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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF THE LEAF EXUDATE OF *DATURA STRAMONIUM*

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Abstract:

This study was carried out with an objective to investigate the antibacterial and antifungal 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: *- Anti-Microbial agent, antibacterial activity, antifungal activity, and Datura stramonium.*

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INTRODUCTION:

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]



Figure -1 Gram stain of *E. coli*.

Table-1 Classification of *E. coli*

Domain	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Enterobacteriales
Family	Enterobacteriaceae
Genus	*Escherichia*
Species	*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.[12] 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.[13]

Epidemiology

Strains of *Escherichia coli* predominate among the aerobic commensal bacteria present in the healthy gut.[14]

***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.[15]

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.[16]

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.[17]

Staphylococcus aureus

Classification

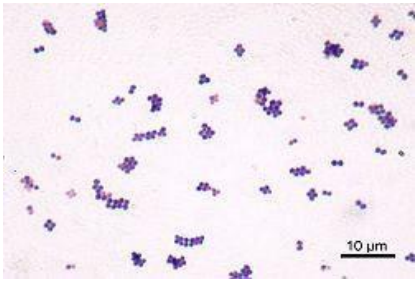
Members of the genus Staphylococcus (staphylococci) are Gram-positive cocci that tend to be arranged in grape-like clusters.[1]

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Table-2 Classification of *S. aureus*

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

Figure -2 Gram stain of *S. aureus*



Morphology and identification

Staphylococci are spherical cells about 1 m in diameter arranged in irregular clusters. Single cocci, pairs, tetrads, and chains are also seen in liquid cultures. Young cocci stain strongly gram-positive; on aging, many cells become gram-negative. Staphylococci are non-motile and do not form

spores.

Staphylococcus aureus is a facultative anaerobe that grows at an optimum temperature of 37ºC and an optimum pH of 7,5.

S. aureus produces white colonies that tend to turn a buff-golden color with time, which is the basis of the species epithet aureus (golden). Most, but not all, strains show a rim of clear β-hemolysis surrounding the colony.

On nutrient agar, following aerobic incubation for 24 hours at 37ºC, colonies are 1 – 3mm in diameter, have a smooth glistening surface, an entire edge and an opaque pigmented appearance. In most strains, pigmentation is golden with orange, yellow and cream varieties. On MacConkey agar, colonies are small to medium in size and pink or pink-orange in colour.

MATERIALS AND METHODS:

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 was 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 were 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.

1. 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- MICROBIAL 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℃ on nutrient agar and potato dextrose agar (PDA) medium (Microcare laboratory, Surat, India), respectively, following refrigeration storage at 4℃. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37℃ (the bacteria were grown in the nutrient broth at 37℃ and maintained on nutrient agar slants at 4℃), whereas the yeasts and molds were grown in Sabouraud dextrose agar and PDA media, respectively, at 28℃. The stock cultures were maintained at 4℃.

Antimicrobial Activity

In vitro antibacterial and antifungal activities were examined for Methanolic extracts. Antibacterial and antifungal 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℃. 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 and antifungal activities against the Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes and the fungi Candida albicans, Aspergillus niger and Aspergillus clavatus. 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 and nystatin and griseofulvin for antifungal activity as standard drugs. The zones of growth inhibition around the disks were measured after 18 to 24 hours of in incubation at 37℃ for bacteria and 48 to 96 hours for fungi at 28℃. 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.

Antimicrobial activity

Agar well diffusion

The plates were inoculated with colonies of the bacteria. Then 8-mm-diameter wells were created with a sterilized cork borer on the plate. These wells were filled with 100 µL of the extract, the positive control, and the negative control. Then incubate the plates at 37 ℃ for 18–24 h for bacterial pathogens and 28 ℃ for 48 h for fungal pathogens. The plant extracts diffuse in the agar medium and inhibit the growth of the microbial strain. The inhibition zone diameters were measured with the ruler, and the means were estimated15–17. Comparing the clearing zone diameters of the extract with the controls is used to determine the antibacterial and antifungal activity18. Ciprofloxacin (5 µg) for bacteria and amphotericin B (12.5 µg) for fungi were the positive controls, while 1% DMSO was the negative control. The experiment was done three times.

