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Phytochemical Analysis and Antimicrobial Activity of Leaf Extract of *Datura stramonium* L.

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ABSTRACT: The adverse environmental and human health impacts of chemical use against microorganisms have increased profoundly. For this reason, alternative methods to minimize the use of chemicals are being explored. One effective approach is the use of plant extracts possessing natural antimicrobial properties. The aim of the present study was to evaluate the ethanolic extract of *Datura stramonium* L. (leaves) against selected microorganisms. The results revealed that the mean zone of inhibition for bacterial species ranged from 23 ± 0.72 to 27 ± 1.24 mm.

The present study focuses on the phytochemical analysis, antibacterial activity, and antioxidant activity of leaf extracts of *Datura stramonium*. The extraction of active phytochemicals from *D. stramonium* leaves was carried out using **decoction and maceration methods** with different solvents, including petroleum ether, benzene, solvent ether, chloroform, acetone, ethanol, and methanol. Qualitative phytochemical screening of the leaf extracts revealed the presence of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins, and saponins.

Furthermore, the phytochemicals of *D. stramonium* were evaluated for their in vitro antibacterial activity against selected bacterial pathogens using the disk diffusion method. The leaf extracts exhibited considerable antibacterial activity even at low concentrations. Among the different solvent extracts, the methanolic extract showed the maximum inhibitory effect. The leaf extracts of *D. stramonium* also demonstrated potent antioxidant activity in in vitro studies.

The significance of this study lies in the fact that the use of many medicinal plants is still confined to limited tribal communities, and their proper management and planned utilization remain largely unexplored for economic and health development. This paper aims to study the ethnomedicinal plant species found in the village of Bassi, located in the Chittorgarh district of Rajasthan.

KEYWORDS: *Datura stramonium*, phytochemicals, antibacterial activity, antioxidant activity, disk diffusion

I. INTRODUCTION

Datura stramonium L. is a well-known medicinal plant traditionally used in many parts of the world for treating asthma, pain, inflammation and various microbial infections. The rise of multidrug-resistant (MDR) bacteria has increased the need to explore natural sources for new antimicrobial agents. Plants contain several important secondary metabolites such as alkaloids, flavonoids, tannins and phenolic compounds, which are known for their strong antimicrobial and antioxidant properties (Mohammed et al., 2021). Because of these bioactive compounds, medicinal plants have gained significant scientific attention.

Datura stramonium contains pharmacologically important tropane alkaloids—mainly atropine, scopolamine and hyoscyamine—which are widely used in modern medicine for pupil dilation, motion sickness, muscle spasms and pre-anesthetic procedures (Waza et al., 2015). Scientific studies have also shown that the leaf extracts of this plant exhibit notable antibacterial activity, supporting its traditional uses (Odeleye & Obameso, 2022; Ajadi et al., 2024). These findings highlight the therapeutic potential of the plant.

Although *Datura stramonium* is distributed across tropical and temperate regions, studies comparing the antimicrobial activity of its leaves from different geographical locations remain limited. In particular, there is very little information on the phytochemical composition and antimicrobial properties of *Datura stramonium* collected from Rajasthan, India. Therefore, the present study aims to evaluate the phytochemical constituents and antimicrobial activity of *Datura*

stramonium leaf extracts collected from this region. This work will help better understand the plant's bioactive profile and its potential for developing plant-based antimicrobial formulations.

Problem Statement

Problem statement The practice of ethnomedicine had been in existence for as long as the history of man, prior to the discovery of synthetic drugs and antibiotics. The inappropriate usage and misuse of antimicrobials has resulted to resistance by various microorganisms due to the ability of the microorganisms to develop certain resistant factors. Most antibiotics also present undesirable side effects to the host when consumed which include; emetic, nausea, oedema, bone marrow depression, thrombocytopaenia purpura and agranulocytosis. This led to the development of new and more cost effective antimicrobials from medicinal plant extracts which may help to treat microbial infections that may present little or no side effect according to the bioactive compound of the plant extract and investigating the antimicrobial sensitivity of the different extractants of *D. metel* on selected clinical organisms with respect to control antibiotics.

