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Study the antibacterial and antibiofilm activities of ethanolic extract of Datura stramonium L. against clinical isolates of *Pseudomonas aeruginosa*

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Abstract :

This research investigates the phytochemical composition, antibacterial properties, and antibiofilm efficacy of *Datura stramonium* L leaf extract against clinical isolates of *Pseudomonas aeruginosa*. The active phytochemicals *from D. stramonium* leaves were extracted using a 90% ethanol solvent. Numerous essential oils exhibiting various biological characteristics were found using HPLC analysis. All concentrations of *D. stramonium* ethanolic extracts (15, 30, 45, and 60 mg/ml) exhibited antibacterial efficacy against the investigated pathogens, as indicated by the results of the antibacterial activity against clinical isolates of *P. aeruginosa*. The tissue culture plate method (TCP), a quantitative technique for biofilm development, demonstrated that all tested isolates were capable of biofilm formation. Despite a reduction in biofilm formation from moderate and strong to weak, the antibiofilm activity of the extracts indicated no significant difference in biofilm formation before and after the application of the extracts. The Cohen's d test was utilised to ascertain the Effect Size. The cell division of onion root tips is cytotoxically influenced by ethanol extracts; in vivo, the mitotic index (MI) of Allium cepa diminished to 79.5% at 15 mg/ml and increased to 100% at 30 mg/ml, recording 102.60%. Chromosomal abnormalities were observed. The ethanolic extract of Datura may serve as an antibacterial and antibiofilm agent against clinical isolates of *P. aeruginosa* and exhibit cytotoxic effects.

Keywords: Datura Antibiofilim Cytotoxic Pseudomonas aeruginosa TCP Mitotic index.

دراسة الفعالية الضد بكتيرية وضد الغشاء الحيوي للمستخلصات الايثانولوية للداتورة ضد عزلات سريرية من الزوائف الزنجارية

رشا كريم محمد[1] ، علياء رزوقي حسين[2]
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مستخلص:

بحث هذا البحث في التركيب الكيميائي النباتي والخصائص المضادة للبكتيريا وفعالية مضادّة للأغشية الحيوية لمستخلص أوراق نبات الداتورة. ضد العزلات السريرية لبكتيريا الزائفة الزنجارية Pseudomonas aeruginosa.. وتم استخلاص المركبات الكيميائية النباتية الفعالة من أوراق الداتورة باستخدام مذيب الإيثانول بتركيز ٪90. وأظهر تحليل HPLC وجود العديد من الزيوت الأساسية التي تمتلك خصائص حيوية متنوعة. أوضحت نتائج النشاط المضاد للبكتيريا أن جميع تراكيز المستخلص الإيثانولي لنبات الداتورة (15,30,45, 60ملغم/ مل) أظهرت فعالية مضادة للبكتيريا ضد الممرضات المدروسة من عزلات الزائفة الزنجارية السريرية. وبيّنت طريقة اطبق لزراعة الأنسجة (TCP) بوصفها تقنية كمية لتكوين الأغشية الحيوية أن جميع العزلات المختبرة كانت قادرة على تكوين الغشاء الحيوي. وعلى الرغم من انخفاض تكوين البيوفيلم من مستويات متوسطة وقوية إلى ضعيفة، فإن النشاط المضاد للغشاء الحيوي للمستخلصات لم يُظهر فرقًا معنويًا في تكوين الغشاء الحيوي قبل وبعد تطبيق المستخلصات. تم استخدام اختبار Co-hen's d لتحديد حجم التأثير. كما أظهرت مستخلصات الإيثانول تأثيرًا سامًا خلويًا على انقسام خلايا قمم جذور البصل؛ إذ انخفض مؤشر الانقسام الخيطي (MI) لنبات البصل في الظروف الحية إلى ٪79.5 عند تركيز 15 ملغم/ مل وارتفع إلى ٪100 عند تركيز 30 ملغم/ مل مسجّلًا ٪102.60. ولوحظت تشوهات كروموسومية. تشير النتائج إلى أن المستخلص الإيثانولي لنبات الداتورة قد يعمل كعامل مضاد للبكتيريا ومضاد للأغشية الحيوية ضد العزلات السريرية لبكتيريا الزائفة الزنجارية، إضافةً إلى امتلاكه تأثيرات سامة خلوياً..

الكلمات المفتاحية : داتورة، ضد الغشاء الحيوي، سام خلويا، الزائفة الزنجارية، طريقة الطبق لزراعة الانسجة، معامل الانقسام الخلوي.

