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EVALUATION OF ANTIMICROBIAL ACTIVITY OF DIFFERENT EXTRACTS OF *DATURA STRAMONIUM* AGAINST *MYCOBACTERIUM KANSASII*

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Keywords:

Datura stramonium, Minimum inhibitory concentration assay, *Mycobacterium kansasii*, Antimicrobial activity

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ABSTRACT: The aim of this study is to assess the antibacterial efficacy of the *Datura stramonium* seeds and leaves extracts against *Mycobacterium kansasii*. The antimicrobial experiments were performed using five doses (0.25µg/ml, 0.50µg/ml, 1µg/ml, 2µg/ml, 4µg/ml) of seeds and leaves extracts. The results showed notable inhibitory effects on *Mycobacterium kansasii.* The results indicated that the extract of *D. stramonium* exhibited action against all the tested bacterial isolates, with the level of activity varying depending upon the concentration. The hexane extract of seeds DSS-1H exhibited antibacterial activity, with mean zones of inhibition measuring 98.94±50.25.%, 120.98±56.92%, 120.499±69.60%, 125.752±133.18% and 89.494±180.63%, at the doses of 0.25, 0.50, 1, 2, and 4 µg/ml and while treating with DSS-2A the percent cell viability of the microbial cell was found to be 128.02±25.64%, 86.79±14.95%, 34.61±16.33%, 26.29±29.76% and 32.56±19.48% at the doses of 0.25, 0.50, 1, 2 and 4µg/ml, respectively, with incubation period of 48 hours. Similarly, the microbial cells were treated (0.25, 0.50,1,2,4 µg/ml) with DSS-3ET with incubation period of 48 hours, indicating a more substantial decrease in cell proliferation (75.09±39.45, 60.16±44.46, 28.19±20.73, 42.24±32.80% and 46.93±69.00%). In a similar manner the percent cell viability of plant extract (DSL-M and DSL-E) also found to be decreased upon treatment with the same concentration (0.25, 0.50, 1, 2, 4µg/ml) with incubation period of 48 hours, which were 146.05±15.24%, 117.63±36.36%, 59.88±95.54, 16.45±58.76 and 42.89±7.23% and 89.52±23.73%, 102.93±28.55%, 60.66±18.13, 58.86±28.90% and 50.91±8.83% respectively, as compared to the control. The findings of this study provided evidence for the conventional utilization of wildly growing plant *Datura stramonium* and emphasize the necessity for further comprehensive research to explore potential alternatives to current antibacterial medications.

INTRODUCTION: *Mycobacterium kansasii* is a group I non-tubercular mycobacterium (NTM) [1]. Seven genotypes or subtypes have been identified. It is one of the most virulent and prevalent NTM. This species of bacteria is widely distributed throughout the world[2,3], often found in aquatic environment, besides this in other environmental sources such as soil, house dust and it may infect wild or domestic animals as well as humans especially in immunocompromised patients [4]. The intensity and frequency of infection depends on the occurrence of this Mycobacterium in the environmental resources.

Sites of infection reported include soft tissue, bone and joints, and the genitourinary system, but pulmonary disease leading to lung infection [5] which is clinically and radiologically similar to tuberculosis and lympho- denitis are the common sites which cause the most important and prominent clinical problems. Detection of these bacteria in human system cannot be relied on simple diagnosis because of the non-specificity of mycobacterium antigens.

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Usually, most strains of *M. kansasii* are resistant to isoniazid and pyrazinamide. Fortunately *M. kansasii* is almost always sensitive to ethambutol, so the addition of this very useful drug should be considered if a patient is infected with an environmental mycobacterium. Recently, the treatment involves the combination of rifampicin[6] with other antimicrobial drugs (i.e., ethambutol and isoniazid or ethambutol and clarithromycin) [7]. The selection of drug and course of treatment is usually determined depending upon the immune system of the patient. Besides this the toxicity of the drug and multi resistant bacterial strains is always a challenge and one of the deciding factors for the drug. These long treatments, which requires the treatment to be continued for about one year even after the culture is negative often raises additional problems, for example patient no adherence and adverse effects. Thus, new alternatives which are also natural in origin are urgently needed to shorten the duration of these therapies.

