IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

Exploring the Potency of Leaf Extracts from Selected Medicinal Plants in Different Solvents: A Comparative Analysis of Their Antibacterial Activities against E. coli Bacteria

[1]Shiv Kumar, [2]Sanjay Bhardwaj, [3]Gazala Praveen
[1]Assistant professor, [2]Professor, [3]Assistant Professor
[1]Department of Chemistry, S.S.V. College, Hapur
[1]CCS University Meerut, India.

Abstract : Plants contain the capacity to produce a diverse array of bioactive chemicals that hold pharmacological importance, either as possible therapeutic agents or as significant resources for the exploration of new drugs. The incorporation of naturally occurring substances continues to be an ongoing imperative within the realm of pharmaceutical investigation. This study aimed to compare the antibacterial characteristics of four medicinal plants belonging to distinct plant groups. The plant species identified in the study were *Adhatoda vasica, Datura innoxia, Calotro pisprocera,* and *Tridex procumbens.* The extraction process involved the use of three solvents: ethanol, chloroform, and acetone. These solvents were selected for their ability to yield leaf extracts from the four plant samples by chromatography. The extracts demonstrated different levels of effectiveness in inhibiting Escherichia coli bacteria. The choice of solvent employed in the extraction process exerted a significant influence on the outcomes obtained. The chloroformic extract of *T. procumbens* demonstrated the most potent antimicrobial activity, while ethanolic extract of *Datura innoxia* showed the most persistent antimicrobial activity.

IndexTerms **-** *Escherichia coli*; *Adhatoda vasica, Datura innoxia; Calotropis procera; Tridex procumbens;* Antimicrobial activity; Leaf extraction; Tropane alkaloids; Zone of inhibition.

1. INTRODUCTION

In today's era of increasing antibiotic resistance and the continuous emergence of new and resilient bacterial strains, the search for alternative sources of antimicrobial agents has become paramount. Medicinal plants, rich in bioactive compounds, offer a promising avenue for the development of novel antibacterial agents. This research paper embarks on a journey to systematically investigate and compare the antibacterial potential of selected medicinal plants: *Adhatoda vasica, Datura innoxia, Calotropis procera,* and *Tridex procumbens*, against E. coli bacteria. By delving into the intricate realm of natural compounds and their biological activities, this study aims to contribute to the expanding knowledge of potential alternatives to conventional antibiotics. Through a meticulous exploration of the antibacterial properties of these plants, we endeavor to uncover new insights that could pave the way for innovative therapeutic strategies in the ongoing battle against bacterial infections. During secondary metabolism, phytochemicals are produced by plants, which are responsible for numerous activities not only occurring in the plant itself but also in other organisms. These are able to perform potential treatments for a wide range of diseases against both plants and animals [1–6]. *D. innoxia* is a member of the family *Solanaceae.* The *Solanaceae* family contains a variety of medicinally important and environmentally friendly plants found worldwide. The active constituents of *D. innoxia* are tropane alkaloids such as atropine, hyoscyamine, and scopolamine. Scopolamine is the main alkaloid. Alkaloid makes up 0.5% of the leaves. Scopolamine is a hypnotic medication [7, 8]. In the context of ample applications of antibacterial materials obtained from plants in diverse fields, development is continually progressing [9–11]. In India, it is used to treat epilepsy, hysteria, insanity, heart conditions, fever with catarrh, diarrhea, skin conditions, and other conditions. The crushed leaves of *D. innoxia* are applied as a poultice to alleviate pain [12]. E. coli (26.0 ± 1.4 mm, MIC of 3.125 mg/ml) and *Micrococcus luteus* (23.8 ± 1.6 mm, MIC of 6.25 mg/ml) has also been tested for the potent reducing, antioxidant, antimicrobial, and cleaning properties of *D. innoxia*. Gas chromatography mass spectrometry has been used to analyze a number of photochemicals found in *D. innoxia*, including scopolamine, heneicosanoic acid, 11, 14, 17-eicosatrienoic acid, methyl (Z)-5, 11, 14, 17-eicosatetraenoate, and others. In addition, FTIR is used to analyze functional groups like amines, esters, lactones, and terpenes. Withanolides (steroid glycosides) and tropane alkaloids were discovered by proton NMR spectroscopy [13]. The presence of phenolic group (72.35 ± 0.52 mg GAE/g), flavonoid group (29.21 ±1.25 mg EQ/g), and DPPH radical cleaning action (IC50 value 91.398 µg/ml) was found highest in the ethanolic extract of D. innoxia leaves as compared to the extract prepared by methanol, ethanol, hexane, and