Introduction

Plants with medicinal properties are utilized to mitigate the symptoms of many microbial and non-microbial conditions owing to their advantageous effects on medical therapy. Medicinal plants have achieved global acceptance and appeal in medical therapy due to their low toxicity in therapeutic uses, availability, affordability, and reliability. Undoubtedly, the initial substance employed in alternative medicine to treat various ailments was botanical matter. Numerous plants demonstrate antimicrobial, antioxidant, anti-infective, and tumour fighting agents characteristics [1, 2]. Akinyemi et al. [3] assert that herbal therapy is extensively utilised as a fundamental element of healthcare in numerous countries. Considering the aforementioned information and the recorded antimicrobial strains. The Solanaceae family includes the bushy annual plant Datura stramonium, also called thorn apple, prickly burr, jimson weed, moon flower, devil's weed, devil's cucumber, and devil's trumpet. Specific traditional chemical constituents included in this plant have fundamental applications that are commonly utilised. The primary psychotropic chemicals found in all D. stramonium species are alkaloids, scopolamine, tannins, flavonoids, saponins, and phenols. These chemicals exhibit resistance to germs and cancer and are incapable of proliferation. Owing to their remarkable attributes, they may also be prescribed to patients with diabetes, cancer, and asthma [5]. Bacterial biofilms, a virulence factor that facilitates persistent and recurrent infections, exhibit significant resistance to both pharmacological treatments and the host's immunological response [6]. Biofilm inhibition is a crucial therapeutic target for the treatment of several bacterial infections. Identifying naturally occurring plant combinations that can inhibit biofilm development is a possible alternative [7]. The objectives of this work were to analyse the phytochemical constituents and evaluate the ability of D. stramonium ethanolic leaf extract to inhibit bacteria and biofilms in clinical isolates of P. aeruginosa. The cytotoxic effect was additionally investigated.

Materials and Methods

2.1. Obtaining Samples

The plant was gathered from AL-Jadiryaa city, as depicted in figure 1 at the Baghdad College of Science. After ten days of drying at room temperature, the leaves were crushed with a sanitized pestle and mortar, and a sieve was used to reduce the size of the powdered plant. The fine powder was kept at room temperature in an airtight container to prevent the effects of humidity [8].

Figure1. The leaves of D. stramonium

2.2. Ethanolic Extract Preparation

According to Girmay [9], leaves were removed with a variety of alterations. Five hundred grammes of powdered leaves were repeatedly extracted with 90% ethanol using a separating funnel and stored in a dark room for five days. The extracts were subsequently filtered using Whatmann filter paper No. 1 and concentrated at 35°C with a vacuum rotary evaporator. After that, the mixture was weighed and put into glass flasks.

2.3. Making Datura extracts at different concentrations

To create stock solutions, 100 milli-

liters of distilled water were combined with ten (10 g) grams of plant powder for leaves. A given volume from the stock solution (100 mg/ml) was then combined with the following equation, C1*V1 = C2*V2, to create various concentrations using distilled water and the formula [10].HPLC: Method of operation: Varian stationary phase XFs 3µ C-18250*4.6mm, practical diameter 5 µm. Phase of mobility: 50:50 acetoniytile: water, 0.8 ml/min flow rate, 35°C temperature, 280 nm wavelength, and 25 µ l sample volume.

2.4. Isolated bacteria

The University of Baghdad's Biology Department provided bacterial isolates of P. aeruginosa that were resistant to antibiotics and isolated from wounds and burn illnesses. Biochemical assays and the Viteck 2 system were used to establish the clinical isolates' identities.

2.5. Antimicrobial qualities

The antibacterial activity of four concentrations of crude leaf ethanolic extracts of D. stramonium (15, 30, 45, and 60 mg/ml) was assessed using the diffusion technique on agar wells. A culture of Pseudomonas aeruginosa (about 10^6 CFU/ml) is inoculated onto Mueller Hinton agar (MHA) plates with a sterilised cotton swab. The contaminated plates were allowed to air dry at ambient temperature for 20 minutes. Subsequently, six (6) mm diameter wells were created in the agar via a sterilised cork borer, and 100 µl of each concentration extract was introduced into the wells. Ethyl alcohol served as a control. The plates were incubated at 37°C for eighteen hours. The existence of a distinct inhibition zone encircling each well indicated antibacterial activity. The diameter of this area was measured and documented in millimetres [11]. This assay was conducted on two occasions.

2.6. Assay for biofilm development

The tissue culture plate method, as described by Atshan et al. [12], was utilised as a quantitative tool to assess the biofilm production capacity of P. aeruginosa isolates. Bacteria were cultivated in 96-well tissue culture plates using Tripticase soy broth (TSB) supplemented with 1% glucose. The samples were subsequently incubated for 24 hours at 37°C in an aerobic atmosphere. The adherent bacterial cells in each well were fixed for 20 minutes

with 200µl of 100% ethanol following the rinsing of non-adherent cells three times with distilled water (D.W). The plates were dried and cleared. Following a 15-minute incubation of the adhering cells with 0.1 percent crystal violet in 200 µl, the surplus stain was eliminated through washing. Following the rinsing of the plates with distilled water, the adhered cell-bound crystal violet dye was solubilised in 200 µl of 96% ethanol each well. The absorbance at 490 nm was assessed via a spectrophotometer. The absorbance of wells with sterile TSB served as a negative control in the triplicate experiment. Utilising the optical density (O.D.) of the biofilm relative to the control and the measurements, the isolates were classified and categorised as presented in Table 1.

