materials were dried in shade and powdered in a mechanical grinder. The powder (25.0 g) of the plant materials were initially defatted with petroleum ether (60-80°C), followed by 900 ml of Methanol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatmann filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in desiccators. The hydro alcoholic extract yields a dark greenish solid residue weighing 5.750 g (23.0% w/w). More yields of extracts were collected by this method of extractions. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use. The dry weight of the plant extracts was obtained by the solvent evaporation and used to determine concentration in mg/ml. The extract was preserved at 2- to 4o C. This crude extracts of hydro alcohol was used for further investigation for potential of antimicrobial properties.

Phytochemical analysis

The Methanolic extract of *Datura stramonium* were subjected to qualitative phytochemical tests for different constituents such as alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, proteins, and free amino acids and triterpinoids.

Test for carbohydrate

Small quantity of extract was dissolved in 5ml of water and filtered.

Molisch test

The filtrate was treated with a few drops of α-napthol (20% in ethyl alcohol). Then 1 ml of concentrated H_2SO_4 was added along the sides of inclined test tube and observed for formation of violet colored ring at the interface.

1. Test for glycosides and anthroquinones

Borntrager's test

A small amount of Methanolic extract was hydrolysed with hydrochloric acid for few hours on water bath and the hydrosylate was extracted with benzene. The benzene layer was treated with dilute ammonia solution and observed for the formation of reddish pink colour.

Legal test

The extract was dissolved in pyridine and made alkaline with few drops of 10% NaOH and freshly prepared sodium nitroprusside was added and observed for formation of blue colour.

1. Test for flavonoids Ammonia test

Filter paper strips were dipped in the dilute solution of the extract, ammoniated and observed for colour change from white to yellow.

1. Test for Tannins and Phenolic compounds

The extract was dissolved in distilled water and dissolved into three portions. Sodium chloride (10%) was added to one portion, 1% gelatin to second portion and gelatin salt reagent to third portion. Precipitation with later or both gelatin salt reagents was indicative of the presence of tannins. Precipitation with salt solution indicates a false positive test. Positive tests were further confirmed by the addition of a few drops of dilute ferric chloride (1%FeCl3) to the test extract which gave blue or green black coloration.

Test for Proteins and Amino acids

Small amount of extract was dissolved in distilled water and filtered.

Biuret's test

To the ammoniated alkaline filtrate add 2-3 drops of 0.002% copper sulphate and observed for appearance of red or violet colour.

Millon's test

To 2 ml of filtrate 5-6 drops of millons reagent (1 g of mercury + 9 ml of fuming nitric acid solution) was added and observed for red precipitates.

Ninhydrin test

To the filtrate lead acetate solution was added to precipitate tannins and filtered. The filtrate was spotted on paper chromatogram and sprayed with ninhydrin reagent and heated at 110ºC for five minutes and observed for red or violet colour.

Xanthoprotein test

To the filtrate a few drops concentrated nitric acid was added bythe side of test tube and observed for appearance of yellow colour.

Test for sterols and triterpenes

The extract was refluxed with alcoholic potassium hydroxide until the completion of saponification. Then the mixture was diluted with distilled water and extracted with diethyl ether. The ethereal extract was evaporated and the unsaponifiable matter was subjected to the following tests.

Libermann- Buchard's test

The ether soluble residue was dissolved in chloroform and a few drops of acetic anhydride was added followed by a few drops concentrated sulphuric acid form sides of the test tube and observed for the formation of blue to blue- red colour.

Salkowski's reaction

To the ether soluble residue 2 ml of concentrated sulphuric acid was added and observed for the formation of yellow ring at the junction which turns red after one minute.

ANTI-BACTERIAL ACTIVITY

Test Microorganisms and Growth Media

Staphylococcus aureus (MTCC 96), Streptococcus pyogenes (MTCC 442), Escherichia coli (MTCC 443), Pseudomonas aeruginosa (MTCC 424) and fungal strains Aspergillus niger (MTCC 282), Aspergillus clavatus (MTCC 1323), Candida albicans (MTCC 227) were chosen based on their clinical and pharmacological importance were used for evaluating antimicrobial activity. The bacterial and fungal stock cultures were incubated for 24 hours at 37°C on nutrient agar and potato dextrose agar (PDA) medium (Microcare laboratory, Surat, India), respectively, following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), whereas the yeasts and molds were grown in Sabouraud dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°C.

Anti-bacterial Activity

In vitro antibacterial activities were examined for Methanolic extracts. Antibacterial activities of plant part extracts against four pathogenic bacteria (two Gram-positive and negative) and three pathogenic fungi were investigated by the agar disk diffusion method. Antimicrobial activity testing was carried out by using agar cup method. Each purified extracts were dissolved in dimethyl sulfoxide, sterilized by filtration using sintered glass filter, and stored at 4°C. For the determination of zone of inhibition, pure Gram-positive, Gram-negative, and fungal strains were taken as a standard antibiotic for comparison of the results.

