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Evaluation of antimicrobial activity of plant extracts and column fractions of hexane extract against *Mycobacterium kansaii*

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Abstract

The aim of this study is to assess the antibacterial efficacy of aerial parts of the *Ageratum conyzoides* plant extracts and column fractions against *Mycobacterium kansaii*. The antimicrobial experiments were performed using five doses (0.1 µg/ml, 1 µg/ml, 10 µg/ml, 100 µg/ml, 1000 µg/ml) of plant extracts and column fractions. The results showed notable inhibitory effects on *Mycobacterium kansaii.* The results indicated that the extract of *A. conyzoides* exhibited action against all the tested bacterial isolates, with the level of activity varying depending on the concentration. The hexane extract exhibited antibacterial activity, with mean zones of inhibition measuring 97.88±9.46 and 27.1±3.33, while the ethanol extract showed a mean zone of inhibition of 100.6±3.42 and 36.77±1.74 against *M.kansaii,* respectively. Nevertheless, the hexane extract's three column fractions exhibited average inhibition zones of 98.60±1.0 and 23.11±4.35, 90.02±10.22 and 21.94±6.77, 99.32±3.04 and 17.23±5.26 for the third, fourth, and fifth (last sample) respectively. The findings of this study provide evidence for the conventional utilization of the Ageratum plant and emphasize the necessity for further comprehensive research to explore potential alternatives to current antibacterial medications.

Keywords: *Ageratum conyzoides*; Minimum inhibitory concentration assay; *Mycobacterium kansaii; E*xtract

1. Introduction

Mycobacterium kansaii is a non-tuberculous species of bacteria that belongs to the *Mycobacterium* genus. It is frequently encountered in environmental reservoirs such as soil and water, and can infect humans through inhalation of aerosols, as well as causing infection in other animals. Upon human contact, it has a profound impact on the development of pulmonary diseases, such as lung tuberculosis, in individuals with impaired immune systems, including those who have undergone organ transplantation and are co-infected with HIV and silicosis [1-4].

Several risk factors have been identified for *M.kansasii* infection, including pneumoconiosis, chronic obstructive lung disease (COPD), previous mycobacterial disease, malignancy, and alcoholism [1,2,5-8]. The symptoms of this bacterial infection can vary, but typically include cough, shortness of breath, chest pain, and fatigue. The standard treatment for the infection caused by *M.kansaii* usually consists of a combination of antibiotics, such as clarithromycin and rifampin. There is still much to be learned about the precise mechanism of action of antibiotics. It is widely accepted that these antibiotics disrupt the production of vital proteins or enzymes in the bacteria, effectively halting their ability to grow and reproduce. Diagnosing *Mycobacterium kansaii* infection can pose a challenge due to its resemblance to other mycobacterial infections. In general, the diagnosis of *Mycobacterium kansaii* involves a series of laboratory tests. These tests include acid-fast staining of sputum or tissue samples, culturing of the bacteria, and the use of molecular techniques such as polymerase chain reaction to detect specific genetic markers. Recent studies have uncovered genetic

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diversity within *M. Kansaii* isolates have been discovered in various locations across the globe. The values [9,10] are provided.

Herbal medicinal supplies have been essential components of nature since ancient times. Researchers have been greatly interested in novel active ingredients, especially those derived from natural sources, for many years. This is due to their unique chemical structures and powerful bioactivities. The significance of plant metabolites in drug development is evident from the fact that most of the therapeutic agents discovered and approved in recent years have originated from plants or natural sources [11]. Based on the information provided, it can be inferred that natural compounds have the potential to be highly effective in both preventing and treating a wide range of diseases. In addition, numerous extracts or secondary metabolites such as terpenoids, oils, alkaloids, flavonoids, etc. have been proven to be efficient antibacterial agents [12]. Ideally, modern herbal antibacterial agents should offer enhancements in their ability to specifically target bacteria, have a wide range of effectiveness against different types of bacteria, employ several methods of action, and not be susceptible to resistance like synthetic antibacterial agents currently available.

Previous thorough study has shown that the antibacterial chemicals found in *Ageratum conyzoides* can be utilized to create safer, more cost-effective, and environmentally friendly alternatives to currently available antibacterial medications. *A. conyzoides* is a fragrant herb with a long history of medicinal benefits in traditional medicine worldwide. 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 essential oil of the herb. These include precocene I, precocene II, and ageratochromene dimer, as well as coumarin, kaempferol, quercetin, quercetin-3-rhamnopiranoside, caffeic acid, echinate, phytol, and pyrrolizidine alkaloids. Other compounds found include stigmasterol, β-sitosterol, and friedeline, as well as α-pinene, β-pinene, phenols, and eugenol. 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,[13] antibacterial, antidiabetic, antimicrobial, anti-inflammatory, antioxidant, anticancer, and numerous more.[14-17] In addition to examining the chemical and pharmacological characteristics, the researchers have also investigated the allelopathy and invasiveness of *A. conyzoides* [18,19]. Nevertheless, there has not been a comprehensive review to date on all aspects pertaining to the potential utilization of this medicinal plant against diverse bacterial pathogens.