Table 1: The biofilm degree of P. aeruginosa isolates [12]

Mean of OD	Biofilm degree
OD ≤ ODc	Non-adherent
ODc < OD ≤ 2*ODC	Weakly adherent
2*ODc < OD ≤ 4*ODc	Moderately adherent
4*ODc < OD	Strongly adherent

OD= optical density, ODc= cut off value

2.7. Antibiofilm activity of crude ethanolic extract of Datura

The identical procedure outlined previously was employed to assess biofilm inhibition. Subsequently, 100 µl of alcoholic leaf extract (20 mg/ml) of *D. stramonium* was introduced to each well. After a 24-hour incubation at 37°C, all wells were cleaned and stained, and an ELISA reader measured the absorbance at 490 nm. Control wells containing crystal violet were utilised to assess binding to the bacterial culture medium.

2.8. Investigating the cytotoxic effects of alcoholic extracts

Onion bulbs were purchased at the local market. The desiccated scales and aged roots were extracted and deposited in vials containing tap water for

germination. After 48 hours, when the roots attained a length of 2-3 cm, the root meristem was exposed to varying concentrations of leaf extract during the peak mitotic phase. The cytotoxicity of four doses (0.0, 15, 30, 45, and 60 mg/ml) was evaluated using the onion test over a duration of four hours [13,14]. The technique employed by Sharma and Sharma [15] for the preparation of mitotic squash root tips was enhanced. For each parameter, a minimum of five micro-glass slides were produced, and each experiment was conducted three times.

2.9. Statistical Analysis

The gathered data and paired samples were analysed with the Statistical Package for Social Sciences version 26. The T test was employed to compare data on biofilm formation prior to and following extract administration. The effect size of biofilm inhibition after the application of datura ethanolic extract was assessed using Cohen's d test.

Results and discussion

As indicated in table (2) and figure (2), HPLC investigation indicated that the extract of D. stramonium leaves contains several essential oils (trepans), such as -picene, caryophyllene, chamazulene, and p-cymene.

3.1. Antibacterial activity

As illustrated in figure 3, the experiment's findings proved that D. stratomonium ethanolic extracts at all concentrations (15, 30, 45, and 60 mg/ml) exhibited antibacterial effectiveness against the examined pathogens. Additionally, this ethanolic extract's impact varied with dose.

The results demonstrated that the diameter of the inhibitory zone expanded with higher extract concentrations, signifying that the effect intensified proportionally with increased concentrations. The inhibitory zone diameter varied between 11 and 23 mm. Our findings concurred with those of Kumar et al. [16], who assessed the ability of D. stramonium leaf ethanolic extract to inhibit the growth of three bacterial strains: Pseudomonas aeruginosa, Bacillus amyloliquefaciens, and Escherichia coli. Pseudomonas aeruginosa exhibited antibacterial action, but neither B. amyloliquefaciens nor E. coli did. The flavonoids found in D. stra-

monium leaves showed the strongest antibacterial action against P. aeruginosa, according to Sharma and Sharma [17]. Furthermore, they discovered that methanolic leaf extract exhibits enhanced antibacterial efficacy against P. aeruginosa, E. coli, and S. aureus. The current findings were consistent with those of Khalaf and Hussein [18], who examined the antibacterial activity of eucalyptus and sesame seed oil against P. aeruginosa. They demonstrated that sesame oil exhibited no antibacterial activity in both the disc diffusion method and the agar well diffusion method. They demonstrated that eucalyptus oil exhibited antibacterial activity against clinical isolates of P. aeruginosa at doses ranging from 20% to 100%.

Our results opposed those of Hussain et al. [19], who reported that P. aeruginosa demonstrated resistance to methanol extracts from D. inoxia leaves, stems, roots, and seeds at a dosage of 2 mg. Nonetheless, the identical extracts exhibited inhibitory effects against Salmonella typhi, Escherichia coli, Klebsiella pneumoniae, Proteus species, Streptococcus pneumoniae, and Staphylococcus aureus, employing a well diffusion approach with inhibition zones ranging from 6 mm to 24 mm.

3.2. Formation of Biofilms

Table 3 and Figure 4 suggest, the results of the tissue culture plate method used to detect biofilm formation by the P. aeruginosa clinical isolates showed that every isolate possessed the ability to generate biofilm., with two (S1&S6) exhibiting moderate biofilm formation and three (S2, S7, and S8) exhibiting strong biofilm formation.