Natural product has evolved over millions of years and attains a unique chemical diversity, which ultimately results in the diversity of their biological activities and drug alike properties. Even before the rise of the modern chemical pharmacology natural products have been used by the people for centuries as components of traditional medication, particularly the active components of herbal remedies [8, 9]. These days some of the traditional healing practices, such as Indian Ayurveda and other traditional medicinal practices around the globe remain the primary treatment option for many people due to several reasons such as economic reasons, personal belief or to the difficulty in accessing pharmaceutical products. In modern pharmacology too, natural products have become one of the most important source for developing new lead drugs and scaffolds [10].

The use of plants by man to treat common ailments since time immemorial and many of the traditional medicines are still included as part of the habitual treatment of various maladies. *D. stramonium* is commonly known as Jimson weed and it belongs to family Solanaceae. It is 60-120 cm tall, branched and pubescent plant. The morphological features of different parts of the plant including root, stem, leaves, number of leaves, and biomasss exhibited considerable variations [11].

The leaves are 8-17x4-13 cm, ovate, sinuately, dentate and minutely puberulose *D. stramonium* commonly found in waste ground, in fertile soils in fields, and roadsides at altitudes of 600-2800m. This herb is originated in Tropical North America, now it is a cosmopolitan weed [12]. Also it is widely used in phytomedicine to cure diseases. *Datura* was known to the ancient Hindu Physicians who regarded it as antispasmotic, intoxicant, germicidal, antipyretic, antiseptic, antiphlogistic, antiproliferative narcotic, sedative, tonic, antidiarrhoeal, antihelmintic, and useful in leucoderma, skin disorders, ulcers, bronchitis, jaundice, piles, lung cancer [13] and breast cancer [14]. Although antibacterial activity have been investigated by various researchers [15, 16]. In the present study we have compared the phytochemical contents and antibacterial activity of extract of the *Datura* seeds and leaves using different solvents of varied polarity against *M. kansaii*.

Previous thorough study have shown that the antibacterial chemicals found in *Datura stramonium* can be utilized to create safer, more cost-effective, and environmentally friendly alternatives to currently available antibacterial medications. The many secondary metabolites from different chemical classes have been identified and described. Furthermore, investigations have also discovered a wide range of phytoconstituents in the seeds. The tropane alkaloids such as scopolamine, hyoscyamine, atropine, flavonoids, withanolides, and sesquiterpenes [17] are found in this plant, primarily in its seeds. Some other compounds isolated and identified were N-trans-feruloyl tryptamine, hyoscyamilactol , scopoletin, umckalin, daturaolone, daturadiol, N-trans-ferulicacyl-tyramine, cleomiscosin A, fraxetin, scopolamine, 1-Acetyl-7-hydrox-beta-carbol-ine, 7-hydroxy-beta-carbolinel-propionic acid.

The concentrations of these compounds may vary depending on the location. The afore mentioned secondary metabolites are purported to possess many therapeutic effects, such as anti-oomycete, antibacterial, antidiabetic, antimicrobial, anti-inflammatory, antioxidant, anticancer, and numerous more. In addition to examining the chemical and pharmacological characteristics, the researchers have also investigated the allelopathy and invasiveness of *Datura stramonium*.

On literature survey it was found that no work has been done or activity reported of *Datura stramonium* against Mycobacterium kansasii hence it was thought worthwhile to screen the different extracts prepared from the seeds and leaves of the plant against *Mycobacterium kansasii*.

MATERIAL AND METHOD: The seeds and leaves were manually detached from the plant during the month of April and were air-dried for duration of at least seven days at room temperature. The shade air dried materials were pulverized into a fine powder using an electric blender. The crude extract of the seeds and leaves of *Datura stramonium* were produced by the maceration process, followed by extraction with solvents with increasing polarity hexane, acetone and ethanol for seeds and methanol and ethanol for leaves as the extraction solvents. Approximately 1000 ml of hexane was introduced into a percolator containing approximately 200g of seeds, which was subsequently covered with aluminum foil. The procedure was performed three times, with manual shaking occurring every 24 hours. The crude extract was concentrated under reduced pressure using water bath and finally dried to obtain a thick green paste which was suitably diluted and used for the experiments. After being extracted with hexane, the remaining plant material was subjected to extraction with approximately 1000 ml of acetone then lastly with ethanol. This procedure was repeated three times. Apart from seeds, leaves were also extracted with methanol and ethanol with same procedure.