2. Material and method

The stem, flowers, and leaves were manually detached from the roots. All the aerial plant material was air-dried for a duration of 4 days at room temperature. The shaded dry materials were pulverized into a fine powder using an electric blender. The crude extract of the aerial part of Ageratum conyzoides was produced by the maceration process, employing hexane and ethanol as the extraction solvents [20,21,22,23]. Approximately 1000 ml of hexane was introduced into a percolator containing approximately 200 g of dehydrated plant material, 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 at 50 and finally dried in a Rota vapor to obtain a thick green paste which was suitably diluted and used for the experiments. After being extracted with hexane, the remaining substance was subjected to treatment with approximately 1000 ml of ethanol. This procedure was repeated three times. The choice of solvent is determined by factors such as the type of plant, the characteristics of the bioactive chemicals, and the accessibility of the solvent. Typically, polar solvents are employed to extract polar compounds, while non-polar solvents are used to extract non-polar compounds.

2.1. Column chromatography

On obtaining the separation 1g of hexane extract subjected to column chromatography where it involves silica gel for column. (1:25). The column was eluted with the following solvent system as hexane, hexane-ethyl acetate (1%), hexane-ethyl acetate (5%), hexane -ethyl acetate (30%), hexane – ethyl acetate (1:1), pure ethyl acetate lastly by hexane-methanol (5%).

2.2. Antibacterial assay

Antibacterial analyses were conducted on ethanol and hexane of *A.conyzoides*. The hexane extract was subjected to column chromatography fractionation using various solvents, and the resulting fractions were tested against the bacterial species *Mycobacterium kansaii.* The study utilized the Broth Dilution technique to ascertain the minimum inhibitory concentration (MIC). The antimicrobial investigation was conducted at the Department of Chemistry, Isabella Thoburn College, located in Lucknow, India. A 0.5 McFarland Standard dilution of microbes will be utilized for the study. 500µl 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 24 hours. Following incubation, the entire contents were transferred to a 96-well plate. Subsequently, readings were obtained using an Elisa Plate Reader (iMark Biorad) at wavelengths of 490nm and 595nm. The positive control utilized in the experiment was Ciprofloxacin at a concentration of 100µg.

3. Results

3.1. Tables and graphs

Table 1 IC_{50} data of different test samples of against M.kansaii

SAMPLE	IC_{50} (µg/ml)
SPP-H-1	11.48
SPP-EtOH-2	11.79
SPP-CC-3	22.98
SPP-CC-4	16.57
SPP-CC-5	4.98

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 hexane extract has a value of 11.48 µg /ml, while the ethanol extract has a value of 11.79 µg/ml. The inhibition value for the third sample is 22.98µg/ml, for the fourth sample it is 16.57µg/ml, and for the fifth sample it is 4.98 µg /ml. From the data it can be concluded that the fifth sample had showed most promising IC_{50} value and therefore maximum activity against the bacteria.

Table 2 Antibacterial assay of hexane extract of *A. conyzoides*

Sample	Concentration(µg/ml)	Exposure Period (in hours)	mic assay (mean ±sd)
SPP-H-1	0	24 h	100±5.09
	0.1	24 h	97.88±9.46
	1	24 h	72.97±3.70
	10	24 h	50.16±1.60
	100	24 h	31.20±2.01
	1000	24 h	27.11±3.33
	PC	24 h	-12.83±4.36

Table 2 and graph 1 shows the seven different concentrations of the sample coded SPP-H-1 that- were utilized in the MIC assay experiment. The MIC value after a 24-hour exposure period is 100±5.09 when no sample is added or at zero concentration, which is considered the negative control. In group 2, the sample is added to the bacterial assay at a concentration of 0.1 µg/ml. The observed value for the MIC assay is 97.88±9.46, which is only 3% lower than the value of the negative controls after a 24-hour exposure period. In group 3, the sample concentration was raised from 0.1µg/ml to 1µg/ml. As a result, the MIC assay value decreased to 72.97±3.70, which is 28% lower than the negative control value after a 24-hour exposure period. In group 4, the concentration is 10 µg/ml, and the MIC assay value is 50.16±1.60, indicating a 50% decrease from the negative control value after a 24-hour exposure period. In group 5, the sample concentration was 100 µg/ml. After 24 hours of exposure, the MIC assay value decreased to 69%, specifically 31.20±2.01. In group 6, the sample concentration was 1000 µg/ml. The MIC assay value was 27.20±2.01, indicating a significant decrease of 73% 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 a duration of 24 hours. The resulting MIC assay value was recorded as -12.83±4.36.