chloform. This study also demonstrated a strong correlation between antioxidant activity with total phenolic (DPPH, R2 = 0.41; PPM, R2 = 0.25) and total flavonoid contents (DPPH, R2 = 0.39; PPM, R2 = 0.23). E. coli and *S. aureus* have been successfully tested for it [14]. *D. stramonium agglutinin* demonstrated that four human glial tumor cells had a decreased rate of proliferation. This clearly demonstrates the medicinal significance of *D. stramonium* for the non-toxic treatment of gliomas [15]. Tropane alkaloids are produced by the various members of the *Datura genus, i.e., D. innoxia, D. stramonium,* and *D. tatula*. The hairy roots of all of these have been treated with 0.1 m M of acetyl salicylic acid, and *D. tatula* showed the highest HS (hyoscyamine) content (17.94 ± 0.14 mg/g D.W.) [16]. The antioxidant and antimicrobial properties of the methanolic extract of *D. stramonium* seeds against *B. subtilis, E. coli, P. aeruginosa,* and *S. aureus* have been demonstrated, and the presence of alkaloids, flavonoids, amino acids, tannins, saponins, carbohydrates, and terpenoids was confirmed. *E. coli* and *P. aeruginosa* demonstrated the maximum and minimum bactericidal behavior, respectively. Hence, the seeds of *D. stramonium* were suggested to be used in the production of health supplements [17]. It was also concluded that the plant as a whole can be used to kill bacteria and synthesize drugs [18]. The toxicity of leaves and seeds of *D. innoxia* in aqueous and methanolic extracts on rats was also investigated, and results demonstrated that the toxicity of both is nearly identical. Up to 40 mg/kg of the extracts were found to be safe when taken orally [19–21]. *A. vasica,* a member of the *Acanthaceae* family, was used as the second plant in this research. Bioactive pyrroloquinazoline alkaloids are active constituents of *A. vasica*. Some more are aliphatic hydroketones (hydroxyhexateracont-l-en-15 one and 37 hydroxy hentetracontam-19-one) and other alkaloids (vasicol, adhatonine, vsicinone, and vasiciolone) [22]. The antimicrobial, antitussive, and anticancer properties of A. vasica are among its well-known medicinal properties. *T. procumbens*, the third plant, is a member of the *Heliantheae* family. Its leaves are used to treat dysentery, bronchial catarrh, and hair loss. Its flowers and leaves are also used to check for haemorrhage from cuts, bruises, and other wounds because they have antiseptic, insecticidal, and parasiticidal properties. The extract from it stops bleeding. It has been found to be very helpful for treating diarrhea, malaria, and stomachaches [23, 24]. The *Apocynaceea* family includes *C. procera*, which is the fourth plant used in this research. The leaves contain several active chemicals, including three toxic glycosides; calotropin, uscharin, and calotoxin. Although the plant is poisonous, it has been used in small amounts as a folk remedy for a variety of ailments, and its anti-coagulant and anti-cancer properties are still under investigation [25]. The objectives of the current study are to identify and select medicinal plants with a reputation for antibacterial properties, extract these plants' bioactive components, and then further examine those chemicals. It also aims to evaluate the potency of the isolated compounds against *E. coli* bacteria using a series of antibacterial assays using standardized procedures and the antibacterial effects of several isolated plant components on the E. coli bacteria.

1. Materials and Methods

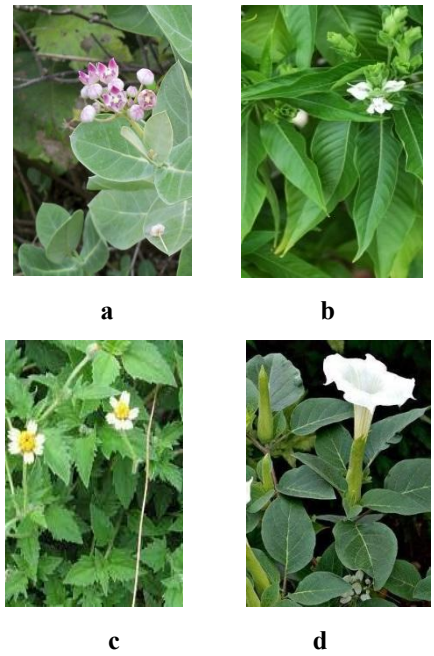


Figure 1. *The medicinal plants used in preparing extracts:* ***a)*** *Calotropis procera* ***b)*** *Adhatoda vasica* ***c)*** *Tridex procumbens* ***d)*** *Datura innoxia*

Four medicinal plants (**Figure 1**) were chosen and used to test their antimicrobial effects: *Adhatoda vasica, Datura innoxia, Calotropis procera,* and *Tridex procumbens.* Ethanol, chloroform, and acetone were used separately as solvents to form leaf extracts from all of these plants one by one.

1.1. Collection of plant material

Based on descriptions given by the local villagers, who use the extract to treat wounds and prevent infection, the four medicinal plants were chosen. During the springtime, all four plants were collected and identified. The young plants were the ones whose leaves were taken. The leaves were stored in a cotton cloth after drying in the shade so they could be used later. The village Chitauli district Hapur, U.P. is the sole study area for this research investigation.