Determination of MIC

The broth microdilution method was done using a 96-well micropipette plate to determine the MIC. A 64 mg/ mL stock solution of the extract was prepared with 1% DMSO. From this, 100 µl of the extract, positive and negative controls were put on the wells. Then two-fold serial dilutions with multichannel micropipettes were illustrated. 5×105 CFU/ml of bacterial suspension and 5×104 CFU/ml of fungal suspension were prepared from a refreshed culture. 100 µl of suspension was

inoculated into each well and incubated. After incubation, 40 µl of a 0.2 mg/ml solution of 2, 3, and 5-triphenyltetrazolium chloride was placed in each well, and incubated at 37 ℃ for 30 min. After incubation, the MIC values were visually shown by observing the presence or absence of pink. When there was no pink color at the lowest concentration of each extract, it indicated MIC values. These values were determined in triplicate.

Determination of MBC

Incubating 10 mL of the microtiter plate well for 24 h, which is larger than or equal to the lowest minimum inhibitory concentration on Mueller Hinton agar, determined the minimum bactericidal concentration. Following incubation of the Petri dish for 24 h, the minimum bactericidal concentration was determined as the minimum concentration of extracts or fractions from which no visible growth occurred. The experiment was conducted in triplicate.

Data analysis

The data were analyzed using SPSS (Statistical Package for Social Science) software version 25.0. The results were presented as the mean±standard error of the mean. A one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied to compare differences in mean among the treatment and control groups. P-values

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-MICROBIALB ACTIVITY:

Microbial activity

The antimicrobial 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. Antibacterial potential of extracts was 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	-	15	17	20	25
P. aeruginosa	-	12	15	19	22
S. pyogenes	-	8	11	17	20
S. aureus	-	10	15	17	18

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

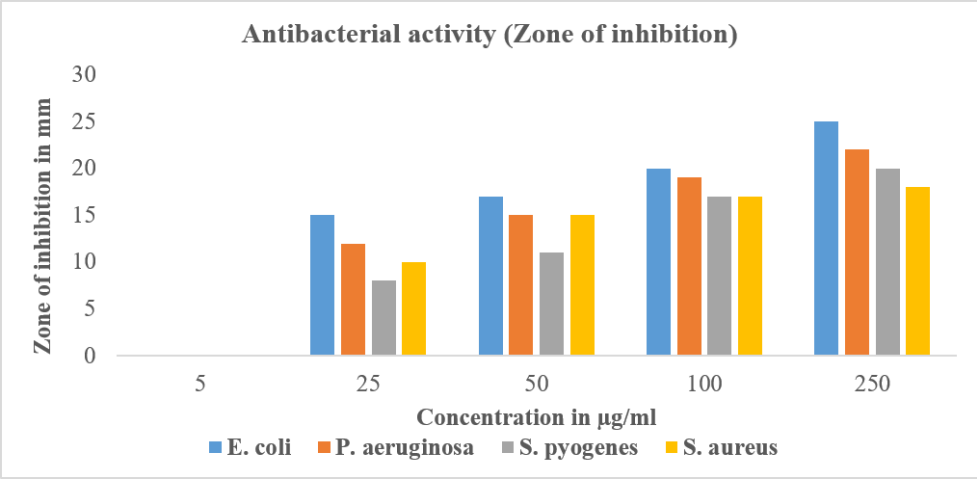


Figure 7.1: Antibacterial activities of Methanolic extracts of leaves of *Datura stramonium* against bacterial test organism

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	-	17	18	19	22
A. clavatus	-	19	20	21	22
C. albicans	-	21	22	23	23