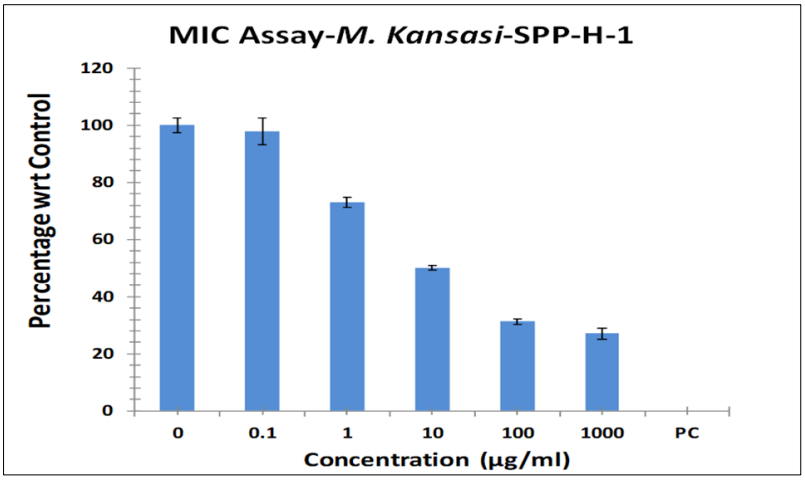

Figure 1 Graphical data of mic assay for hexane extract of *A.conyzoides*

Table 3 Antibacterial assay of ethanolic extract of *A. conyzoides*

Sample	Concentration(µg/ml)	Exposure Period (in hours)	mic assay (mean±sd)
SPP-EtOH-2	0	24 h	100±3.19
	0.1	24 h	100.6±3.42
	1	24 h	66.37±1.74
	10	24 h	48.21±1.74
	100	24 h	42.06±12.32
	1000	24 h	36.77±1.74
	PC	24 h	-10.02±1.18

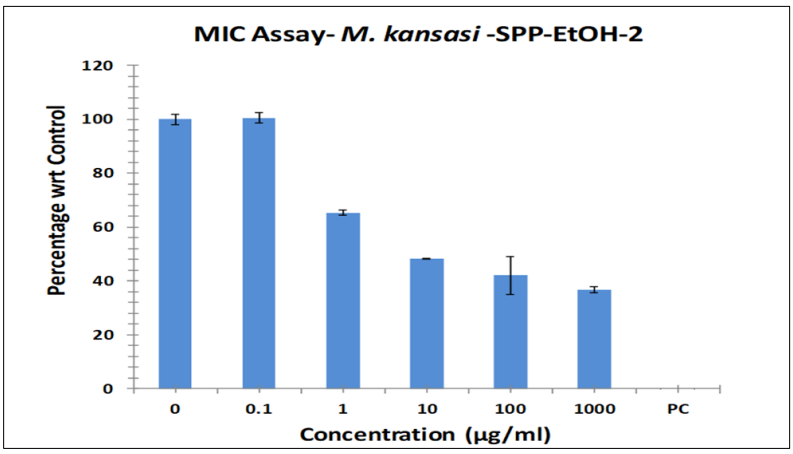

Figure 2 Graphical data of mic assay for ethanolic extract of *A.conyzoides*

When the ethanolic extract was subjected to a MIC assay experiment the results showed in table3 and graph 2 shows that the growth of M.kansaii was inhibited at different levels for each concentration (0.1µg/ml, 1µg/ml, 10 µg/ml, 100 µg/ml, 1000 µg/ml). After 24 hours of exposure, the inhibitory percentages were 100±3.19, 100.6±3.42, 66.37±1.74, 48.21±1.74, 42.06±12.32, and 36.77±1.74 for the respective concentrations. These findings are summarized in table 3. At a concentration of 1000 µg/ml, the growth of bacteria was significantly reduced ($p<0.05$) to 36.77±1.74, compared to the negative control (0 µg/ml) at 100±3.19.