Preparation of extract

The leaves were washed with water and dried under shade. Each of 10 gm of leaves powder was extracted for 72 hours in 100 ml of ethanol, acetone and of chloroform each separately at 60 °C. Each of the crude extract was filtered with Whatman filter paper and then allowed to evaporate to obtain concentrated extract.

1.2. Collection of bacteria

Escherichia coli bacteria were cultivated in a nutrient broth medium for 24 hours. Subsequently, fresh inoculums were obtained from the culture in order to assess antimicrobial activity. The preparation of sterile test discs involved immersing filter paper discs in concentrated plant extract solutions, followed by placement in a hot air oven maintained at a temperature below 50 °C. The discs were then weighed with utmost precision. Negative control discs were created by immersing the sterilised filter paper discs individually into ethanol, acetone, and chloroform, followed by drying them in the sterilized Petri dishes. Positive control discs were created by immersing sterilised filter paper discs into solutions of amikacin sulphate with a concentration of 50 mg/mL of distilled water, as well as ampicillin and cyloxacillin with varying concentrations (10 mg/mL of ethanol, 30 mg/mL of ethanol, and 50 mg/mL of ethanol). This process resulted in dried discs with varying levels of antibiotic absorption.

1.3. Culture media and inoculums

A solid medium consisting of nutrient agar (*Luria Britaini* media) was made by dissolving the appropriate amount of agar powder, tryptone, yeast extract, and sodium chloride in 100 ml of distilled water. The resulting mixture was tightly sealed with a cotton plug, and the flask's neck was covered with aluminum foil. The combination underwent autoclaving at a temperature of 150°C and a pressure of 15 pounds for 30 minutes. Following the process of cooling to around 45°C, a volume of 25 ml of the nutritional media was carefully transferred into a Petri dish. The E. coli inoculum was generated through the cultivation of a substantial quantity of bacteria in a 250-ml conical flask with 100 ml of nutritional broth (*L.B. broth*) and subsequent overnight incubation at a temperature of 37°C. The agar plates utilized in the assay were appropriately labeled with the corresponding names of the discs, denoting the specific plant extract absorbed within each disk. The bacterial inoculum, each measuring 500 microliters, was aseptically deposited onto solid nutritional agar media in petri dishes using a sterile swab. The use of a swab facilitated the even distribution of germs across the media, resulting in a confluent lawn. The rotation of the petri dish at a 90° angle was employed to facilitate the assessment of bacterial proliferation. The entire process of inoculation was carried out within a laminar flow chamber that had been thoroughly decontaminated to minimize the presence of bacteria.

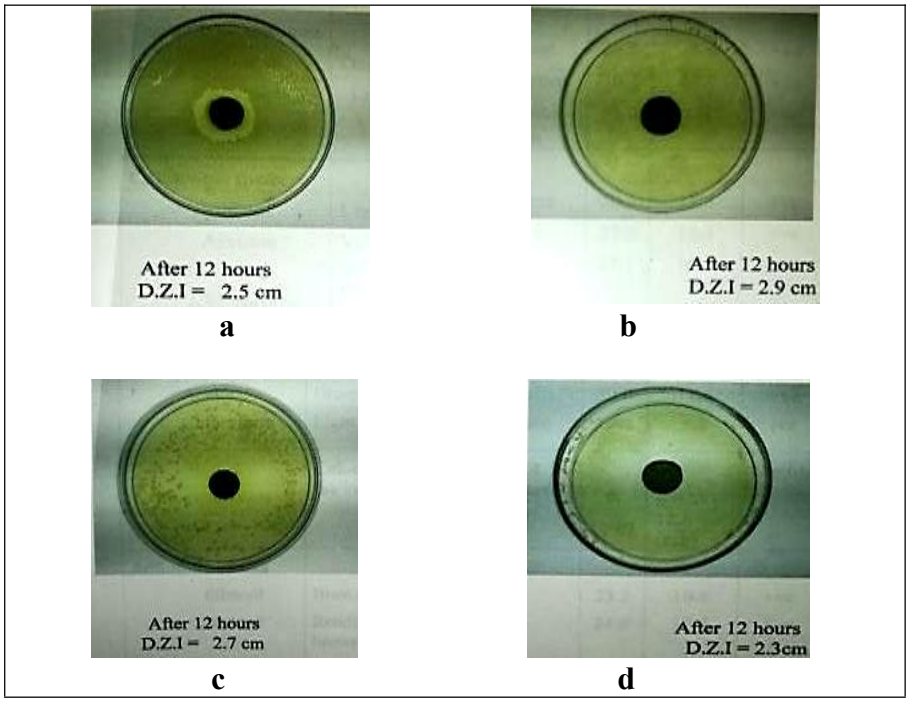


Figure 2. *Ethanolic extracts of* ***a)*** *D. innoxia (21 mg),* ***b)*** *T. procumbens (15.6 mg),* ***c)*** *C. procera (17.8 mg) and* ***d)*** *A. vasika(10.6 mg) showing D.Z.I. after 12 hours*

1.4. Antimicrobial activity of the plants

The disc diffusion assays were used to screen the antimicrobial activity of the plant extract. Completely dried discs were transferred on a bacterial lawn under aseptic conditions using sprit lamp-sterilized forceps each time within the laminar flow chamber. Each disc was placed gently on the surface of the agar media and patted with the forceps so that it stuck well to the surface of the media. The Petri dish was incubated at 37 °C for 12 hours. The resulting zone of inhibition was observed and noted as +ve or -ve results. Where positive results were obtained, the diameters of zones of inhibition (D.Z.I.) were noted. The D.Z.I. around the test paper disc indicates the absence of bacterial growth, and that was noted as a positive result and the absence of the zone as a negative result.