Research Gap

The determination of the phytochemical constituents of plant extracts is essential in order to ensure the reliability and repeatability of pharmacological and clinical research, and to understand their bioactivities and possible side effects of the active compounds through toxicity evaluation protocol. *Datura stramonium L.* is widely distributed in Ethiopia and known for its folk medicine including the treatment of bacteria. Thus, this study addresses on phytochemical analysis and evaluation of antibacterial potential of leave extracts of *Datura stramonium L.* against selected human pathogens

II. MATERIALS AND METHODS

DATURA STRAMONIUM

Datura stramonium also called datura is an erect annual plant, on average 30 to 150 cm (1-5 feet) tall with erect, forking and purple stems. The leaves are large 7 to 20 cm (3-8 in) long and have irregular teeth similar to those of oakleaves. The flowers are one of the most distinctive characteristics of *D. stramonium*. They are trumpet-shaped, white to purple and 5-12.5 cm (2-7 in) long. The flowers open and close at irregular intervals during the evening, earning plant the nickname Moonflower. The fruit are walnut-sized, egg-shaped and covered in prickles. They split into four chambers each with a few kidney-shaped seeds. All parts of the plant emit a foul odour when crushed or bruised (Willard & Meritt, 1986)[5]. The plant is strongly narcotic, but has a peculiar action on the human frame which renders it very valuable as a medicine. The whole plant is poisonous, but the seeds are the most active neither drying nor boiling destroys the poisonous properties. The usual consequences of the poison when taken in sufficient quantity are dimness of sight, dilation of the pupil, giddiness, delirium and sometimes amounting to mania, but its action varies greatly on different persons (Yoshikawa K et al., 1989) [9]. *D. stramonium* is known to have several benefits to human health care as a wonderful therapeutic agent. The *D. stramonium* were collected from the surrounding areas of Urdigere, situated in the regions of Devarayana durga hills of Tumkur district which has a rich ecological resource.

2.1 Collection of Plant Material and Authentication

Fresh and mature leaves of *Datura stramonium L.*, a plant widely distributed throughout India, were collected from the Bassi Wildlife Sanctuary, Chittorgarh (Rajasthan), India, during October 2024. Healthy and disease-free specimens were carefully selected from naturally growing populations. The collected plant material was identified and authenticated by Dr. Shadab Hussain, Assistant Professor, Department of Botany, Sangam University, Bhilwara (Rajasthan). Herbarium sheets were prepared and deposited in the Department for future reference.

In the laboratory, the freshly collected leaves were first washed thoroughly with clean tap water and then rinsed with sterile distilled water to remove dust and surface contaminants. The leaves were shade-dried at room temperature to preserve thermolabile phytoconstituents. After complete drying, they were finely powdered using a mechanical grinder, and the prepared powder was used for phytochemical screening and antimicrobial evaluation.

**Figure 1: *Datura stramonium* plant**

2.2 Preparation of leaf Extracts

The powdered leaves of *Datura stramonium* L. were subjected to two extraction techniques: the decoction method and maceration. In the decoction method, the leaf powder was boiled in ethanol to obtain the ethanolic extract. For maceration, the powdered material was soaked in the selected solvent at room temperature to allow thorough extraction of phytochemicals. The extracts obtained from both methods were filtered, concentrated, and stored appropriately for subsequent phytochemical screening and antimicrobial evaluation.

In the decoction method, 10 g of the finely powdered leaf material was extracted with 100 mL each of ethanol and petroleum ether. The extraction was continued until the solvent became clear, indicating exhaustive extraction of phytoconstituents (Gul, Singh, & Singh, 2022). The obtained extracts were filtered through Whatman No. 1 filter paper, and the filtrates were evaporated at room temperature to remove the solvents. The dried crude extracts were collected and stored at 4 °C in airtight containers for further phytochemical screening and antimicrobial evaluation.