All the extracts were screened for their antibacterial activities against the Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes. The sets of five dilutions (5, 25, 50, 100, and 250 µg/ml) of *Datura stramonium* extract and standard drugs were prepared in double distilled water using nutrient agar tubes. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains (108 cfu) and allowed to stay at 37o C for 3 hours. Control experiments were carried out under similar condition by using ampicillin, chloramphenicol, ciprofloxacin, and norfloxacin for antibacterial activity as standard drugs. The zones of growth inhibition around the disks were measured after 18 to 24 hours of in incubation at 37°C for bacteria. The sensitivities of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms.

7. RESULTS AND DISCUSSION:

Table 7.1: Qualitative phytochemical Evaluation of *Datura stramonium*

Parameters	value
1. Alkaloid	+
2. Carbohydrates	++
3. Glycosides	-
4. Flavonoids	+
5. Tannins & Phenolic compounds	++
6. Proteins & Amino acids	+
7. Saponins	++
8. Sterols or Triterpenes	+

++: High content, +: moderate, - : Negative,

From the qualitative phytochemical analysis of Methanolic extract of *Datura stramonium*. It content alkaloids, Tannins, phenolic compound, sterols, saponins, protein and amino acids and high amount of flavonoids.

RESULTS AND DISCUSSIONS ON ANTI-BACTERIAL ACTIVITY:

Anti-bacterial Activity

The Anti-bacterial Activity of the extracts of *Datura stramonium* were studied in different concentrations (5, 25, 50, 100, and 250 µg/ml) against four pathogenic bacterial strains, two Gram-positive (Staphylococcus aureus MTCC 96, Streptococcus pyogenes MTCC 442) and two Gram-negative (Escherichia coli MTCC 443, Pseudomonas aeruginosa MTCC 424). These strains have been selected for the basis of its application purpose of further formulation study. Anti-bacterial potential of extracts were assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial activities are presented in Tables. The antibacterial activities of the extracts increased linearly with increase in concentration of extracts (µg/ml). As compared with standard drugs, the results revealed that in the extracts for bacterial activity, S. pyogenes and S. aureus were more sensitive as compared with E. coli and P. aeruginosa. The growth inhibition zone measured ranged from 11 to 20 mm for all the sensitive bacteria. The results show that the extracts of *Datura stramonium* were found to be more effective against all the microbes tested.

Table 7.2: Antibacterial activities of Methanolic extracts of leaves of *Datura stramonium* against bacterial test organism

Antibacterial activity (Zone of inhibition)					
Microorganism	***Datura stramonium* - Zone of inhibition in mm**				
	Concentration in µg/ml				
	Methanolic extracts (µg/ml)				
	5	25	50	100	250
E. coli	-	13	16	18	22
P. aeruginosa	-	10	14	17	20
S. pyogenes	-	9	12	16	19
S. aureus	-	12	16	18	19

Values are mean ± SD of three parallel measurements - = No zone of inhibition

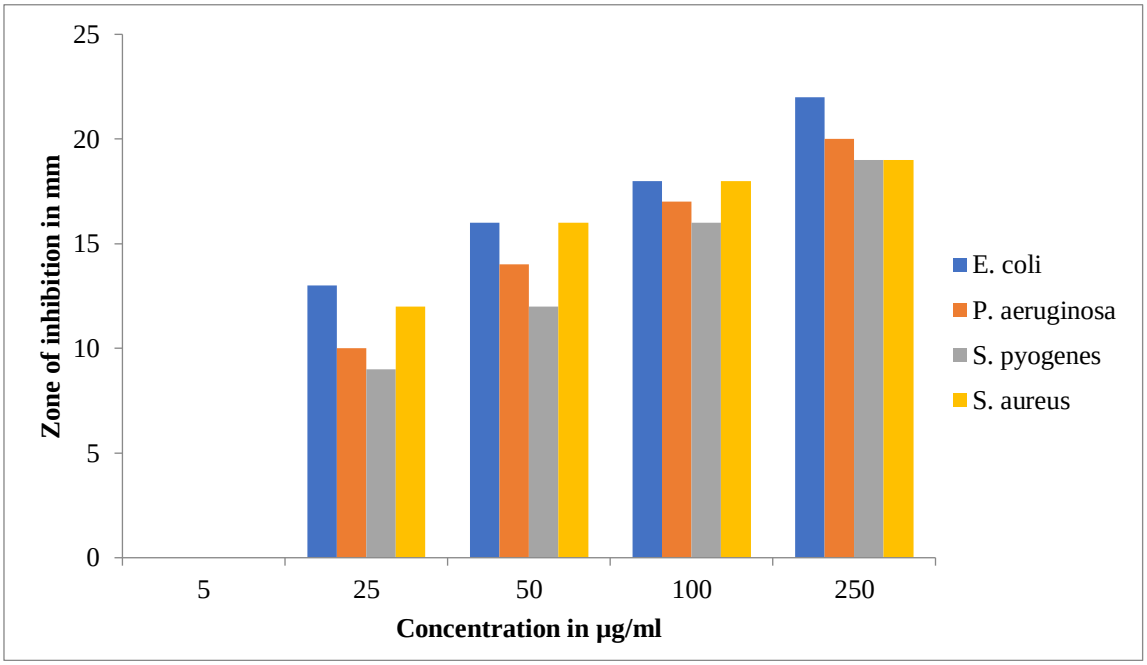

Figure 7.1: Antibacterial activities of Methanolic extracts of leaves of *Datura stramonium* against bacterial test organism