Kamali et al. [20] reported that 83.75% of P. aeruginosa clinical isolates produced biofilm, categorised as follows: 16.25% of isolates exhibited robust biofilm formation, 33.75% shown moderate biofilm production, and 33.75% displayed weak biofilm formation, whereas 16.25% did not create any biofilm. The results were in reasonable concordance with the current study, as our local clinical isolates exclusively generate robust and moderate biofilm.

Table (2). Datura stramonium leaf extract was analyzed using HPLC

	Compounds	Ret. Time of Test (min) Area of Test	Ret. Time of Test (min) Area of Test	Ret. Time of standard	Area of Standard	conc. of test
1	α-picene	3.257	10686208	3.232	1639319.625	32.59342424
2	caryophylene	4.132	828848.625	4.273	5078418.5	0.8160499425
3	chamazulene	4.29	3462834.5	4.932	2144605	8.073361994
4	p-cymene	7.315	4432682	6.498	12517030	1.770660452

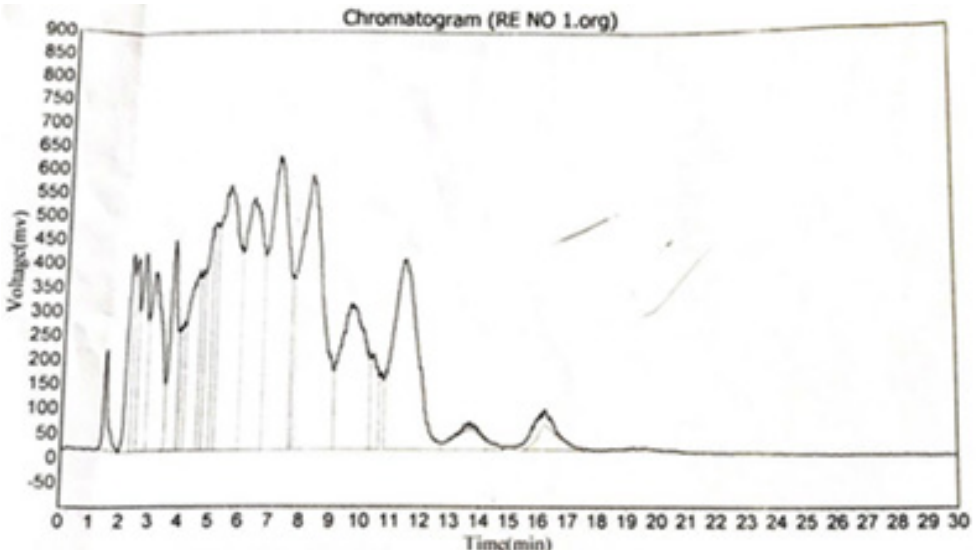

Figure (2). HPLC profile of essential oils of leaves extract of *D.stramonium*

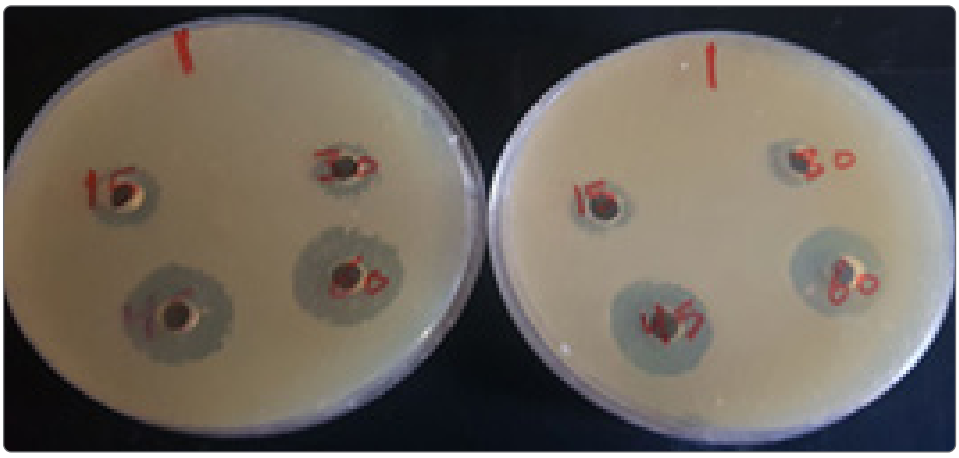

Figure 3: Antibacterial activity of datura

Table (3): Biofilm formation of *P.aeruginosa* clinical isolates

Isolates of *P. aeruginosa*			Control			
Number		S1	S2	S6	S7	S8
R1	0.126	0.521	0.158	0.270	0.548	0.031
R2	0.182	0.383	0.174	0.450	0.224	0.072
R3	0.201	0.472	0.184	0.174	0.664	0.065
Mean	0.17	0.46	0.17	0.30	0.48	0.06
SD	0.04	0.07	0.01	0.14	0.23	0.02
Ability	M	S	M	S	S	-

ODc =0.06 2*ODc=0.12 4*ODc=0.24

3.3. Antibiofilm action

As seen in table 4 and figures 4 and 5, the results of this test showed no discernible change in the production of biofilms before and after the use of natural alcoholic extracts. Despite this, one isolate went from producing biofilm to not producing any, while four isolates went from strong or moderate to weak stages. This might be because, in the antibiofilm activity assay, we employ the extract as a crude material rather than refined components.