1.5. Detection of presence of alkaloids in ethanolic extract

5 ml of ethanolic extract was evaporated to dryness. This solvent-free residue was taken from 5 ml of 2% (v/v) hydrochloric acid, saturated with sodium chloride, and filtered. The filtrate was tested separately to detect the alkaloids in the ethanolic extract with two alkaloid reagents, Dragendorff's reagent and Mayer's reagent.

- **Preparation of Dragendorff's reagent**

Solution was prepared by dissolving 0.17 gm of bismuth nitrate in 2 ml of acetic acid and 8 ml of distilled water.
Another solution, by dissolving 4 gm of potassium iodide in 10 ml of distilled water, was prepared. Both solutions were mixed with each other and then volume was made up to 100 ml with water [35].

- **Preparation of Mayer's reagent**

Solution by dissolving 1.358 grams of $HgCl_2$ in 60 milliliters of water, and pouring that solution into a solution of 5 grams of KI in 10 milliliters of water was prepared.

1.6. Extraction of the active compound

Slurry of 8 grams of silica gel per 20 milliliters of ethanol was packed into a glass column with a diameter of 1.5 centimeters and a height of 25 centimeters. The packing was done until a height of 20 centimeters was reached, with a layer of cotton placed at the bottom of the column. Subsequently, a volume of 5 ml of the concentrated ethanolic extract was transferred onto the column and subjected to elution using 5 ml of ethanol at regular intervals. The crude mixture of alkaloids, which had a yellowish appearance, was subjected to elution. The mixture was let to undergo evaporation of ethanol, resulting in the acquisition of approximately 2 ml of a concentrated solution. This solution was characterized as a white mixture containing crude alkaloids. Subsequently, the crude alkaloid mixture was subjected to a similar elution process using ethanol, resulting in the acquisition of another crude alkaloid mixture. Subsequently, a crude combination of alkaloids was assessed for the presence of alkaloids using thin-layer chromatography [36, 37].

1.7. Development of the thin layer chromatogram

The slurry of 1 g of silica gel (13% calcium sulphate) per 2 ml of distilled water was layered uniformly over a clean 5 x 6" glass plate to prepare a thin layer with width of about 0.55 mm. This plate was activated by heating it at 110°C for 30 minutes in a hot oven. 100 microliters of concentrated solution of the mixture of alkaloids in methanol were spotted in the middle and 2 cm above the bottom of the thin layer. Then the thin-layer chromatogram was developed for 20 minutes in the solvent methanol-water-acetic acid in a ratio of 16:3:1 [38]. The layer was allowed to dry at room temperature.

S. N.	Name of Plants	Solvents Used	Colour of the Extracts	W (gm)	w= W-12.6 (mg)	Result	D.Z.I. (cm)
1.	*Tridax Procumbens*	Ethanol	Dark green	28.2	15.6	+ve	2.9
		Acetone	Light green	30.3	17.7	+ve	2.5
		Chloroform	Yellowish green	21.0	8.4	+ve	2.9
2.	*Calotropis Procera*	Ethanol	Light green	30.4	17.8	+ve	2.7
		Acetone	Yellowish green	29.0	16.4	+ve	2.2
		Chloroform	Light green	23.1	10.5	-ve	-
3.	*Datura Innoxia*	Ethanol	Dark green	33.6	21.0	+ve	2.5
		Acetone	Green	30.0	17.4	+ve	1.7
		Chloroform	Light green	19.8	7.2	-ve	-
4.	*Adhotoda Vasika*	Ethanol	Brown	23.2	10.6	+ve	2.3
		Acetone	Reddish green	24.9	12.3	+ve	2.2
		Chloroform	Light green	34.0	21.4	-ve	-

Table 1. *It represents the plants/solvents used in extraction, color of the extract obtained after keeping at 65°C for 72 hours:*

2. Results

Right after a period of 12 hours following the placement of the ethanolic extract-absorbed disk on the inoculum within the Petri dish, it was observed that all four plants presented antibacterial activity against E. coli. Subsequently, photographs were captured to document these findings (**Fig. 2**).