Table 7.3: Antibacterial activity of standard drugs against bacterial test organism

Antibacterial activity (Zone of inhibition)					
Drug	**Concentration (µg/ml)**	**Zone of inhibition in mm**			
		E. coli	**P. aeruginosa**	**S. pyogenes**	**S. aureus**
Ampicillin	5	16	16	10	11
	25	18	19	14	13
	50	20	21	18	17
	100	22	24	19	19
	250	24	25	20	20
Chloramphenicol	5	15	15	11	12
	25	19	18	13	15
	50	20	20	17	18
	100	23	22	18	19
	250	23	23	19	20
Ciprofloxacin	5	17	19	15	16
	25	20	21	18	19
	50	23	24	19	21
	100	25	25	21	22
	250	26	27	22	23
Norfloxacin	5	20	18	17	16
	25	22	22	21	19
	50	24	24	23	20
	100	26	26	25	23
	250	28	28	27	25

Table 7.4: Antibacterial activities of Methanolic extracts of leaves of *Datura stramonium* against fungal test organism

Antifungal activity (Zone of inhibition)					
Microorganism	***Datura stramonium* - Zone of inhibition in mm**				
	Concentration in µg/ml				
	Methanolic extracts (µg/ml)				
	5	25	50	100	250
A. niger	-	16	17	18	21
A. clavatus	-	18	19	20	21
C. albicans	-	20	20	21	21

Values are mean ± SD of three parallel measurements; - = No zone of inhibition

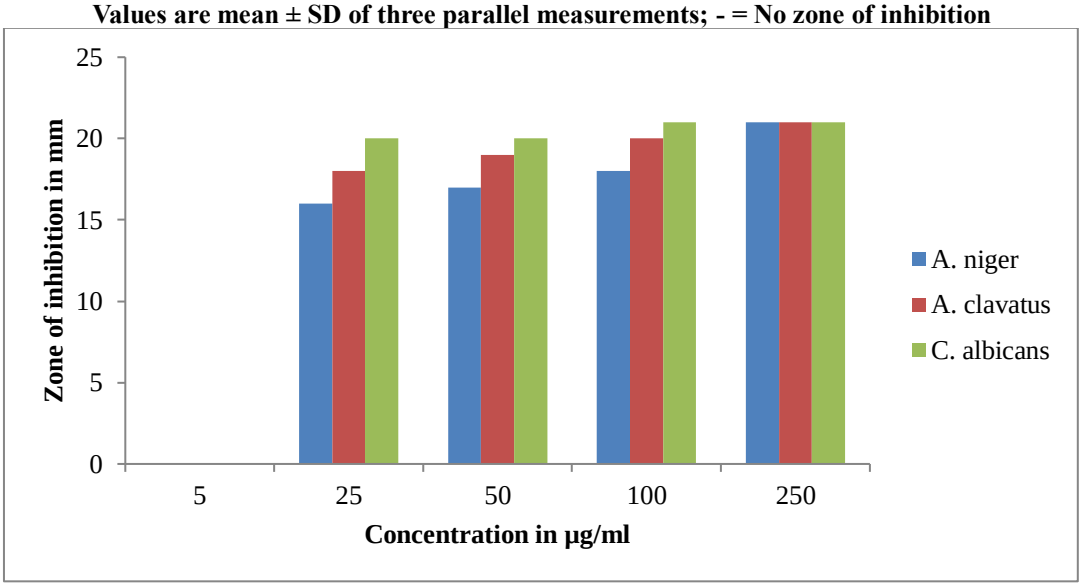

Figure 7.2: Antibacterial activities of Methanolic extracts of leaves of *Datura stramonium* against fungal test organism

The above results show that the activity of Methanolic extracts of *Datura stramonium* shows significant antibacterial activities. This study also shows the presence of different phytochemicals with biological activity that can be of valuable therapeutic index. The result of phytochemicals in the present investigation showed that the plant contains more or less same components like saponin, triterpenoids, steroids, glycosides, anthraquinone, flavonoids, proteins, and amino acids. Results show that plant rich in tannin and phenolic compounds have been shown to posse's antimicrobial activities against a number of microorganisms.

8. CONCLUSION:

In the current investigation, the Methanolic extract in the ratio of 80:20 has been selected after study of such a selected plant with methanolic extracts, extract gave higher yield of chemical constituents expected for this research work; the originality of this work is that good results have been found with methanol ratio, and it will be helpful to carry out other data with MIC and other formulation study, because in comparison of methanolic extracts is more suitable for clinical study. The methanolic extracts of *Datura stramonium* were found to be active on most of the clinically isolated microorganism and fungi, as compare with standard drugs. The present study justified the claimed uses of leaves in the traditional system of medicine to treat various infectious disease caused by the microbes. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Further studies which aimed at the isolation and structure elucidation of antibacterial active constituents from the plant have been initiated.

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