For maceration, 10 g of dried leaf powder was placed in separate glass containers, and 20 mL each of hexane, chloroform, ethyl acetate, and methanol were added individually. The mixtures were kept undisturbed at room temperature (25 ± 2 °C) for 72 hours with intermittent shaking every 3–4 hours to facilitate solvent penetration and diffusion of soluble phytoconstituents. After completion of the maceration period, the extracts were filtered through Whatman No. 1 filter paper. The filtrates were allowed to air-dry at room temperature to obtain the respective crude extracts. The dried extracts were stored at 4 °C in airtight containers until further phytochemical and antimicrobial analyses were performed. This procedure was adopted based on standard plant-extraction protocols described in previous studies (Harborne (1998) and Azwanida (2015), with suitable modifications to suit the current study).

The efficiency of extraction was evaluated by calculating the percent yield using the following formula (Parveen, Sultana, & Sharma, 2014)

$$\text{Percent yield (\%)} = (\text{Weight of dried crude extract (g)} / \text{Weight of initial dried plant material (g)}) \times 100$$

2.3 Phytochemical Screening

The leaf extracts of *Datura stramonium* L., obtained through the decoction and maceration techniques, were subjected to preliminary qualitative phytochemical screening following the standard protocols described by Dubale et al. (2023), Gul, Singh, & Singh (2022), and Harborne (1998). The analyses aimed to identify the presence of major classes of secondary metabolites. The extracts were examined for carbohydrates, glycosides, proteins, amino acids, fats, alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, phenols, and coumarins. The qualitative findings provided essential insights into the chemical composition of *D. stramonium* leaf extracts and established a foundation for their subsequent phytochemical and antimicrobial evaluation.

2.3.1 Qualitative Phytochemical Screening

The leaf extracts of *Datura stramonium* L., obtained through the decoction and maceration techniques, were subjected to preliminary qualitative phytochemical screening following standard protocols (Trease & Evans, 2009; Harborne, 1998). The analyses aimed to identify the presence of major classes of primary and secondary metabolites. Carbohydrates were detected using Molisch's and Barfoed's tests, in which the extracts reacted with α -naphthol and ferric solutions, producing violet rings and red precipitates, respectively. Reducing sugars were confirmed by Fehling's and Benedict's tests, which yielded brick-red or red coloration, while non-reducing polysaccharides produced a blue coloration with dilute iodine solution. Glycosides were identified by treatment with NaOH and distilled water, resulting in a yellowish layer. Proteins and amino acids were detected using Biuret and Ninhydrin tests, giving violet or pink, and purple-bluish coloration, respectively. Fats and fixed oils were assessed through the Saponification test, which resulted in soap formation.

Secondary metabolites were further evaluated to assess the chemical diversity of the extracts. Tannins were detected using the Ferric Chloride test, producing a blue-black coloration. Alkaloids were confirmed by Dragendorff's test, yielding an orange-brown precipitate. Saponins formed a stable foam in the foam test. Flavonoids were identified by the appearance of yellow-green coloration with dilute NaOH. Steroids were confirmed using the Liebermann-Burchard test, producing a bilayer with a red top layer and greenish bottom layer. Terpenoids gave a reddish-brown color when treated with chloroform and concentrated sulfuric acid. Phenols formed a blue-black precipitate with ferric chloride. Coumarins produced yellow-green coloration with alkaline reagent, and anthraquinones yielded a pink color upon treatment with chloroform and ammonia. These qualitative findings provided comprehensive insights into the chemical composition of *D. stramonium* leaf extracts, forming a basis for their subsequent antimicrobial and pharmacological evaluation.

2.4 Antimicrobial Assay

2.4.1 Test Bacteria

Pure cultures of *Staphylococcus aureus* (MCC2043T), *Escherichia coli* (MCC3671T), *Klebsiella pneumoniae* (MCC2451T), and *Pseudomonas aeruginosa* (MCC2080T) were procured from the National Centre for Microbial Resource (NCMR), Pune. After acquisition, the bacterial strains were maintained as stock cultures at 4 °C for routine laboratory experiments. Prior to antimicrobial testing, each bacterial isolate was freshly sub-cultured and incubated at 37 °C for 48 hours to achieve active growth and prepare a standardized inoculum for the assays.