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

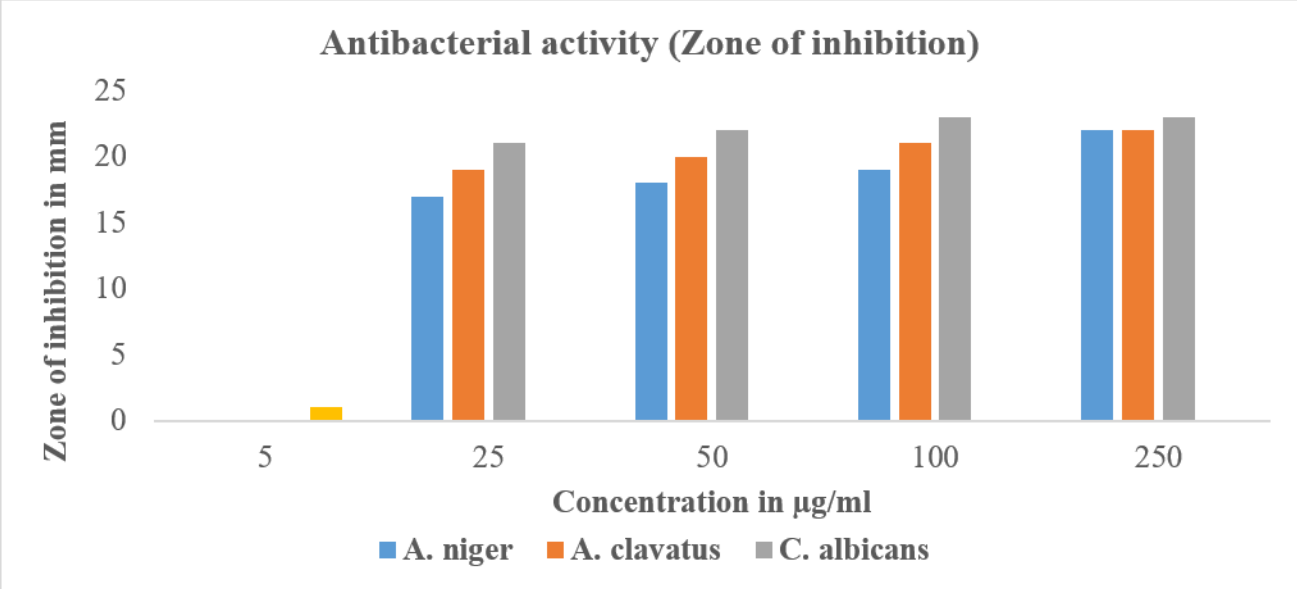


Figure 7.2: Antibacterial activities of Methanolic extracts of leaves of *Datura stramonium* against fungal test organism

Table 7.5: Antifungal activity of standard drugs against fungal test organism

Antifungal activity (Zone of inhibition)				
Drug	**Concentration in (µg/ml)**	**Zone of inhibition in mm**		
		A. niger	**A. clavatus**	**C. albicans**
Griseofulvin	5	19	20	21
	25	21	19	20
	50	25	23	24
	100	27	25	26
	250	30	28	28
Nystatin	5	18	17	18
	25	21	20	19
	50	24	25	24
	100	28	26	25
	250	29	27	28