Table 4 Antibacterial assay of column fraction eluted with hexane from hexane extract of *A. conyzoides*

Sample	Concentration (µg/ml)	Exposure Period (in hours)	mic assay (mean±sd)
SPP-CC-3	0	24 h	100±2.31
	0.1	24 h	98.60±1.0
	1	24 h	72.76±9.21
	10	24 h	59.03±7.23
	100	24 h	36.55±8.02
	1000	24 h	23.11±4.35
	PC	24 h	-12.74±8.74

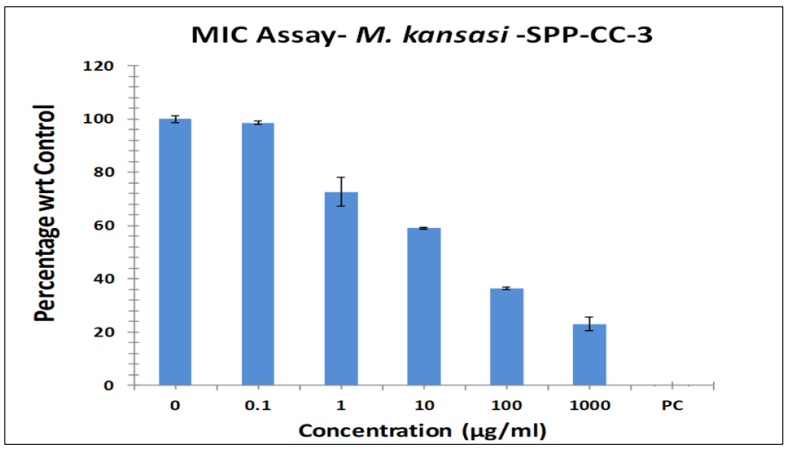

Figure 3 Graphical data of mic assay of column fraction eluted with hexane from hexane extract of *A. conyzoides*

The outcomes in table 5 and graph 3 indicated that the hexane extract of *A. conyzoides* obtained from column fractionation using hexane as the solvent exhibited notable antimycobacterial activity. The inhibition percentages against Mycobacterial tuberculosis ranged from 1.4 %, 27.74%, 59.03%, 40.97%, 63.74%, 76.89% across six different concentration groups: 0 µg/ml, 0.1 µg/ml, 1 µg/ml, 10 µg/ml, 100 µg/ml, and 1000 µg/ml, respectively. The results were like those of the conventional medication Ciprofloxacin. The p-value is less than 0.05.

Table 5 Antibacterial assay of column fraction eluted with hexane from hexane extract of *A. conyzoides*

Sample	Concentration (µg/ml)	exposure period (in hours)	mic assay (mean±sd)
SPP-CC-4	0	24 h	100±7.04
	0.1	24 h	90.02±10.22
	1	24 h	69.69±10.56
	10	24 h	56.60±4.23

	100	24 h	33.92±3.85
	1000	24 h	21.94±6.77
	PC	24 h	-12.53±2.19

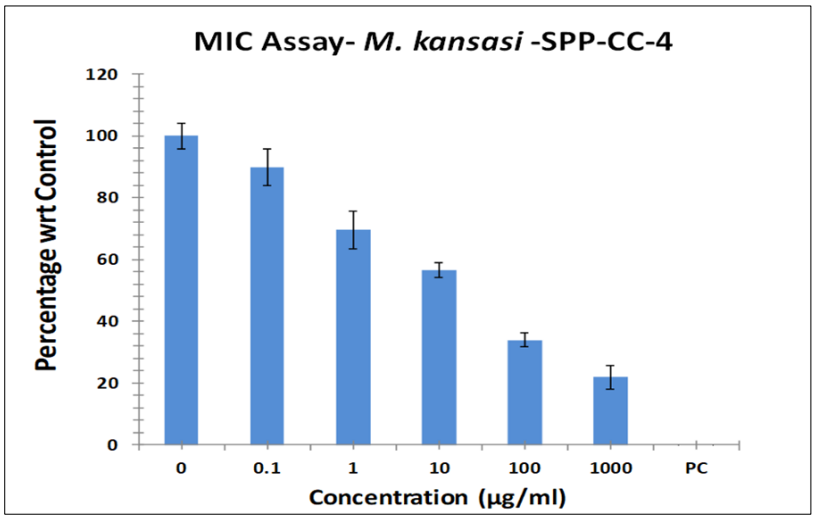

Figure 4 Graphical data of mic assay of column fraction eluted with hexane from hexane extract of *A.* conyzoides

In table 6 and graph 4, the MIC assay experiment was conducted according to the guidelines set by the Clinical and Laboratory Standards Institute. A column fraction was used with hexane as the eluting solvent against bacteria in 96 well u-bottomed microliter plates. The plant extracts were diluted in a series of concentrations: 0.1 µg/ml, 1 µg/ml, 10 µg/ml, 100µg/ml and 1000 µg/ml. In group 6, the MIC assay result was the lowest at 21.94±6.77, which represents a substantial decrease of 78.06% 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.