2.4.2 Preparation of Crude Leaf Extract and Antibiotics

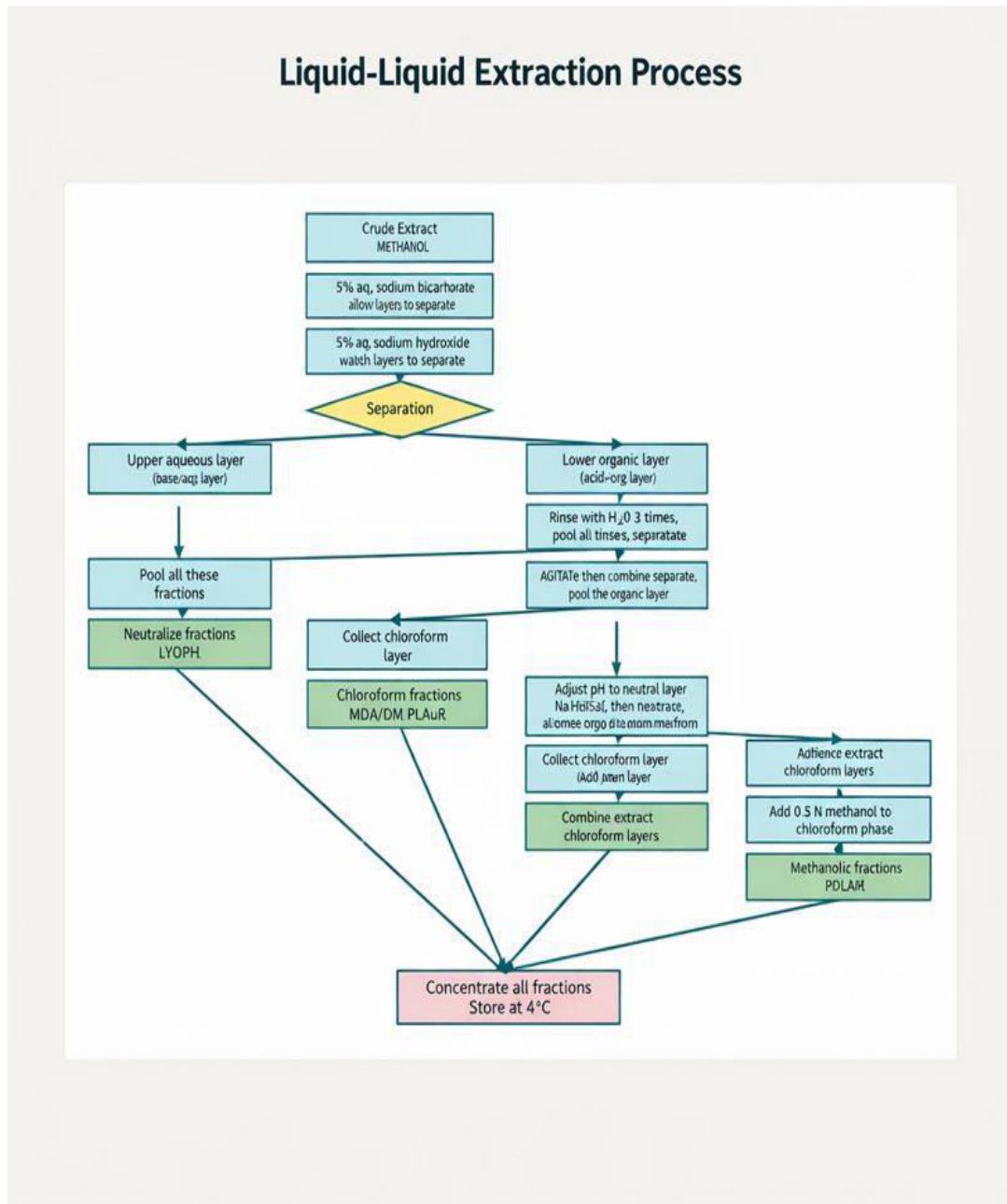
For antimicrobial testing, the crude methanolic leaf extract was prepared at a concentration of 50 mg/mL by dissolving the extract in 50% DMSO. Kanamycin strip was used as the positive control in the form of commercial antibiotic strips. 50% DMSO solution was used as the negative control.