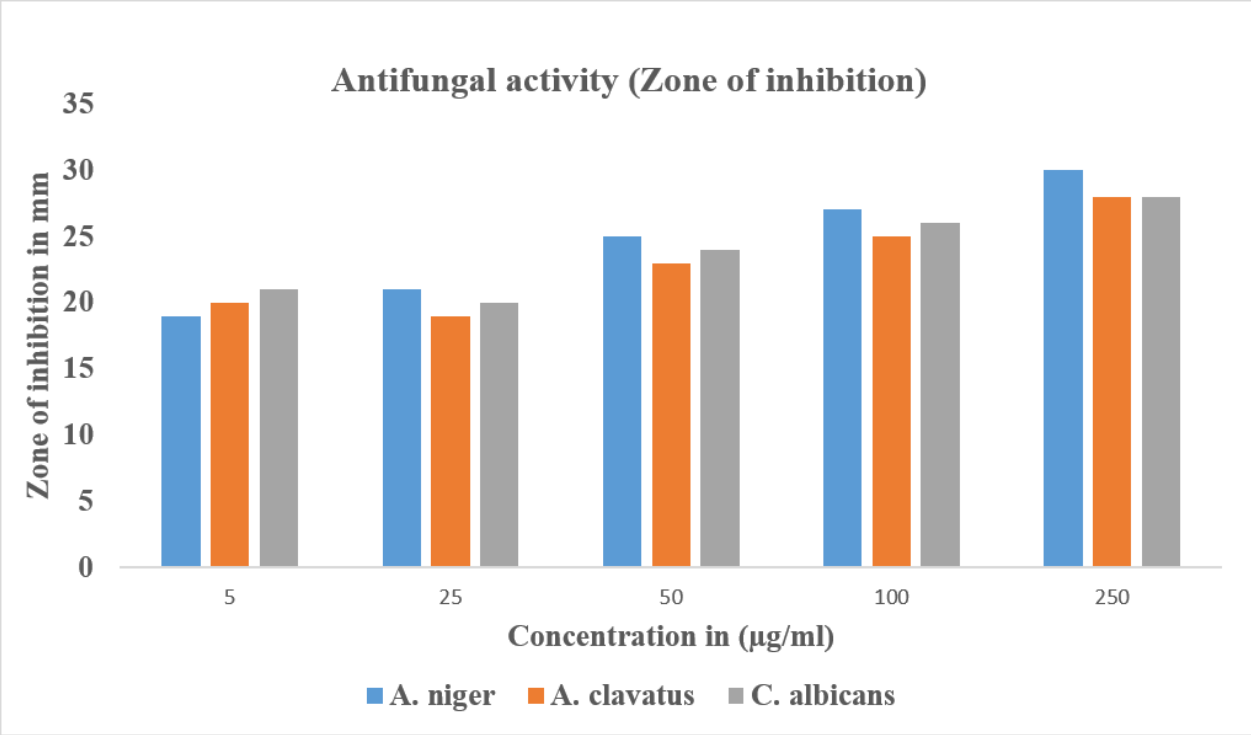


Figure 7.3: Antifungal activity against Griseofulvin

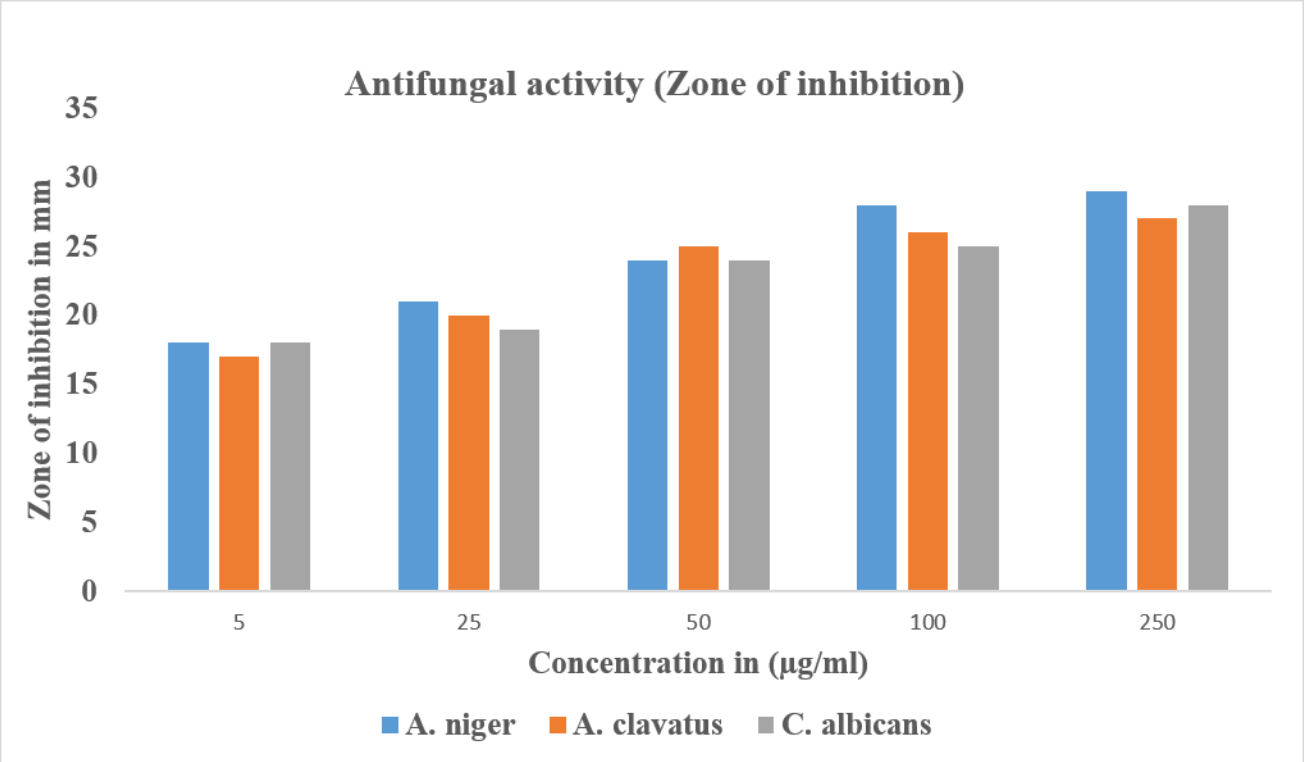


Figure 7.4: Antifungal activity against Nystatin

The above results show that the activity of Methanolic extracts of ***Datura stramonium*** shows significant antibacterial and antifungal 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 leaf contains more or less same components like saponin, triterpenoids, steroids, glycosides, anthraquinone, flavonoids, proteins, and amino acids. Results show that leaf rich in tannin and phenolic compounds have been shown to posse's antimicrobial activities against a number of microorganisms.

Antibacterial activity

Agar well diffusion method

The agar-well diffusion method was employed to investigate the inhibition zone diameter of the crude extract of *Datura stramonium*. According to Table, the hydroalcoholic extract of *Datura stramonium* has antibacterial action against S. epidermis, E. coli, and S. typhimurium by inhibiting zone diameter. Growth of S. epidermis, E. coli, and S. typhimurium was effectively suppressed at 200 mg/mL, with a mean inhibition zone diameter of 24.33 mm, 23.33 mm, and 23 mm, respectively. These bacterial strains were also susceptible to the crude extract at a lower dose. For instance, the clearing zone diameter of the extract of *Datura stramonium* shows 17 mm (for S. epidermis), 16 mm (for E. coli), and 16.67 mm (for S. typhimurium). However, no response was observed for S. aureus, E. faecalis, or K. pneumoniae at both 100 mg/mL and 200 mg/mL. In other words, the mean inhibition zone diameters of the crude extract are equal to the size of the wells (8 mm) against these bacteria.