The chloroformic extracts of *Datura innoxia, Calotropis procera,* and *Adhatoda vasica* did not show any antimicrobial zones of inhibition. However, the chloroformic extract of Tridax procumbens (weighing 8.4 mg) demonstrated the most potent antimicrobial activity, with a zone of inhibition measuring 2.9 cm. The acetonic extracts of *D. innoxia, C. procera*, and A. vasica displayed lower activity compared to their respective ethanolic extracts. The order of activity for the three plants, namely *Datura innoxia, Calotropis procera, and Asclepias vasika*, was determined to be in the following descending order:

Ethanol > Acetone > Chloroform.

On the other hand, the order of activity for *Tridax procumbens* was discovered to be in the following descending order: **Chloroform > Ethanol > Acetone.**

Table 1 displays the plants and solvents employed in the extraction process, the colour of the extract obtained after a 72-hour period at a temperature of 65°C, the weight (W in mg) of the disc plus absorbed extract, the net weight (w in mg) of the extract absorbed calculated by subtracting the weight of the plain paper disc (12.6 mg) from the weight of the disc plus absorbed extract, the result obtained (positive or negative) after 24 hours of applying the disc on the inoculums, and the corresponding diameter of the zone of inhibition (D.Z.I.) measured in centimeters.

Table 2 illustrates the antimicrobial activity of the positive control, which involved the administration of two antibiotics, namely amikacin sulphate and ampicillin (**Fig. 3**). However, the negative control did not exhibit any zone of inhibition in terms of antibacterial activity

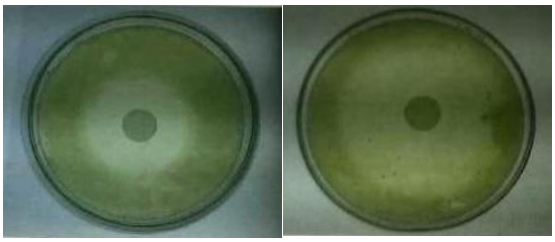
Table 2. *Antimicrobial activity of positive control:*

S. N.	Antibiotic/ concentration used	W (mg)	w (mg)	D.Z.I. (cm)
1.	Amikacinsulphate 50 mg/ml water	18.5	5.9	4.2
2.	Ampicillin/amoxicillin 10 mg/ml EtOH	13.2	0.6	3.3
	30 mg/ml EtOH	17.4	4.8	3.9
	50 mg/ml EtOH	18.5	5.9	4.2

Table 3. *Antimicrobial activity of T. precumbens and D. innoxia over time:*

S.N.	Time (Hours)	*T. precumbens* w=15.6 mg D.Z.I.(cm)	*D. innoxia* w=41.6 mg D.Z.I.(cm)
1	12	2.9	3.0
2	24	2.8	3.0
3	36	2.7	2.9
4	48	2.7	2.9
5	60	2.7	2.9
6	72	2.6	2.9
7	84	2.6	2.9
8	96	2.6	2.9
9	108	2.6	2.9
10	120	2.6	2.9

Antimicrobial activity of *T. precumbens* and *D. innoxia* over time upto 120 hours is shown in **Table 3.** The graphical representation of antimicrobial activity of *T. precumbens* and *D. innoxia* with time up to 120 hours is given in **figure 4**.



a **b**

Figure 3. *Antimicrobial activity of positive control:* ***a)*** *Amikacinsulphate and* ***b)*** *Ampicillin/cyloxacillin*

A consistent pattern of D.Z.I. of *D. innoxia* showed a direction to perform further study of its antimicrobial activity. Hence, six more disks of ethanolic extracts with different concentrations were prepared and observed after 12 hours. It was found that D.Z.I. was almost linearly dependent on the amount (w) of the extract absorbed (**Table 4**.).

The graphical representation of antimicrobial activity of *D. innoxia* with various amount of its extract absorbed versus D.Z.I. after duration of 12 hours is given in **figure 5.**

3. Discussion

Following a 12-hour period, the chloroformic extracts of *Datura innoxia, Calotropis procera,* and *Adhota vasica* exhibited minimal antimicrobial activity against *Escherichia coli*. In contrast, the chloroformic extract of *T. procumbens* demonstrated the most potent antimicrobial activity. Furthermore, the acetonic extracts of D. innoxia, C. procera, and A. vasica displayed lower levels of activity compared to their corresponding ethanolic extracts. The activity of the three extracts was seen to follow a decreasing sequence, as follows:

Ehanolic > Acetonic > Chloroformic,

whereas that for T. procumbens was found to be

Chloroformic > Ethanolic > Acetonic

The order of D.Z.I. showed by ethanolic extract of all four plants against E. coli was found to be

T. procumbens > C.procera > D.innoxia > A.vasika

After 24 hours, only *T. procumbens* and *D. innoxia* were persistent enough to show constant antimicrobial activity. Thus, the antibacterial impact of the chloroformic extract was found to be the least potent, whereas the ethanolic extract exhibited the highest level of antimicrobial activity. The antibacterial activity of *T. procumbens* against E. coli was found to be the highest, as evidenced by the diameter of the zone of inhibition.