Table 6 Antibacterial assay of column fraction eluted with 1:1 hexane: ethyl acetate from hexane extract of *A. conyzoides*

Sample	**Concentration(µg/ml)**	**Exposure period (in hours)**	**mic assay (mean±sd)**
SPP-CC-5	0	24 h	100±2.18
	0.1	24 h	99.32±3.04
	1	24 h	60.32±5.12
	10	24 h	40.85±1.84
	100	24 h	35.75±4.84
	1000	24 h	17.23±5.26
	PC	24 h	-9.96±4.58

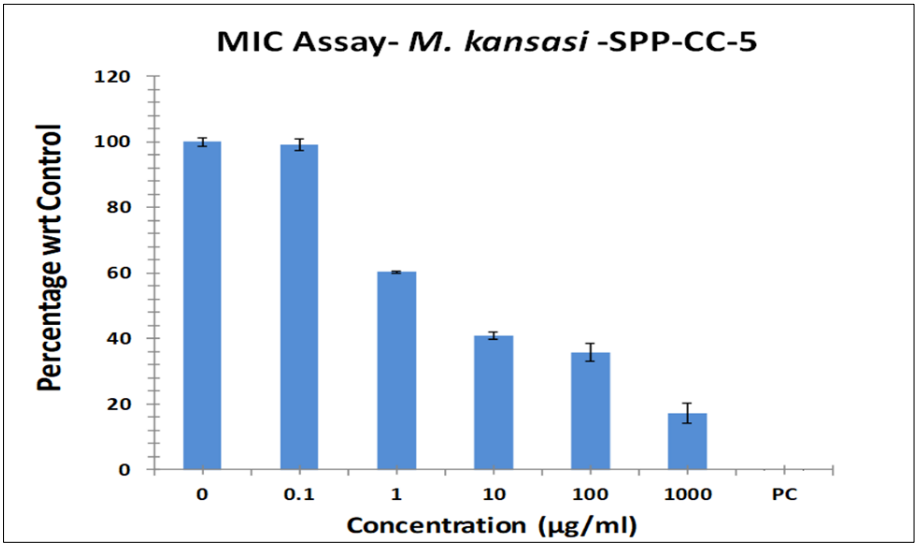

FIgure 5 Graphical data of mic assay of column fraction eluted with 1:1 hexane: ethyl acetate from hexane extract of *A. conyzoides*

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 (SPP-CC-5), which was obtained using column chromatography of a hexane extract employing a 1:1 ratio of ethyl acetate to hexane as the eluting solvent, exhibited inhibitory efficacy against bacteria, as shown in Table 6 and graph 5. The test sample exhibited more antibacterial activity at a concentration of 1000 µg/ml, as indicated by the mean zones of inhibition measuring 17.23±5.26. In comparison, the negative control had a value of 100±2.18. The p-value is less than 0.05.

4. 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 *A.conyzoides* plant [24,25,26,27]. The analysis showed that the hexane and ethanolic extract of *A.conyzoides* 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, anthelmintic, and wound healing activity [28,29]. The hexane and ethanolic extracts of A.conyzoides exhibited inhibitory activity against the tested bacterial strain. Prior studies have documented the varying antibacterial efficacy of distinct *A.conyzoides* plant species. [30,24,27,31] The activity is a result of the presence of phytochemicals in the extracts. Nevertheless, the hexane and ethanol extracts exhibited action against the bacterial strain in a manner that varied with the concentration. The efficacy of the extract diminishes as the concentration of the test samples decreases. 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 and ethanolic extract, as well as the column fractions derived from the hexane extract. The hexane extract exhibited an IC_{50} value of 11.48 µg/ml, indicating a 50 percent inhibition of growth of *M.kansaii* In comparison, the ethanolic extract shown inhibition at a dosage of 11.79 µg /ml, as stated in table 1. The third sample exhibited inhibition at a concentration of 22.98 µg/ml, the fourth

sample at 16.57 µg /ml, and the fifth sample at 4.98 µg mg/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, flowers and stem of A. conyzoides showed concentration-dependent activity against all the tested bacterial isolates. The activity of the extracts decreased as the concentration decreased and vice versa. This result confirms the earlier report that

plant extracts of A.conyzoides have shown antibacterial activity against certain bacterial strains. [31] The active component of this plant may be due to its high nonpolar compounds.

5. 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. kansaii.* 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 should 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 and column fractions of *A. conyzoides* can be applied in medicine formulations. To further our knowledge of *Mycobacterium kansaii*, 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.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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