As shown in Fig. 1, dose-dependent activity was observed against S. epidermis, E. coli, and S. typhimurium. As the dose of the extract increases, the growth of these bacteria is effectively inhibited. The positive control achieved noteworthy ($p<0.001$) antibacterial activity in contrast to all doses of the extract.

MIC and MBC

The antibacterial activity of the extract was also investigated by determining the MIC and MBC. As indicated in Table, the crude extract showed inhibitory activity against S. epidermis, E. coli, and S. typhimurium with an MIC of 4 mg/mL and MBC of 32 mg/mL for the first and second bacteria.

Table 7.6. The mean inhibition zone diameter (mm) of the hydroalcholic root extract of *Datura stramonium* against selected bacteria. Values are presented as mean ± SEM; n = 3; means followed by a different letter indicate significant differences between different doses of the same treatments, negative control and positive control in the same row. a = negative control, b = positive control, c = 100 mg/mL, d = 200 mg/mL. The diameters = size of wells (8 mm) + wells of the extract.

Test organisms	Mean inhibition zone diameter (mean ± SEM)			
	Crude extract (100 mg/mL)	Crude extract (200 mg/mL)	Ciprofloxacin (5 µg)	1% DMSO
Staphylococcus aureus	10 ± 0.00b	10± 0.00b	25.14 ± 0.14acd	10 ± 0.0 cd
Staphylococcus epidermis	18 ± 0.00abd	25.36 ± 0.24abc	35 ± 0.00acd	10 ± 0.0bcd
Enterococcus faecalis	10 ± 0.00b	8.33 ± 0.33b	22 ± 0.00acd	10 ± 0.0 cd
Escherichia coli	18 ± 0.12abd	25.33 ± 0.12abc	35.14 ± 0.33acd	10 ± 0.0bcd
Salmonella typhimurium	18.12 ± 0.41abd	25 ± 0.00abc	35.25 ± 0.33 acd	10 ± 0.0bcd
Klebsiella pneumonia	10 ± 0.00b	10.01 ± 0.14b	25 ± 0.00acd	10 ± 0.0 cd