Antibacterial Assay: Antibacterial analysis against *Mycobacterium kansasii* were conducted on hexane, acetone and ethanol extracts of seeds and the methanol and ethanol of the leaves of *Datura stramonium*. The study utilized the Broth Dilution technique to ascertain the minimum inhibitory concentration (MIC).

The antimicrobial investigation was conducted by the Aakaar Biotechnology Private limited, located in Lucknow, India. A 0.5 McFarland Standard dilution of microbes was utilized for the study. 500 µl of diluted bacterial cultures were added to a micro centrifuge tube. Then, 10µl of prepared treatment dilutions with varying concentrations were added to specific tubes. The tubes were incubated for 48 hours. Following incubation, the entire contents were transferred to a 96-well plate. Subsequently, readings were obtained using an Elisa Plate Reader (iMarkBiorad) at wavelengths of 490 nm and 595nm. The positive control utilized in the experiment was Ciprofloxacin at a concentration of 4µg.

RESULTS:

TABLE 1: IC_{50} DATA OF DIFFERENT TEST SAMPLES AGAINST *M. KANSAII*

Sample	IC_{50} (µg/ml)
DSS-1H	cannot be calculated
DSS-2A	1.500
DSS-3ET	0.992
DSL-M	2.332
DSL-E	3.171

The half-maximal inhibitory concentration (IC_{50}) is a commonly used and highly informative measure of a drug's effectiveness. The term "half maximal inhibitory concentration" (IC_{50}) refers to the amount of a drug required to block a biological process by 50%. This measurement is used to determine the potency of an antagonist medication in pharmacological research. The acetone extract of seeds has a value of 1.500µg /ml, while the ethanol extract of seeds has a value of 0.992µg/ml. The inhibition value for the methanolic extract of leaves sample is 2.332µg/ml, while for the ethanolic extract of leaves is 3.171µg/ml. From the data it can be concluded that the ethanolic extract of seeds sample had showed most promising IC_{50} value and therefore maximum activity against the bacteria.

TABLE 2: ANTIBACTERIAL ASSAY OF HEXANE EXTRACT OF SEEDS OF *D. STRAMONIUM*

Sample	Concentration (µg/ml)	Exposure Period (In Hours)	MIC Assay (MEAN ±SD)
DSS-1H	0	24 h	100±11.23
	0.25	24 h	98.94±50.25
	0.50	24 h	120.98±56.92
	1	24 h	120.499±69.60
	2	24 h	125.752±133.18
	4	24 h	89.494±180.63
	PC	24 h	-42.83±22.36

In **Table 2** it shows the seven different concentrations of the sample coded DSS-1H that were utilized in the MIC assay experiment. The MIC value after a 24 hour exposure period is 100±11.23 when no sample is added or at zero concentration, which is considered to be the negative control. In group 2, the sample is added to the bacterial assay at a concentration of 0.25µg/ml. The observed value for the MIC assay is 98.94±50.25, which is only 2% lower than the value of the negative controls after a 24-hour exposure period. In group 3, the sample concentration was raised from 0.25µg/ml to 0.50µg/ml. As a result, the MIC assay value increase to 120.98±56.92, which is 20% higher than the negative control value after a 24-hour exposure period. In group 4, the concentration is 1 µg/ml, and the MIC assay value is 120.499±69.60, indicating a 20% increase from the negative control value after a 24 hour exposure period. In group 5, the sample concentration was 2µg/ml. After 24 hours of exposure, the MIC assay value increase to 25%, specifically 125.7512±133.18. In group 6, the sample concentration was 4µg/ml. The MIC assay value was 89.494±180.63, indicating a significant decrease of 11% compared to the negative control value after a 24 hour exposure period. The statistical significance level was found to be less than 0.05. Finally, Ciprofloxacin, used as a positive control, was introduced into the bacterial assay and monitored for duration of 24 hours. The resulting MIC assay value was recorded as -42.83±22.36.