2.4.3 Antimicrobial Assay

Agar Well Diffusion Method

The antibacterial activity of *Datura stramonium* crude leaf extract was evaluated using the agar well diffusion technique as described by Perez (1990). Sterilized Mueller–Hinton agar served as the growth medium and was cooled to 45–50 °C before use. A pre-standardized bacterial suspension was aseptically mixed into the molten agar and poured into sterile Petri plates to ensure uniform solidification. Wells measuring 6 mm in diameter and approximately 3 mm in depth were created using a sterile cork borer. Different concentrations of the crude leaf extract (100 μ L and 50 μ L) were added to the wells using a micropipette. Commercial Kanamycin strips were used as the positive control, while 50% DMSO (50 μ L) served as the negative control. The plates were left undisturbed for 2 hours to allow diffusion of the test samples into the agar. After sealing with parafilm, the plates were incubated at 37 °C for 48 hours. Antibacterial activity was assessed by measuring the diameter of the inhibition zones using an antibiotic zone scale, following Sharmin et al. (2012). All assays were performed in triplicate to ensure reproducibility and minimize experimental error.

Liquid-Liquid Extraction of Crude Extracts



Disc Diffusion Method

The disc diffusion assay was performed according to CLSI (2018) guidelines. Sterile paper discs (6 mm diameter) were impregnated with 50 μ L of each plant extract and placed carefully on Mueller–Hinton agar plates previously inoculated with standardized bacterial suspensions. Commercial Kanamycin strips were used as the positive control, while 50% DMSO served as the negative control. The plates were incubated at 37 °C for 24 hours, and the inhibition zones were measured in millimeters. All experiments were performed in triplicate to ensure reproducibility and statistical reliability (Balouiri et al., 2016; Dubale et al., 2023).

2.5 Statistical analysis

Statistical analysis was performed using SPSS software (version 16.0). Data were analyzed through one-way ANOVA following a completely randomized design, and Tukey's HSD test was applied to identify any significant differences among the samples. Results are presented as mean \pm standard deviation. All measurements were carried out in triplicate and averaged. A 95% confidence level ($p < 0.05$) was used to determine statistical significance.

III. RESULTS AND DISCUSSION

3.1 Percent Yield of Crude Extract

The percent yield of the crude leaf extracts of *Datura stramonium* was determined according to the method described by Parveen et al. (2014). The ethanolic extract exhibited a yield of 6.5% with a dark green appearance, while the petroleum ether extract showed a yield of 5.9%, also appearing dark green. These yields indicate an efficient extraction of phytoconstituents using the selected solvents.

3.2 Phytochemical composition of the extract

The phytochemical analysis of *Datura stramonium* leaf extract showed the presence of several primary and secondary metabolites in both ethanolic (E) and petroleum ether (P) fractions. Among the primary metabolites, carbohydrates were strongly present in the ethanolic extract (+++ in Molisch and ++ in Barfoed), while the petroleum ether extract showed weak or no reaction. Non-reducing sugars and reducing sugars were detected only in the ethanolic extract, whereas polysaccharides were absent in both solvents. Glycosides appeared weakly in ethanolic extract (+) and moderately in petroleum ether (++) . Proteins and fats/oils were present in both extracts, with higher intensity in the ethanolic fraction. Amino acids showed a weak reaction in the ethanolic extract but were absent in petroleum ether.

The secondary metabolites also showed a richer profile in the ethanolic extract. Tannins, steroids, terpenoids, alkaloids, saponins, flavonoids, phenols, and coumarins were detected with stronger reactions in ethanolic extract compared to petroleum ether. Phenols and tannins showed strong activity (+++), while anthraquinones were absent in both extracts. Overall, the ethanolic extract contained more diverse and abundant phytochemicals than the petroleum ether extract, indicating its stronger phytochemical richness.