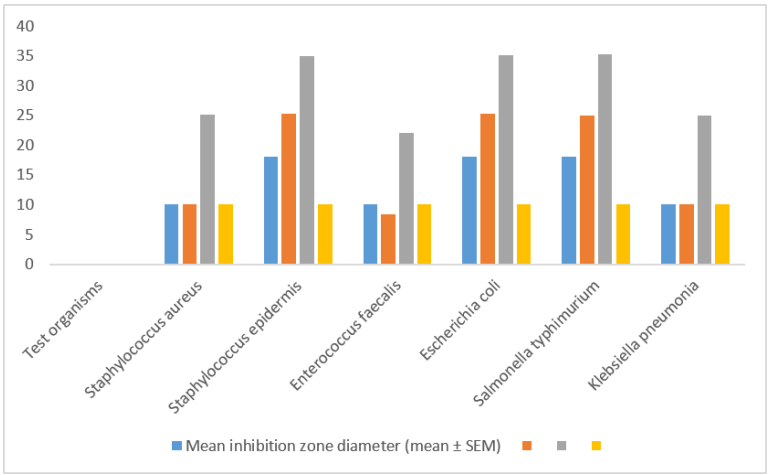


Fig. 7.5. Zones of inhibition (mm) Vs inhibitory activity of the treatment and control groups against bacterial strains.

Table 7.7: The MIC and MBC (mg/mL) of the leaf extract of *Datura stramonium* against bacterial strains.

No	Name of bacteria	MIC	MBC	MIC (mg/L) for ciprofloxacin
1	*S. aureus*	> 32	66	1.30
2	*S. epidermis*	4	35	1.30
3	*E. faecalis*	> 32	65	1.30
4	*E. coli*	4	33	1.30
5	*S. typhimurium*	3	17	1.30
6	*K. pneumoniae*	> 32	66	1.30

Table 7.8. Diameters of zone of inhibition (mm) of the leaf extract of *Datura stramonium*, DMSO and amphotericin B against selected fungi. Values are expressed as Mean ± SEM: n = 3, 8 ± 0.0 = no inhibition (well diameter), a = compared to DMSO, b = compared to 100 mg/mL, c = compared to 200 mg/mL, d = compared to Amphotericin B 12.5 µg/mL, p1 < 0.005.

Microorganism	Diameter of zone of inhibition in mm(Mean ± SEM)			
	Leaf extract (100 mg/mL	Leaf extract (200 mg/mL)	DMSO	Amphotericin B (12.5 µg/mL)
C. albicans	$10.12 \pm 1.31.0d^1$	$11.2 \pm 2.8d^1$	$9 \pm 0.0d^1$	$26.0 \pm 0.0a^1b^1c^1$
A. niger	$9.65 \pm 0.86d^1$	$11.02 \pm 0.23d^1$	$9 \pm 0.0d^1$	$21.1 \pm 0.0a^1b^1c^1$
T. rubrum	$19.12 \pm 1.15\ a^1$	$23.2 \pm 7.1\ a^1$	9 ± 0.0	$26.0 \pm 0.0\ a^1b^1$
T. mentagrophytes	$22.14 \pm 5.1a^1d^1$	$29.24 \pm 3.12\ a^1d^1$	$9 \pm 0.0d^1$	$31.4 \pm 0.0a^1b^1c^1$

Antifungal activity

The leaf extract of *Datura stramonium* was investigated for its antifungal activity using the agar well diffusion method. The leaf extract showed an antifungal activity against most of the fungal strains tested at a concentration of 100 and 200 mg/mL, as shown in Table. Of all the tested fungal strains, T. mentagrophytes (zone inhibition = 28.65 ± 2.65 mm) was found to be the most sensitive fungal strain to the leaf extract, followed by T. rubrum (zone inhibition = 22.7 ± 6.0 mm) at a concentration of 200 mg/mL, as shown in Table. However, the root extract did not show any antifungal activity against A. niger at a concentration of 100 mg/mL. As the leaf extract concentration increased to 200 mg/mL, the extract showed antifungal activity against A. niger with a mean inhibition zone diameter of 10.15 ± 0.85 mm.

In all fungal strains, as the dose of the extract increased from 100 to 200 mg/mL, the inhibitory activity also increased. Amphotericin B significantly ($p < 0.005$) inhibited the growth of all the tested fungal strains except T. rubrum at both doses of the extracts (Fig. 2).

The broth dilution method was employed to determine the MIC and MFC of the leaf extract of *Datura stramonium*. As shown in Table, the extract achieved the highest inhibitory activity against T. rubrum with MIC and MFC values of 2 and 2.35 mg/mL, respectively. Besides this, the extract also has activity against T. mentagrophytes (MIC = 6 mg/mL, MFC = 12 mg/mL). However, I. rothii did not show any activity against C. albicans and A. niger (MIC and MFC > 64 mg/mL).