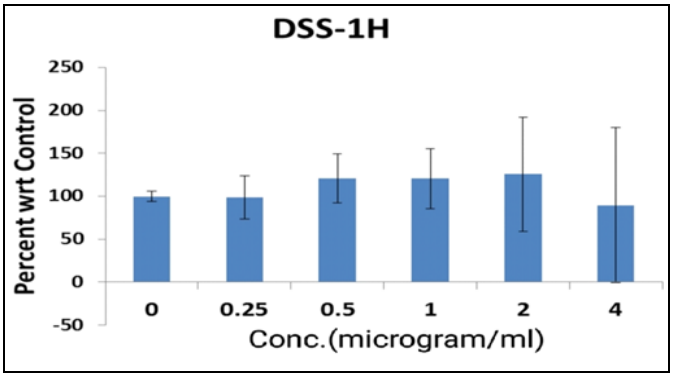

FIG. 1: GRAPHICAL DATA OF MIC ASSAY FOR HEXANE EXTRACT OF SEEDS OF *D. STRAMONIUM*

TABLE 3: ANTIBACTERIAL ASSAY OF ACETONE EXTRACT OF SEEDS OF *D. STRAMONIUM*

Sample	Concentration (µg/ml)	Exposure Period (In Hours)	MIC Assay (Mean±SD)
DSS-2A	0	24 h	100±11.23
	0.25	24 h	128.02±25.64
	0.50	24 h	86.79±14.95
	1	24 h	34.61±16.33
	2	24 h	26.29±29.76
	4	24 h	32.56±19.48
	PC	24 h	-20.02±12.23

When the acetone extract was subjected to a MIC assay experiment the results showed in **Table 3** that the growth of *M. kansaii* was inhibited at different levels for each concentration (0.25µg/ml, 0.50µg/ml, 1µg/ml, 2µg/ml, 4µg/ml). After 24 hours of exposure, the inhibitory percentages were 128.02±25.64, 86.79±14.95, 34.61±16.33, 26.29±29.76, 32.56±19.48 for the respective concentrations. These findings are summarized in **Table 3**. At a concentration of 4µg/ml, the growth of bacteria was significantly reduced ($p<0.05$) to 32.56±19.48, compared to the negative control (0µg/ml) at 100±11.23.

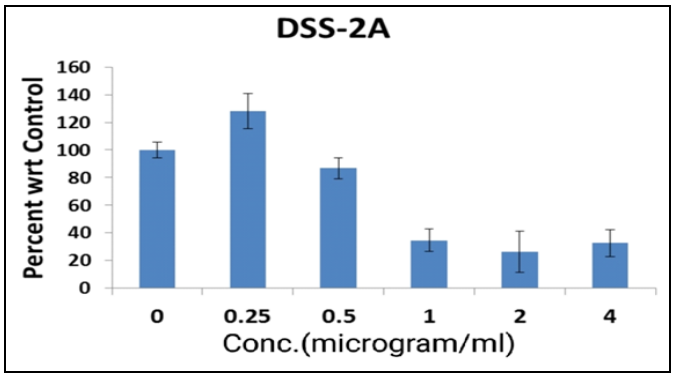

FIG. 2: GRAPHICAL DATA OF MIC ASSAY FOR ACETONE EXTRACT OF SEEDS OF *D. STRAMONIUM*

TABLE 4: ANTIBACTERIAL ASSAY OF ETHANOLIC EXTRACT OF SEEDS OF *D. STRAMONIUM*

Sample	Concentration (µg/ml)	Exposure Period (In Hours)	MIC Assay (MEAN±SD)
DSS-3ET	0	24 h	100±11.23
	0.25	24 h	75.09±39.45
	0.50	24 h	60.16±44.46
	1	24 h	28.19±20.73
	2	24 h	42.24±32.80
	4	24 h	46.93±69.00
	PC	24 h	-20.23±14.23

The outcomes in **Table 4** indicated that the ethanolic extract of seeds of *D. stramonium* as the solvent exhibited notable antimycobacterial activity. The inhibition percentages against *Mycobacterium kansasii* ranged from 1%, 2.5%, 40%, 71%, 58%, and 54% across six different concentration groups: 0µg/ml, 0.25µg/ml, 0.50µg/ml, 1µg/ml, 2µg/ml, and 4µg/ml, respectively. The results were like those of the conventional medication Ciprofloxacin. The p-value is less than 0.05.

