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INTEGRATED ASSESSMENT OF HEAVY METAL BIOACCUMULATION, HUMAN HEALTH RISKS, AND ANTIMICROBIAL ACTIVITY IN MEDICINAL PLANTS FROM PUNJAB, PAKISTAN

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Abstract

Citrullus colocynthis (L.) Schrad., *Capparis decidua* (Forssk.) Edgew., and *Calotropis procera* (Aiton.) W.T. Aiton were selected as medicinal plants from the environmentally diverse locations of Punjab province, Pakistan, such as Bahawalpur, Bahawalnagar, Rahim Yar Khan, and Lodhran. Variation in the levels of eight heavy metals (As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn) was found among different species. *Capparis decidua* accumulated more heavy metals than other medicinal plants, particularly Hg (6.566 mg/kg), Pb (3.666 mg/kg), Ni (5.424 mg/kg), Cd (1.344 mg/kg), Zn (43.23 mg/kg), while *Calotropis procera* accumulated more As and Cu. Significant non-carcinogenic health risks (THQ > 1) were assessed by Hazard Index (HI), Target Hazard Quotient (THQ), and Estimated Daily Intake (EDI), which exceeded safety limits in all the plant species, indicating health hazards related to the consumption of medicinal plants. The antibacterial potential against *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus* was checked using the agar well diffusion method. *Calotropis procera* exhibited the strongest inhibitory effects against *S. aureus* (22.0 ± 0.6 mm), *B. subtilis* (19.5 ± 0.5 mm), and *E. coli* (14.5 ± 0.4 mm), followed by *Capparis decidua* and *Citrullus colocynthis.* HPLC analysis identified numerous bioactive compounds, including flavonoids, phenolic acids, alkaloids, and terpenoids. *Capparis decidua* demonstrated the greatest phytochemical diversity, whereas *Calotropis procera* contained higher concentrations of several potent metabolites.

Key words: Medicinal plants; Heavy metals; *Citrullus colocynthis*; *Capparis decidua*; *Calotropis procera*; Punjab; Pakistan

Introduction

Medicinal plants constitute an important part of the health care system worldwide due to their medicinal and therapeutical uses. It is believed that approximately 80% of the population of the world uses herbal medicine as their primary health care provider, according to the Anon., (2024). While the therapeutic properties of medicinal plants stem from their content of chemical compounds, e.g., alkaloids, flavonoids, terpenoids, glycosides, phenolics, etc. These compounds give rise to many different pharmacological actions, such as anti-inflammatory, anti-bacterial, antioxidant, and anti-cancer effects. Unfortunately, with respect to medicinal plants, quality and safety issues are becoming increasingly important to investigators (Ahmad Nejhad *et al*., 2023). The reason for this phenomenon is that many medicinal plants are contaminated with environmental pollutants and heavy metals, which may negatively impact their medicinal value (Sevindik *et al.,* 2025).

Therapeutic properties and uses of medicinal plants: Research suggests that medicinal plants serve as an important source of numerous bioactive compounds, leading to activities that are used for healing purposes. An example of a plant that meets these criteria is *Citrullus colocynthis*, which has been reported to have numerous medicinal uses throughout the world, though a significant number of scientific papers and reports have reviewed *C. colocynthis*' medicinal uses (Table 1). The four primary therapeutic properties attributed to *C. colocynthis* include antidiabetic, anti-inflammatory, antimicrobial or antifungal, and antioxidant. This plant has been classified as a traditional herb to treat gastrointestinal disorders such as rheumatism and skin diseases. However, more recent studies have also noted its cytotoxicity, genotoxicity, and ability to induce apoptosis, which shows that this plant can potentially have further biomedical value (Sevindik *et al.,* 2024). The therapeutic potential of the species arises from its diversity of phytochemicals: flavonoids, phenolic compounds, alkaloids, and terpenoid content. Due mainly to the presence of bioactive compounds within this plant, the use of traditional medicine continues to play an essential role within medicinal systems and provides the source of current research for the development of new pharmaceuticals (Alsalihi *et al.,* 2025).

Heavy metal contamination of medicinal plants: A family of naturally occurring chemical substances with high molecular weights and densities is known as heavy metals. Both natural sources, such as worn rocks, and man-made sources, such as industrial and commercial pollution, farming, and irrigating crops and plants with unclean water, are common ways that heavy metals enter the

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environment (Kolapo & Omoboyede, 2018). They remain in soil and stream/river systems indefinitely due to their inability to be deconstructed. They can enter plants through being taken up from soil via roots or through being distributed via the air onto the surface of leaves, leading to their accretion in plant tissues.

The accumulation of heavy metals in plants has adverse effects on the growth and development of the plant and ecosystem balance and pose serious health threats to humans in case these toxic plants are ingested as food or medicine (Yang *et al.,* 2021). In contrast to pharmaceutical products, medicinal plants are mostly extracted directly from the environment without proper regulation and quality control due to the less processed nature of medicinal plants (Dahiya *et al.,* 2025). Many researchers have established that plants grown in agricultural, industrial, and urban areas contain high levels of heavy metals that are higher than the safety limits recommended by the international community (Sevindik *et al.,* 2024). Studies done in Pakistan have revealed that certain types of medicinal plants contain nickel (Ni), copper (Cu), lead (Pb), and other heavy metals above the permissible limits set by the World Health Organization (WHO). Moreover, worldwide surveys have revealed that lead (Pb) and cadmium (Cd) concentrations in herbs are above the acceptable safety limits (Geronimo *et al*., 2021).