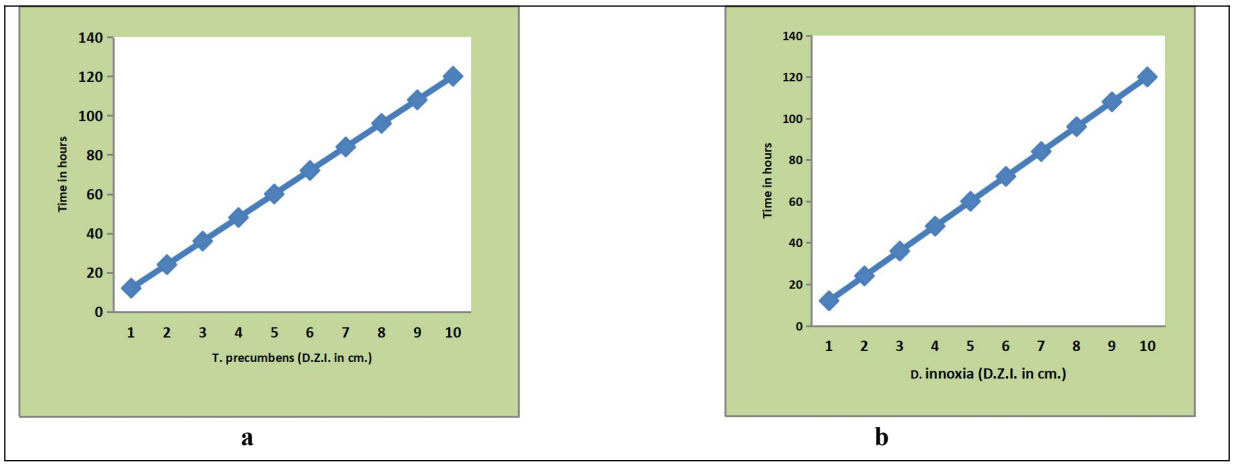
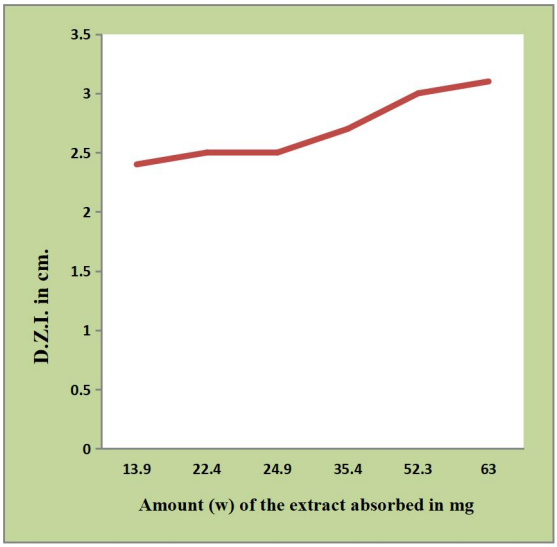


Figure 4. *Graphical representation of antimicrobial activity of* ***a)*** *T. precumbens and* ***b)*** *D. innoxia over time upto 120 hours.*

Table 4: *Various amount of extract of D. innoxia absorbed with D.Z.I:*

D. innoxia			
S.N.	**W (mg)**	**W (mg)**	**D.Z.I. (cm)**
1	75.6	63.0	3.1
2	64.9	52.3	3.0
3	48.0	35.4	2.7
4	37.5	24.9	2.5
5	35.0	22.4	2.5
6	26.5	13.9	2.4



Nevertheless, it was observed that only *T. procumbens* and *D. innoxia* exhibited sustained antibacterial activity throughout the duration of the study. Conversely, the effectiveness of C. procera and A. vasika declined progressively over time. A positive linear relationship was seen between the diameter of the zone of inhibition (D.Z.I.) and the quantity of absorbed extract. Nevertheless, it was shown that leaf extracts of *D. innoxia* persistently exhibited antibacterial activities.

The study has certain limitations that should be acknowledged. The study's scope might be constrained by the choice to examine a particular bacterial strain for experimentation, thereby limiting the generalizability of the findings. It is plausible that there may exist additional strains or developing pathogens that were not encompassed within the scope of this study that would exhibit distinct responses to the extracts under investigation. The presence of extraction variability may result in variations in the quantity and quality of bioactive chemicals extracted, which in turn could impact the measured antibacterial activity.

The complexity of bioactive mixtures is a common characteristic observed in medicinal plant extracts, where a combination of many bioactive chemicals is present. The process of isolating specific components for testing may not comprehensively capture the potential synergistic effects that may be present in the natural extract.

Potential areas for future research may belong to clinical studies of the chloroformic *T. procumbens* and the ethanolic extract of *D. anoxia* or involve the implementation of their clinical trials to evaluate the safety, effectiveness, and possible negative consequences of the most favorable bioactive substances or botanical extracts on human participants.

Ethics approval and consent to participate:
Not applicable.