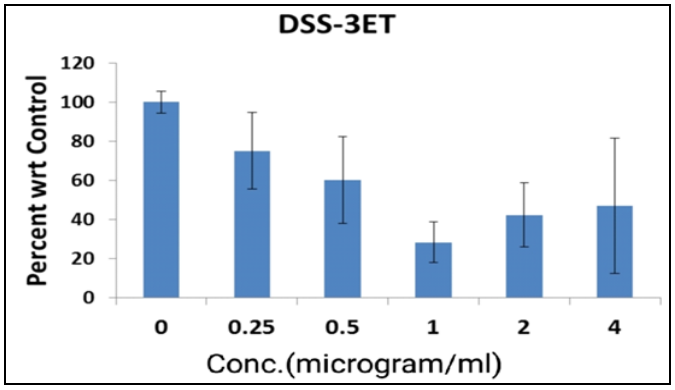

FIG. 3: GRAPHICAL DATA OF MIC ASSAY OF ETHANOLIC EXTRACT OF SEEDS OF *D. STRAMONIUM*

TABLE 5: ANTIBACTERIAL ASSAY OF METHANOLIC EXTRACT OF LEAVES OF *D. STRAMONIUM*

Sample	Concentration (µg/ml)	Exposure Period (In Hours)	MIC Assay (MEAN±SD)
DSL-M	0	24 h	100±28.34
	0.25	24 h	146.05±15.24
	0.50	24 h	117.63±36.36
	1	24 h	59.88±95.54
	2	24 h	16.45±58.76
	4	24 h	42.89±7.23
	PC	24 h	-12.53±5.19

In **Table 5,** the MIC assay experiment was conducted according to the guidelines set by the Clinical and Laboratory Standards Institute. The column fraction was used with hexane as the eluting solvent against bacteria in 96 well u-bottomed micro liter plates. The plant extracts were diluted in a series of concentrations: 0.25, 0.50 µg/ml, 1µg/ml, 2 µg/ml and 4µg/ml. In group 5, the MIC assay result was the lowest at 16.45±58.76, which represents a substantial decrease of 84.06% compared to the value of group 1 (negative control) after 24 hours of incubation. The statistical significance level is less than 0.05. The experiment is conducted in triplicate using Ciprofloxacin as the standard medication or positive control.

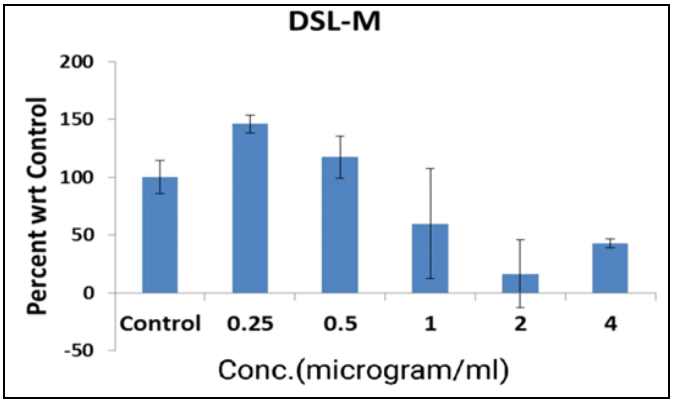

FIG. 4: GRAPHICAL DATA OF MIC ASSAY OF METHANOLIC EXTRACT OF LEAVES OF *D. STRAMONIUM*

TABLE 6: ANTIBACTERIAL ASSAY OF ETHANOLIC EXTRACT OF LEAVES OF *D. STRAMONIUM*

Sample	Concentration (µg/ml)	Exposure Period (In Hours)	MIC Assay (MEAN±SD)
DSL-E	0	24 h	100±15.66
	0.25	24 h	89.52±23.73
	0.50	24 h	102.93±28.55
	1	24 h	60.66±18.13
	2	24 h	58.86±28.90
	4	24 h	50.91±8.83
	PC	24 h	-30.96±4.58

The well-diffusion approach was employed for conducting antimicrobial susceptibility testing. Following a 24 hour incubation period, each group was assessed for inhibition levels. All experiments were conducted simultaneously, and the results were the mean of a minimum of three separate studies. The study's findings revealed that the sample (DSL-E), which was obtained from leaves using solvent ethanol, exhibited inhibitory efficacy against bacteria, as shown in **Table 6**. The test sample exhibited more antibacterial activity at a concentration of 0.50µg/ml, as indicated by the mean zones of inhibition measuring 102.93±28.55. In comparison, the negative control had a value of 100±15.66. The p-value is less than 0.05.