Table 7.9: MIC and MFC of the leaf extract of *Datura stramonium* and amphotericin B against fungal strains. MIC and MFC are expressed in mg/mL.

Microorganism	Root extract		Amphotericin B	
	MIC	MFC	MIC	MFC
C. albicans	> 64	> 64	0.038	0.070
T. rubrum	2.0	2.35	0.004	0.008
T.mentagrophytes	6	12	0.002	0.006
A.niger	> 64	> 64	0.006	0.007

DISCUSSION:

Antibacterial activity

Results of the present study support the use of methanolic extract to treat infectious diseases. The leaf extract of *Datura stramonium* has activity against S. epidermis, E. coli, and S. typhimurium. This indicates that some broad coverage of antibacterial compounds is present in the leaf extract of *Datura stramonium* against these infections. The lower the MIC value and the larger the zone of inhibition, the more sensitive the antimicrobial is. As a result, the present study against these bacteria is supported by this information. As the dose of the extract increases, the growth of these bacteria is effectively inhibited. At 200 mg/mL, the extract significantly ($p < 0.001$) possessed activity as compared to 100 mg/mL. However, at high and low doses of the extract, the growth of S. aureus, E. faecalis, and K. pneumoniae was not shown. Due to this, there is no need to enhance the dose. The positive control achieved significant ($p < 0.001$) antibacterial activity as compared to all doses of the extract. This might be due to the presence of more active constituents in the standard drug.

A study showed that the leaf extract of *Datura stramonium* inhibited E. coli by 18.5 mm and S. epidermidis by > 10 mg/mL16 In another study, a 6 mm inhibition zone diameter was observed in the methanolic extract of *Datura stramonium* for this bacteria strain. In the disc diffusion method, this extract provided 9 mm of protection against S. typhimorium. The hydroalcoholic extract of *Datura stramonium* produced a better clearing zone than I. edgeworthii, but it possessed comparable activity to *Datura stramonium* against E. coli. This might be due to the nature of the constituents in the present study. The growth of S. aureus was suppressed (inhibition zone diameter = 18 mm, MIC = 2.5 mg/mL) with a methanol extract of *Datura stramonium*. Impatiens tinctoria is the most sensitive to S. epidermidis, with MIC values of 1 mg/mL and 2 mg/mL, respectively, but in this study, the extract was inactive against these bacteria. The low amount of active constituent(s) present in the crude extract of *Datura stramonium* might be a possible reason.

Antifungal activity

Among the four tested fungal strains, *Datura stramonium* (MIC = 2 mg/mL) and T. mentagrophytes (MIC = 6 mg/mL) were the most susceptible to the crude extract of *Datura stramonium* (Table). Therefore, this extract can be used to treat onychomycosis, which is caused mainly by T. rubrum and rarely by T. mentagrophytes. In all fungal strains, as the dose of the extract increased from 100 to 200 mg/mL, the inhibitory activity also increased (Table). This showed that dose-dependent activity was observed. Ethyl acetate extract of Impatiens sulcate inhibited the growth of A. niger with the inhibition zone diameter of 15 mm. This showed the leaf extract of *Datura stramonium* provides less inhibitory activity against this fungus. This might be due to the difference in the type of extracting solvent, the part of the plant, and the laboratory setup. On the other hand, the growth of C. albicans and A. niger was not inhibited by methanol, water, and ethyl extracts of I. tinctoria. However, these extracts showed antifungal activity against T. rubrum and T. mentagrophytes. These findings were supported by the present study. On the other hand, 80% ethanol flower extracts of Impatiens walleriana had antifungal activity against Aspergillus niger with MIC (0.025–0.05 mg/mL) and MFC (0.05–0.1 mg/mL). This showed that *Datura stramonium* is less active as compared to this study because of the difference in the extracting solvent and plant part. With MIC and MFC values between 0.7 and 32 and 1–64 mg/mL, respectively, the ethyl acetate root extracts of I. tinctoria are the most effective against C. albicans, T. rubrum, T. mentagrophytes, and A. niger. The type of solvents used in this study makes I. rothii less active than I. tinctoria.

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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