Table 6: Antibiofilm activity of datura alcoholic extract against clinical isolates of *P.aeruginosa*

Isolates of *Pseudomonas aeruginosa*			Control			
Number		S1	S2	S6	S7	S8
R1	0.253	0.288	0.175	0.244	0.315	0.125
R2	0.185	0.305	0.228	0.291	0.319	0.295
R3	0.232	0.244	0.216	0.305	0.266	0.230
Mean	0.22	0.28	0.21	0.28	0.30	0.22
SD	0.03	0.03	0.02	0.03	0.02	0.07

ODC=0.22 2*ODC=0.44 4*ODC=0.88.

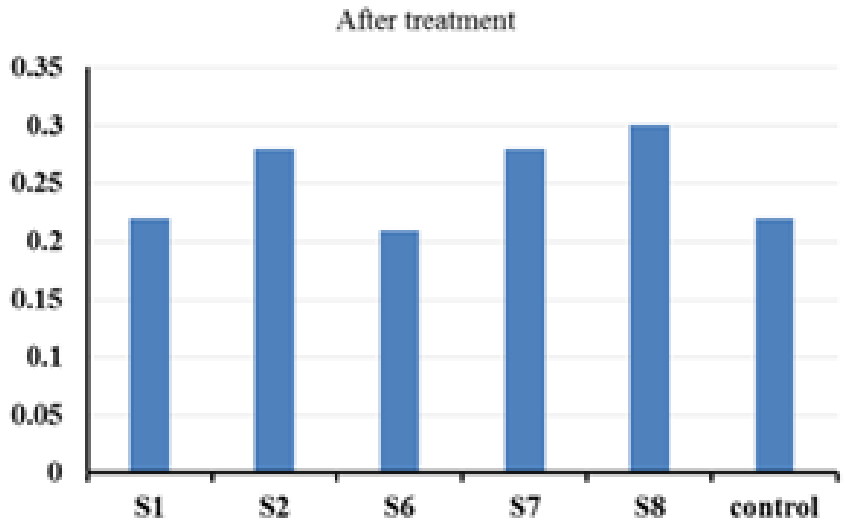

Figure 4: Biofilm formation ability for *P .aeruginosa* isolates

The Effect Size was determined using Cohen's d test. According to table 5, this test revealed that the extract's effect size was medium both before and after use.

Methanolic caper extract significantly inhibited the production of biofilms and exopolysaccharides in Proteus mirabilis, Serratia marcescens, *P. aeruginosa*, and E. coli [21].

Table5: The Effect Size as calculated by Cohen,s d test

Pseudomonas aeruginosa	**Cohen test**					
Isolates Number	S1	S2	S6	S7	S8	Medium
Before (mean)	0.17	0.46	0.17	0.30	0.48	0.51
After (mean)	0.22	0.28	0.21	0.28	0.30	

3.4 In vivo mitotic index (MI) of *Allium cepa* onion root tips treated to alcohol-based datura extracts

In Figure 6 and Table 6, the mitotic index dropped sharply for all datura leaf extract dosages, showing 7.67, 6.1, 7.87, 7.07, and 7.07% for the control (15, 30, 45, and 60 mg/ml, respectively). The mitotic index decreased to 79.5% at 15 mg/ml and increased to 100% at 30 mg/ml, registering 102.60%. (Table 7). Extracts that

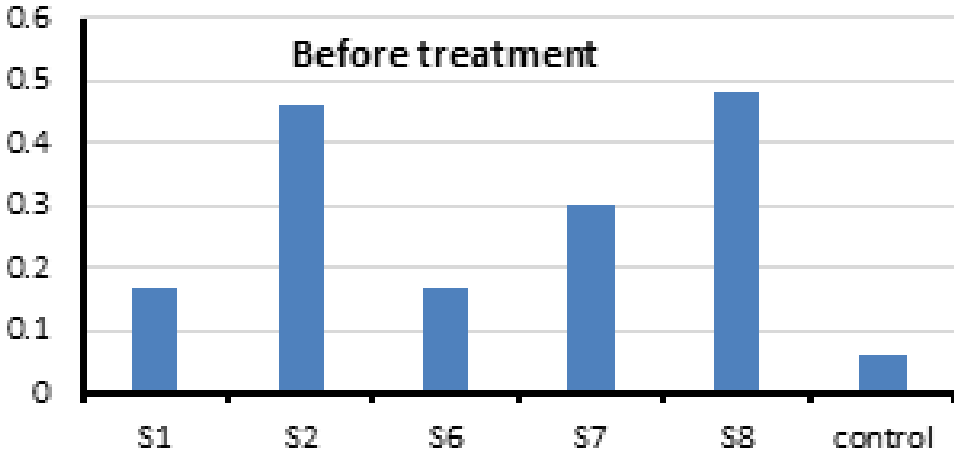

Figure 5: Antibiofilm activity of datura alcoholic extract against clinical isolates of *P.aeruginosa*