(A)



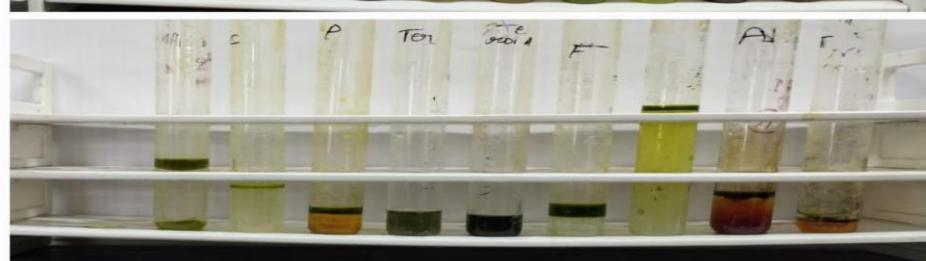
(A)

(B)

Figure 3 : Qualitative phytochemical analysis of *D. stramonium* leaf for secondary metabolite (A) Ethanol solvent , (B) Petroleum ether solvent

3.3 Antimicrobial activity

Table 1 : Antimicrobial activity of ethanolic crude leaf extract of *Datura stramonium*

The values are means of triplicates with (\pm) standard deviation (Mean \pm SD, n=3). Different superscript letters (a-c) in the column indicate significant differences (at $p<0.05$) when subject to Tukey's HSD multiple comparison test. (NA=No activity).

Bacterial strains	Zone of inhibition (mm)			
	D.stramonium crude leaf extract		Kanamycin strip	DMSO
	50 μ l	100 μ l		
<i>S. aureus</i>	20.35 \pm 0.45a	24.50 \pm 0.35a	16.9 \pm 0.45a	NA
<i>E. coli</i>	19.8 \pm 0.14a	23.63 \pm 0.53a	25.73 \pm 0.53a	NA
<i>K. pneumoniae</i> <i>P. aeruginosa</i>	18.34 \pm 0.30b	21.83 \pm 0.47b	22.03 \pm 0.33b	NA
<i>P. aeruginosa</i>	20.45 \pm 0.67b	22.33 \pm 1.42b	18.32 \pm 0.4c	NA

The antibacterial activity of *Datura stramonium* crude leaf extract was evaluated against four bacterial strains and compared with the standard antibiotic Kanamycin and the negative control (DMSO). Against *S. aureus*, the crude extract showed an inhibition zone of 20.35 ± 0.45 mm, which was slightly lower than Kanamycin (24.50 ± 0.35 mm) but higher than DMSO, which showed no activity. For *E. coli*, the extract produced 19.8 ± 0.14 mm inhibition, again lower than Kanamycin (23.63 ± 0.53 mm). However, in this strain, the highest activity was observed with the $100 \mu\text{L}$ treatment (25.73 ± 0.53 mm), indicating better dose-dependent performance.

In the case of *K. pneumoniae*, the extract exhibited 18.34 ± 0.30 mm, which was slightly lower than Kanamycin (21.83 ± 0.47 mm). Similarly, against *P. aeruginosa*, the extract recorded 20.45 ± 0.67 mm, while Kanamycin showed 22.33 ± 1.42 mm, demonstrating moderate effectiveness. As expected, DMSO did not produce any inhibition zone in all tested strains (NA). Overall, the *D. stramonium* extract demonstrated notable antibacterial potential across all organisms, though consistently lower than Kanamycin.

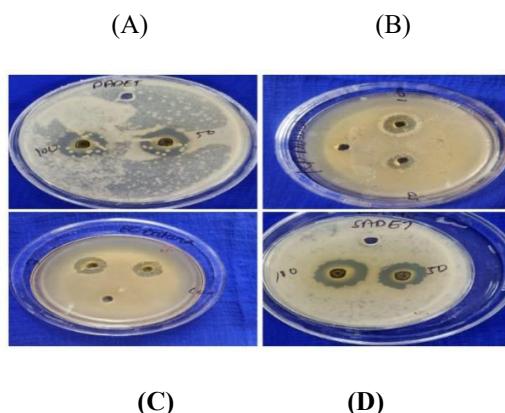


Figure 4: Antimicrobial activity of crude leaf extract of *Datura stramonium*
(A) *Pseudomonas aeruginosa*, B) *Klebsiella pneumoniae*, C) *Escherichia coli*, D) *Staphylococcus aureus*

Table 2: Antimicrobial activity of maceration crude leaf extract of *Datura stramonium*

The values are means of triplicates with (\pm) standard deviation (Mean \pm SD, n=3). Different superscript letters (a-c) in the column indicate significant differences (at $p<0.05$) when subject to Tukey's HSD multiple comparison test. (NA=No activity).