Consent for publication:
Not applicable.

Availability of data and materials:
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Funding
This research received no external funding.

Author Contributions:
SK designed the work, did extraction of alkaloids and was a major contributor in writing the manuscript.
SKB worked on chromatography.
GP prepared culture media and inoculums.
All authors read and approved the final manuscript.

References

1. Eng Soon Teoh, MD, FRCOG, FACS (2015) Secondary Metabolites of Plants. Medicinal Orchids of Asia. 5: 59-73.
2. Sanchez S, Demain AL (2011) Scientific Fundamentals of Biotechnolog. Comprehensive Biotechnology (Second Edition).
3. Usha K, Singh B, Praseetha P, Deepa N, Agarwal DK, Agarwal R, Nagaraja A (2009) A ntifungal activity of Daturastramonium, Calotropisgigantea and Azadirachtaindicaagainst Fusariummangiferae and floral malformation in mango. European Journal of Plant Pathology 124:637–657.
4. Maheshwari NO, Khan A, Chopade BA (2013) Rediscovering the medicinal properties of Datura sp.: A review. Journal of Medicinal Plant Research 7(39):2885-2897.
5. Mbida H,Tsala DE, Aboubakar S, Habtemariam S, Edmond JJ, Bakwo EF, Minkande JZ (2022) Antioxidant Activity of Aqueous Extract of Leaves and Seeds of Daturametel (Solanaceae) in Frog's Heart Failure Model. Evidence-Based Complementary and Alternative Medicine. 12:2022:5318117. https://doi.org/10.1155/2022/5318117.
6. Sharma M, Dhaliwal I, Rana K, Delta AK, Kaushik P (2021) Phytochemistry, Pharmacology, and Toxicology of Datura Species—A Review. Antioxidants (Basel) 10(8):1291. DOI: 10.3390/antiox10081291.
7. Gerlach GH (1948) Datura innoxia-a potential commercial source of scopolamine. Economic Botany, 2:436-454
8. Srivastava R, Srivastava P (2020) The Medicinal Significance of *DaturaStramonium:* A Review. Biomed J Sci & Tech Res 29(2).
9. Olmstead RG, Bohs L, Migid HA, Santiago-Valentin E, Garcia VF, Collier SM (2008) A molecular phylogeny of the Solanaceae. *Taxon* . 57(4)1159-1181.
10. Céspedes-Méndez C, Iturriaga-Vásquez P, Hormazábal E (2021) SECONDARY METABOLITES AND BIOLOGICAL PROFILES OF *DATURA* GENUS, J. Chil. Chem. Soc. 66(2).
11. De Jesus Matias L, Rocha JA, de Andrade Royo V, Menezes EV, de MeloJúnior AF, de Oliveira DA (2019) Phytochemistry in medicinal species of *Solanum* L. (Solanaceae). *Pharmacognosy Research*. 11(1)47.
12. Soni P, Siddiqui AA, Dwivedi J, Soni V (2012) Pharmacological properties of Datura stromonium L.: An overview. Asia Pacific Journal of Tropical Biomedicine. 2 (12):2885-2897.
13. Bagewadi ZK, Muddapur UM, Madiwal SS, MullaSI, Khan A (2019) Biochemical and enzyme inhibitory attributes of methanolic leaf extract of *Datura inoxia* Mill. Environmental Sustainability. 2(1) (https ://doi.org/10.1007/s4239 8-019-00052 -6).
14. George R, Mathur P (2022) Anti-bacterial, Anti-oxidant and other Phytochemical Proper-ties of *Datura innoxia* Mill. Leaves. Plant Science Today. 9(2):262–271 https://doi.org/10.14719/pst.1387.
15. Sasaki T, Yamazaki K, Yamori T, Endo T (2002) Inhibition of proliferation and induction of differentiation of glioma cells with *Datura stramonium* agglutinin. British Journal of Cancer. 87. 918–923.
16. Harfi B, Khelifi L, Khelifi- Slaoui M, Assaf-Ducrocq C, Gontier E (2018) Tropane alkaloids GC/MS analysis and low dose elicitors' effects on hyoscyamine biosynthetic pathway in hairy roots of Algerian *Datura* species. Scientific Reports. 8:17951.
17. Waza SA, Anthony P, Dar S (2015) PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF METHANOLIC EXTRACT OF *DATURA STRAMONIUM* SEEDS. *IJPSR. 6(7): 3021-3026.*
18. Hussain F, Kalim M, Ali H, Ali T, Khan M, Xiao S, Iqbal MN, Ashraf A (2016) Antibacterial Activities of Methanolic Extracts of *Datura inoxia.* PSM Microbiology. 1(1): 33-35.
19. Bhandari YL, Godale CH (2020) Effect of Datura innoxia on disease causing bacteria in Abutilon indicum. IJRAR. 7(1).
20. Partap M, Gupta RC, Pradhan SK (2019) COMPARATIVE ANALYSIS OF MORPHOLOGY AND PHYTOCHEMICAL CONSTITUENTS IN DIFFERENT POPULATIONS AND MORPHOTYPES OF *DATURA INNOXIA* MILL. AND *DATURA METEL* L. FROM PUNJAB PLAINS. Asian J Pharm Clin Res. 12(1):193-199.
21. Eltayeib AA, ANT Matter S (2020) The Effect of *Datura Innoxia*Seeds and Leaves Contents on Albino. Wister Rats. J Pharma Res Dev. 1(1).