include certain compounds that have toxic or semi-lethal effects and lower the mitotic index to 50% or below are the source of the decline. These compounds include, for example, essential oils that include many fractions such as p-cymene, caryophylene, chamazulene, and α-picene, and alkaloids such as hyoscyamine, scopolamine, and atropine in which DNA topoisomerase-I interacted. This could impede the process of cell division by preventing the cell nucleus from entering the phase [22]. Thus, when the extract concentration rose, the MI fell.

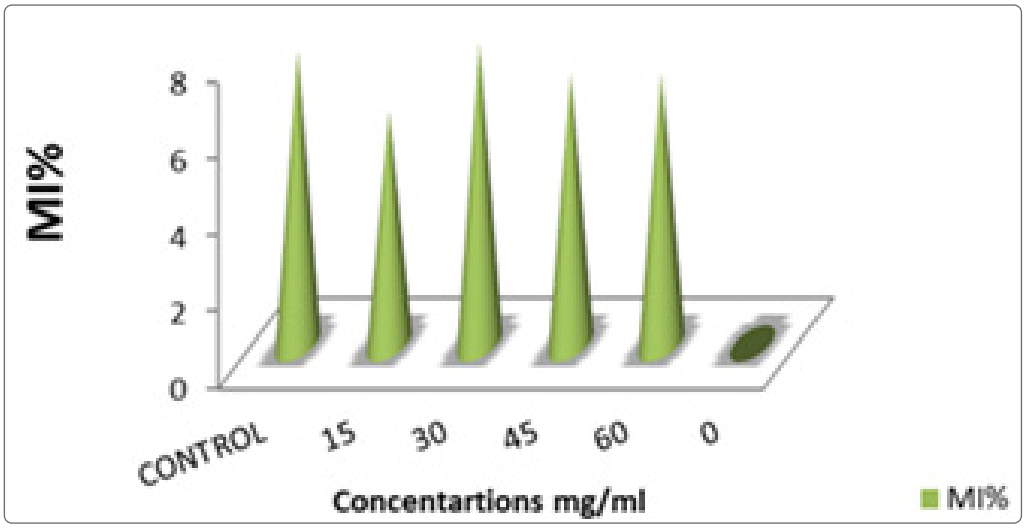

Figure 6 Mitotic index%

Table 6. After four hours of treatment with various quantities of an alcoholic extract of Datura leaves, the mitotic index, phases, and percentage of abnormality in A. cepa root

Concentration mg/ml	MI%	Phase index %				Chromosomal aberration %		
		Prophase	Metaphase	Anaphase	Telophase	C-metaphase	Nuclear-lesion	Micro nuclei
Control	7.67	26.98	23.05	14.21	35.74	0.00	0.00	0.00
15	6.1	26.35	23.93	15.16	35.68	7.44	14.66	0.00
30	7.87	24.86	21.18	14.00	40.22	0.00	17.33	1
45	7.07	24.47	27.37	12.55	35.58	0.00	21.66	2
60	7.07	27.59	22.55	12.46	40.90	0.00	32.33	2

Table7. After four hours, the phases of mitotic division (%control) were observed in the onion root tips that had been exposed to various concentrations of the alcoholic

Concentration mg\ml	MI%	Prophase%	Metaphase%	Anaphase%	Telophase%
15	79.5	97.66	103.81	106.68	99.80
30	102.60	92.25	91.88	98.52	112.53
45	92.17	92.14	118.74	88.31	99.55
60	92.17	102.26	97.83	87.67	114.43

- **Prophase**

According to Table 6, the prophase index recorded was 26.98, 26.35, 24.86, 24.47, and 27.59% in the control, 15, 30, 45, and 60 mg/ml, respectively. In 15 and 30 mg/ml, the percentage of control dropped to 92.25 and 92.14%, respectively, but in 60 mg/ml, it rose to 100%, recording 102.26% (table 7). Figure 7-E illustrates the presence of certain aberrant cells in this stage, including those with unusual cell shapes.

Metaphase: According to table 6, the control group's metaphase index readings were 23.05, 23.93, 21.18, 27.37, and 22.55% at 15, 30, 45, and 60 mg/ml, respectively. Table 7 indicates that the percentage of control dropped to 91.88% at 30 mg/ml.