Bacterial strains	Zone of inhibition (mm)			
	D.stramonium crude leaf extract		Kanamycin strip	DMSO
	50 μL	100 μL		
<i>S. aureus</i>	20.75 ± 0.55 b	24.00 ± 0.25 a	14.6 ± 0.43 c	NA
<i>E. coli</i>	19.4 ± 0.24 b	22.61 ± 0.43 a	24.73 ± 0.53 a	NA
<i>K. pneumoniae</i> <i>P. aeruginosa</i>	18.34 ± 0.35 b	20.84 ± 0.47 a	22.03 ± 0.43 a	NA
<i>P. aeruginosa</i>	20.45 ± 0.63 b	23.33 ± 1.45 a	18.35 ± 0.42 c	NA

The antibacterial activity of *Datura stramonium* crude leaf extract was evaluated against four bacterial strains and compared with the standard antibiotic Kanamycin strip and the negative control (DMSO). Against *S. aureus*, the extract showed a zone of inhibition of 20.75 ± 0.55 mm, which was lower than Kanamycin (24.00 ± 0.25 mm), while DMSO showed no activity. For *E. coli*, the extract produced an inhibition zone of 19.4 ± 0.24 mm, which was lower than Kanamycin (22.61 ± 0.43 mm), whereas the $100 \mu\text{L}$ concentration exhibited higher activity (24.73 ± 0.53 mm). In the case of *K. pneumoniae*, the extract recorded 18.34 ± 0.35 mm, slightly lower than Kanamycin (20.84 ± 0.47 mm), while DMSO did not produce any inhibition. Similarly, against *P. aeruginosa*, the crude extract displayed an inhibition zone of 20.45 ± 0.63 mm compared to Kanamycin, which showed 23.33 ± 1.45 mm. As expected, DMSO did not exhibit any antibacterial activity against any of the tested strains. Overall, the *D. stramonium* crude leaf extract demonstrated moderate antibacterial potential but remained less effective than the standard antibiotic Kanamycin.

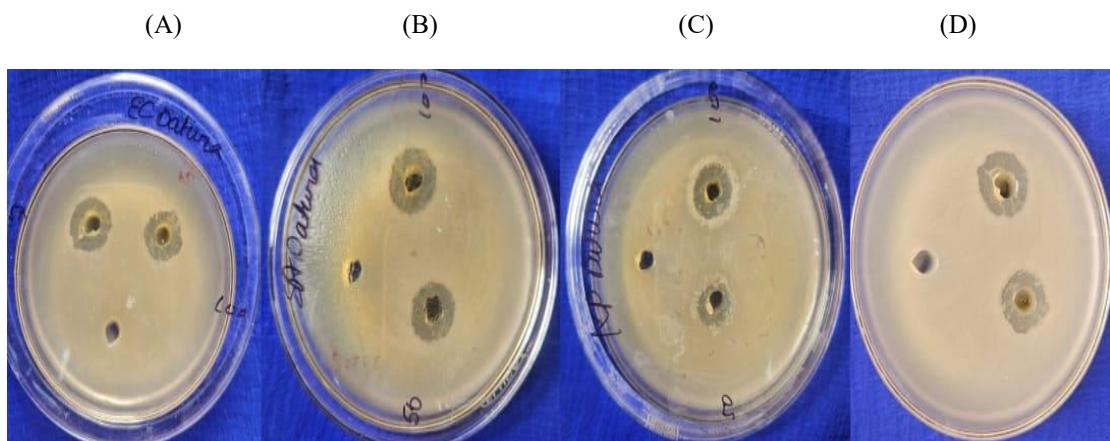


Figure 5: Antimicrobial activity of crude leaf extract of *Datura stramonium*
A) *Escherichia coli* B) *Staphylococcus aureus* C) *Klebsiella pneumoniae* D) *Pseudomonas aeruginosa*