22. Shahzad Q, Sammi S, Mehmood A, Naveed K, Azeem K, Ayub A, Hassan M, Hussain M, Ayub Q, Shokat O (2020) Phytochemical analysis and antimicrobial activity of adhatodavasica leaves. PesquisaAgropecuairaBrasilera 09(2). 1654-1661.
23. Christudas S, Kulathivel TM, Agastian P (2012) Phytochemical and antibacterial studies of leaves of *Tridax procumbens* L. Medicin. Asian Pacific Journal of Tropical Biomedicine 2(1) S159-S161.
24. Holm LR, Doll J, Holm E, Pancho JV, Herberger JP (1997) World Weeds: Natural Histories and Distribution. Wiley and Sons. Hoboken. 1129.
25. Saddiq AA, Tag HM, Doleib NM, Salman AS, Hagagy N (2022) Antimicrobial, Antigenotoxicity, and Characterization of *Calotropis procera* and Its Rhizosphere-Inhabiting Actinobacteria: In Vitro and In Vivo Studies. Molecules. 27(10): 3123.
26. European Food Safety Authority (EFSA). (2008) Tropane alkaloids (from Datura sp.) as undesirable substances in animal feed-Scientific Opinion of the Panel on Contaminants in the Food Chain. *EFSA Journal*, *6*(8), 691.
27. Shonle I, Bergelson J (2000) evolutionary ecology of the tropane alkaloids of *Datura stramonium l.* (Solanaceae). Evolution. 54(3). 778–788.
28. Gomathia M, Rajkumarb PV, Prakasama A, Ravichandran K (2017) Green synthesis of silver nanoparticles using *Datura stramonium* leaf extract and assessment of their antibacterial activity. Resource-Efficient Technologies. 3. 3. 280–284.
29. Singh R, Dubey AK (2020) Isolation and Characterization of a New Endophytic, Actinobacterium Streptomyces californicus Strain, ADR1 as a Promising Source of Anti-Bacterial, Anti-Biofilm and Antioxidant Metabolites. Microorganisms. 8. 6. 929. doi:10.3390/microorganisms8060929.
30. O'Hagan D, Robins RJ, Wilson M, Wong CW, Berry M, Zabetakis I (1999) Fluorinated tropane alkaloids generated by directed biosynthesis in transformed root cultures of *Datura stramonium*. *J. Chem. Soc., Perkin Trans. 1***,** 1999, 2117-2120.
31. Mane S, Narmawalaa R, Chatterjee S (2018) Selective recognition of atropine in biological fluids and leaves of *Datura stramonium* employing a carbon nanotube–chitosan film based biosensor. New J. chem. 42, 10852-10860.
32. Al-Zharani M, Nasr FA, Alqahtani AS, Cordero MAW, Alotaibi AA, Bepari A, Alarifi S, Daoud A, Barnawi IO, Daradka HM, (2021) In vitro cytotoxic evaluation and apoptotic effects of datura innoxia grown in saudiarabia and phytochemical analysis. App. Sci. (Switzerland) 11(6), 2864. DOI: 10.3390/app11062864.
33. Chandan G, Pal S, Kashyap S, Siwal SS, Dhiman SK, Saini AK, Saini RV (2021) Synthesis, characterization and anticancer activities of silver nanoparticles from the leaves of Daturastramonium L. Nanofabrication. 6. 25–35.
34. Ojha AK , Rout J , Behera S, Nayak PL (2013), Green Synthesis and Characterization of Zero Valent Silver Nanoparticles from the Leaf Extract of DaturaMetel. IJPRAS. 2.1. 31-35.
35. Roper G, Blomster RN, Farnsworth NR, Draus FJ (1965) Stability and Sensitivity of Modified Dragendorff's ReagentsE. Planta Med. 13(1). 98-103 DOI: 10.1055/s-0028-1100103
36. Pothier J, Galand N, (2005) Automated multiple development thin-layer chromatography for separation of opiate alkaloids and derivatives. J. Chromatogr A. 1080. 2. 186–191.
37. Li Wei-Xin, Wang H, Dong Ai-Wen (2019) Systematic Separation and Purification of Alkaloids from Euchrestatubulosa Dunn. by Various Chromatographic Methods. Processes. 7. 924. doi:10.3390/pr7120924.
38. Svendsen AB, Vorpoorte R (1983) Chromatography of alkaloids part A: thin-layer chromatography. Journal of chromatography library. 23A.