Telophase and Anaphase:

Anaphase index readings in the control group were 14.21, 15.16, 14, 12.55, and 12.46%, respectively (15, 30, 45, and 60 mg/ml). The control group's telophase index was 35.74 %, whereas the concentrations (15, 30, 45, and 60 mg/ml) were 35.68, 40.22, 35.58, and 40.90 %, respectively (table 6).

Figures 7-A, B, C, D, E, F, G, and H illustrate how the alcoholic extract of the datura leaves under study was tested for cytotoxicity using the onion test. The onion test demonstrated the existence of multiple cytological abnormalities in cells, such as nuclear lesion (chromatin lesion), multinucleated cells, and aberrant cell shape, when compared to control. This crucial in vivo technique predicts potential DNA damage in humans by having the roots grow in close proximity to the material of interest. In addition to producing a variety of nuclear abnormalities, including nuclear lesions (Fig 7D), the most prevalent aberration in prophase and interphase, this extract dramatically decreased MI in onion meristematic cells. The incidence of nuclear lesion increased in proportion to higher concentrations. The toxicity of the detected concentration is demonstrated in Figure 6, where greater dosages could dramatically inhibit cell proliferation in Allium root tip cells [23]. Higher concentrations of Datura alcoholic extract may disrupt cellular DNA replication and impede cell growth. Single and multiple lesions were the most preva-

lent abnormalities in prophase and interphase cells. A halt in the S phase of the cell cycle, when DNA strands are replicated, results in lesions. Prophase lesions are common in irradiated cells, although it is still unknown where they are located and how they are repaired [24].

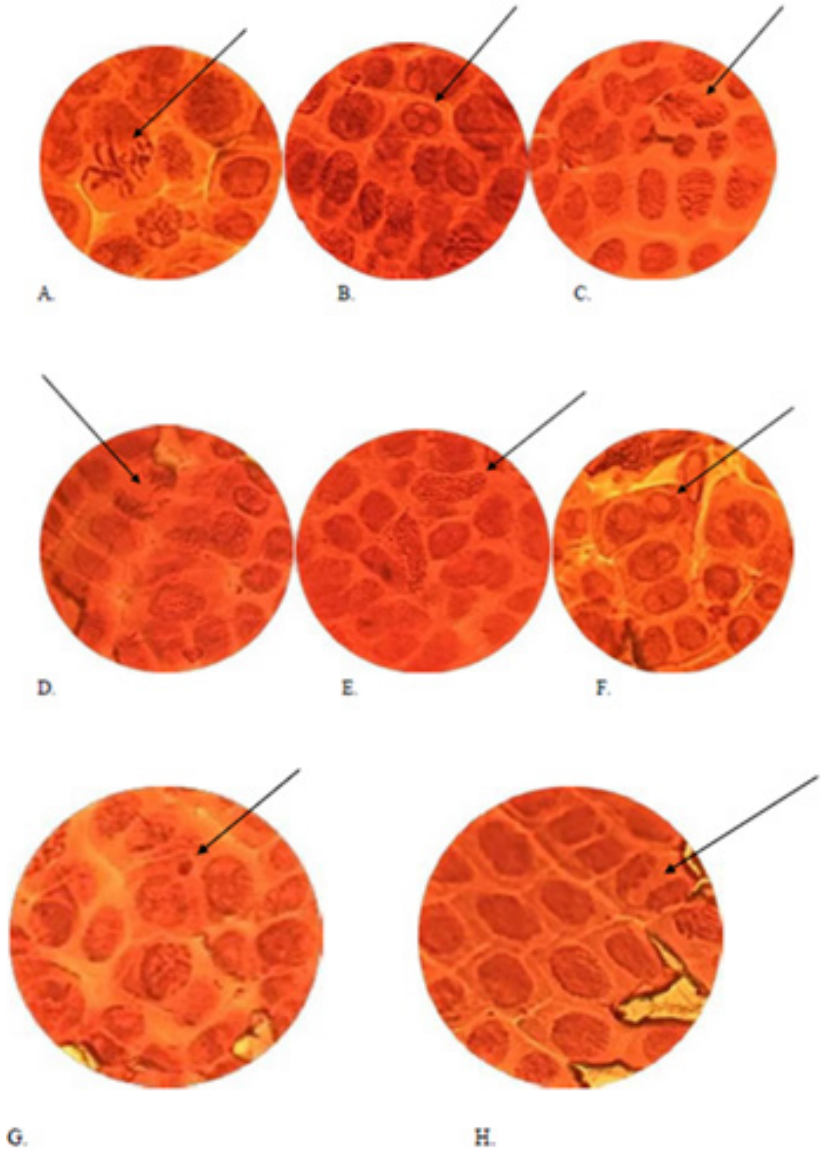

Figure 7 : cytotoxic activity

A. Arrested cell in metaphase
B. Multiple cells with nuclear lesion
C. Abnormal cell shape in Anaphase
D. Late chromosome in Anaphase
E. Abnormal cell shape in prophase
F. Abnormal cell with two nuclei
G. Micronucleus cell
H. Anaphase not completely separa

Discussion:

Jimsonweed, or *D. stramonium*, is a plant that has long been utilized for its therapeutic qualities, such as its analgesic, antibacterial, and anti-inflammatory benefits. Numerous bioactive substances found in the plant, including flavonoids, phenolic compounds, and alkaloids (such as hyoscyamine, scopolamine, and atropine), have been demonstrated to have antibacterial properties [25]. *Pseudomonas aeruginosa* is a well-known opportunistic pathogen that is challenging to treat due to its resistance to several medicines and capacity to build biofilms on host tissues and medical equipment [26].