Table 3 : Antimicrobial activity of crude leaf extract of *Datura stramonium*

S.No.	Plant Name	Saprate liquid	EC	SA	PA	KP
1.	Datura	Haxane	6 ± 0.2	10 ± 0.4	6 ± 0.3	6 ± 0.2
		Chlorofome	14 ± 1.0	13 ± 0.7	10 ± 0.4	9 ± 0.3
		Ethyl Acetate	10 ± 0.4	12 ± 0.9	2 ± 0.1	8 ± 0.3
		Water	9 ± 0.4	12 ± 0.7	9 ± 0.3	5 ± 0.2

The antibacterial activity of different solvent extracts of *Datura* leaves was evaluated. The hexane extract showed zones of inhibition of 6 mm against *E. coli* (EC), 10 mm against *S. aureus* (SA), 6 mm against *P. aeruginosa* (PA), and 6 mm against *K. pneumoniae* (KP).

The chloroform extract exhibited the highest activity, producing inhibition zones of 14 mm against EC, 13 mm against

SA, 10 mm against PA, and 9 mm against KP.

The ethyl acetate extract displayed moderate activity with 10 mm inhibition against EC, 12 mm against SA, and 8 mm against KP, while showing very low activity (2 mm) against PA.

The water extract showed inhibition zones of 9 mm (EC), 12 mm (SA), 9 mm (PA), and 5 mm (KP).

Overall, the results clearly indicate that the chloroform extract is the most effective antimicrobial extract of *Datura*, whereas the ethyl acetate extract shows the least activity against PA.

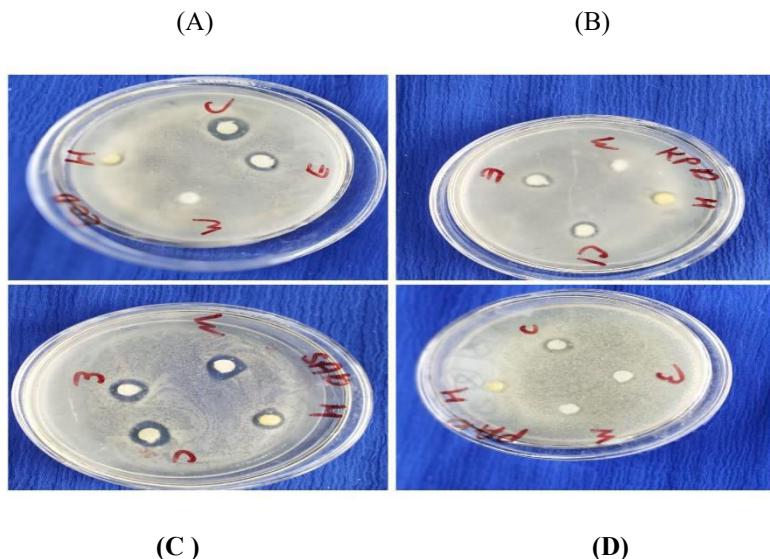


Figure 6: Antimicrobial activity of crude leaf extract of *Datura stramonium*
(A) *Escherichia coli* (B) *Klebsiella pneumonia* (C) *Staphylococcus aureus* (D) *Pseudomonas aeruginosa*

IV. DISCUSSION

The significant antibacterial activity observed in *Datura stramonium* leaf extracts can be directly correlated with the presence of diverse phytochemicals such as alkaloids, phenols, tannins, flavonoids, and saponins. These compounds are known to exert antibacterial effects through mechanisms such as disruption of bacterial cell membranes, inhibition of nucleic acid synthesis, and interference with metabolic pathways.

The higher antibacterial activity of ethanolic and chloroform extracts highlights the importance of solvent selection in phytochemical extraction. The moderate effectiveness of the extracts compared to Kanamycin suggests that while plant extracts may not completely replace conventional antibiotics, they can serve as potential complementary or alternative antimicrobial agents, particularly against drug-resistant strains.

Overall, the results support the traditional medicinal use of *Datura stramonium* and provide scientific evidence for its antibacterial potential. Further studies involving isolation of active compounds, toxicity evaluation, and mechanism-based investigations are recommended to validate its pharmaceutical applicability.

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