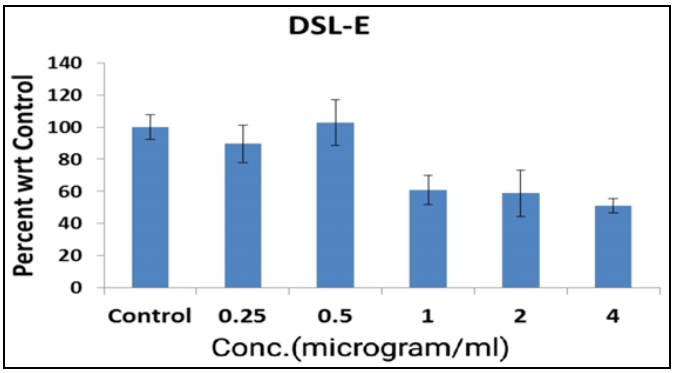

FIG. 5: GRAPHICAL DATA OF MIC ASSAY OF ETHANOLIC EXTRACT OF LEAVES OF *D. STRAMONIUM*

DISCUSSION: Botanical specimens serve as a valuable reservoir of both contemporary and conventional pharmaceutical remedies. Previous studies have examined the secondary metabolites in various extracts of the *D. stramonium* plant. The analysis showed that the hexane, acetone and ethanolic extract of the seeds of *D. stramonium* contains significant amounts of diverse phytochemicals including flavonoids, alkaloids, saponins, chromenes, tannins, phenols, and others. These chemicals have been found to possess pharmacological and physiological properties, such as antibacterial, antifungal, antioxidant, antidiabetic, insecticidal, spermicidal, anticancer, antihelmintic, and wound healing activity. The hexane, acetone and ethanolic extracts of *D. stramonium* seeds and methanolic and ethanolic extract of leaves exhibited inhibitory activity against the tested bacterial strain. Prior studies have documented the varying antibacterial efficacy of distinct *D. stramonium* seeds and leaves species. The activity is a result of the presence of phytochemicals in the extracts. The efficacy of the extract increases as the concentration of the test samples increases. This suggests that the bioactive chemicals, which are responsible for the inhibitory action, become more potent as the dilution or concentration of the test sample declines.

The test bacterial strains exhibited different levels of susceptibility to the hexane, acetone and ethanolic extract of seeds, as well methanolic and ethanolic extract of leaves. The acetone extract of seeds exhibited an IC_{50} value of 1.500µg/ml, indicating a 50 percent inhibition of growth of *M. kansaii* in comparison, the ethanolic extract of seeds shown inhibition at a dosage of 0.992µg/ml, as stated in **Table 1**. The fourth sample of methanolic extract of leaves exhibited inhibition at a concentration of 2.332µg/ml, the fifth sample of ethanolic extract of leaves at 3.171µg/ml. The variation in inhibition values could be attributed to the presence of distinct bioactive compounds in the respective test samples. The sample exhibiting the highest antibacterial activity is clearly indicated by the IC_{50} values. The final Ciprofloxacin was anticipated to exhibit greater efficacy compared to the test samples due to the potential presence of contaminants in the samples, whereas Ciprofloxacin is produced synthetically and undergoes many purification steps. However, extracts of the leaves and seeds of *D. stramonium* showed concentration-dependent activity against all the tested bacterial isolates. This result confirms the earlier report that seeds and leaves extracts of *D.*

stramonium have shown antibacterial activity against certain bacterial strains.

CONCLUSION: The findings of this study validate the long-standing use of this plant in medicinal practices and provide further evidence for the efficacy of its extracts. The plant has the potential to serve as a source for antibacterial agents, making it a viable option for treating *M. kansasii*. Herbal therapies are readily accessible and cost-effective, providing viable alternatives to pricier allopathic medicine. Effective antimicrobial drugs have the potential to mitigate illness and decrease morbidity in bacterial infections, and in some cases, they can be crucial in saving lives during invasive infections. Additional research will be conducted using fractionation techniques to isolate and characterize the active components. There is still much work to be done before the results obtained from various extracts of seeds and leaves of *D. stramonium* can be applied in medicine formulations. To further our knowledge of *Mycobacterium kansasii*, it is important for future research to concentrate on understanding the mechanisms of antibiotic resistance, devising innovative treatment approaches, and enhancing diagnostic tools for precise and prompt identification of this pathogen.

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Author Contribution: Conceptualization, Z.A. and S.; methodology, Z.A. and; validation, M.S.; formal analysis, M.S. and S.; investigation, Z.A.; writing-original draft preparation, Z.A. and S.; writing-review and editing, S. and M.S. All authors have read and agreed.

CONFLICT OF INTEREST: The authors declare that there is no conflict of interests.

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