The duration and chronicity of *P. aeruginosa* infections are greatly increased by biofilms, which are collections of bacterial cells covered in an extracellular polymeric matrix that the bacteria manufacture on their own [27]. Therefore, a crucial field of research is the creation of novel drugs that can both prevent bacterial growth and interfere with the production of biofilms.

Pseudomonas aeruginosa is among the bacterial pathogens against which the ethanolic extract of *D.stramonium* has shown encouraging antibacterial qualities. According to studies, chemicals found in extracts from different plant components, including leaves, seeds, and flowers, can break down bacterial cell membranes or stop vital biological functions [28]. Standard techniques like the disk diffusion assay and minimum inhibitory concentration (MIC) tests are frequently used to evaluate the antibacterial activity.

According to a number of studies, *D. stramonium's* ethanolic extract has strong antibacterial properties against both Gram-positive and Gram-negative bacteria. Nasir et al. [29], for instance, showed that the ethanolic extract of *D. stramonium* suppressed *P. aeruginosa* growth in vitro. According to Bashir et al. [25], the mechanism underlying this antibacterial effect is probably complex and includes destruction of the bacterial cell wall, interference with protein synthesis, and inhibition of enzymes necessary for bacterial growth. Alkaloids, which have been demonstrated to have antibacterial properties in the

past, are thought to be active constituents in the ethanolic extract [30].

The ethanolic extract of *D. stramonium* has been studied for its capacity to break up bacterial biofilms in addition to its antibacterial properties. Because it improves bacterial resistance to medicines and host immunological responses, biofilm development is a crucial component of *P. aeruginosa's* pathogenicity [26]. One useful characteristic in the creation of innovative antimicrobial treatments is the capacity of plant extracts to prevent the formation of biofilms.

The ability of different plant extracts to break up *P. aeruginosa* biofilms was evaluated by Xie et al. [31], who discovered that *D. stramonium* extract was very successful in lowering biofilm biomass. It was demonstrated that the extract disrupted the initial phases of biofilm formation, presumably by preventing the synthesis of extracellular polymeric substances (EPS), which are essential for maintaining the integrity of biofilms. Datura extracts decreased biofilm development in a concentration-dependent manner, with greater concentrations resulting in considerable biofilm eradication, according to a different study by Bashir et al. [25].

The alkaloid component of *D. stramonium* extract, especially scopolamine, which has been demonstrated to have biofilm-inhibitory effects in other bacterial species, may be responsible for the extract's ability to destroy biofilms [32]. Quorum sensing pathways, which control the production of biofilms in P. aeruginosa, may be modified by scopamine and similar substances [33]. Furthermore, the extract might cause biofilm-associated bacteria to experience oxidative stress, which would harm the biofilm matrix and increase their vulnerability to antimicrobial treatments. z

The production of reactive oxygen species (ROS), which can result in oxidative stress and mitochondrial damage, has been connected to the cytotoxic effects of Datura extracts on cells. Research has demonstrated that Datura extracts can cause DNA damage and lipid peroxidation in human cells, pointing to a cellular oxidative damage-based mechanism of toxicity [34]. Apoptosis (planned cell death) or necrosis may be the outcome of ROS-in-

duced damage to cellular constituents such as lipids, proteins, and nucleic acids.

According to certain research, Datura extracts may cause cultured cells, especially cancer cell lines, to undergo apoptosis. These investigations have demonstrated that Datura species enhance the activity of caspases, which are essential enzymes in the apoptotic process [35]. This implies that Datura may be investigated for its anti-cancer qualities, contingent on the concentration and the particular plant species or extract utilized. However, healthy cells exposed to high concentrations of Datura compounds also exhibit cellular damage and apoptosis, demonstrating that Datura's cytotoxicity is not just confined to cancer cells [36].

Conclusion

Ethanolic leaf extracts from D. stramonium L. exhibit strong antibacterial and antibiofilm properties, especially against clinical isolates of P. aeruginosa that are resistant to a wide range of antimicrobial drugs. According to these results, *D. stramonium* may be a viable natural remedy for illnesses brought on by *P. aeruginosa* that is resistant to drugs. It may also aid in the creation of novel treatment approaches that target bacterial biofilms.

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