Cadmium and lead are highly toxic with serious health effects. Cadmium can result in kidney failure, skeletal problems, and cancer. Lead can affect the brain, heart, and kidneys, especially for children and their mental development. Zinc and copper are both essential micronutrients, but high levels can lead to oxidative stress and impaired enzyme function. Nickel is another heavy metal that is linked to lung problems, skin problems, and even cancer (Karahan *et al.,* 2023; Tadi *et al.,* 2024). Organizations like the Food and Agriculture Organization (FAO) and World Health Organization (WHO) have established permissible limits for heavy metals in medicinal plants to safeguard consumers against health complications as a result of high heavy metal levels in medicinal plants. The results of the analysis were compared with the permissible levels established by WHO for medicinal plants, wherever applicable, including Pb (10 mg/kg), Cd (0.3 mg/kg), As (1.0 mg/kg), and Hg (0.1 mg/kg). The above-mentioned guideline values are universal and species-independent for *Citrullus colocynthis, Capparis decidua,* and *Calotropis procera.* Due to human activity, pollution of medicinal plants is a growing environmental and public health concern (Alsalihi *et al.,* 2025). Soil pollution from manufacturing, agriculture, transportation, mining, and the use of polluted water for irrigation is a major environmental and public health concern. Heavy metals can be absorbed through the roots of plants and then moved to the rest of the plant, entering the human food chain through herbal products (Moghaddam *et al.,* 2020). As heavy metals are non-degradable, they build up over time in plant tissues, which increases the potential for adverse health effects (Fig. 1).

Excessive amounts of harmful metals such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), and zinc (Zn) can be found in medicinal plants collected from contaminated locations. Although plants need vital nutrients like zinc and copper, excessive amounts of these elements can cause phytotoxicity and have a detrimental effect on human health. At low concentrations, arsenic, cadmium, mercury, and lead have extremely strong nephrotoxic, neurotoxic, cardiotoxic, and carcinogenic effects.

Some examples of plants with medicinal properties are *Citrullus colocynthis*, *Calotropis procera*, and *Capparis decidua*, which have been used in alternative medicine modalities (Ali *et al.,* 2025). However, the presence of these plant species leads to the accumulation of heavy metals in very different amounts depending on their growing conditions (type of soil, climate, and metal bioavailability). In addition, different species exhibit different heavy metal uptake abilities. Therefore, comprehensive assessments of metal accumulation of medicinal plants are necessary to ensure their safety and efficacy. The transport of heavy metals into and through the plant is an important and complex process that occurs through the inherent mechanisms of plant physiology. Non-essential heavy metals can be absorbed into the plant root systems via routes normally used for regular nutrient absorption. While this relies on the chemical similarity between essential and non-essential heavy metals, it is not always guaranteed that heavy metals will be absorbed in this manner once they are inside the plant. Be that as it may, once heavy metals have entered the plant, they may be chelated (bonded) with phytochelatins and metallothioneins and stored in the vacuoles of plant tissues (Dahiya *et al.,* 2025). The efficiency with which different types of plants detoxify heavy metals varies significantly, and therefore the patterns of heavy metal accumulation within individual plant tissues also vary significantly.

Evaluation of heavy metal health risks: Quantitative risk assessment techniques are frequently employed to examine possible health effects due to growing concerns about the toxicity of heavy metals in medicinal plants. Commonly used indices include the Health Risk Index (HRI) and Estimated Daily Intake (EDI), which compare the level of exposure to defined reference doses (Ebrahimzadeh *et al.,* 2024). HRI values > 1 may indicate potential non-carcinogenic health concerns, while values < 1 suggest tolerable risk levels. The cumulative risks from various metals and routes of exposure were also evaluated by the Hazard Index (HI) and Target Hazard Quotient (THQ). These indices are useful for regulatory decision-making for herbal safety and are critical for the assessment of potential health hazards associated with the ingestion of contaminated medicinal plants (Afzal *et al.,* 2026).

There is an abundance of research on heavy metal accumulation in different food crops and medicinal plant species across South Asia, with studies showing evidence of increased levels of contamination (Daoud *et al.,* 2025). Multiple studies carried out within Pakistan indicate that food crops are irrigated using wastewater containing Pb and Cd levels above permissible limits, causing HRI values to be greater than 1 for both children and adults. While several scientific research projects have been directed on ethnobotany in Pakistan to date, many studies are limited to one species or region within the country, thus highlighting an overall lack of broad-based ecological assessments of these species (Javeed *et al.,* 2024). Conditions such as soil properties, agricultural practices, and anthropogenic factors vary by region, leading to differences in heavy metal accumulation. Additionally, differences between plant species require risk assessments related to the biophysical and physiological characteristics of the species being studied.

Bahawalpur, Bahawalnagar, Rahim Yar Khan, and Lodhran are all part of southern Punjab, Pakistan, which is an ecologically diverse area with semi-arid to arid climates, intensive agriculture, and growing industrial activity (Afzal *et al.,* 2026). The area is heavily dependent on chemical fertilizer and uses high volumes of surface water, groundwater and waste water for irrigation which greatly contributes to soil contamination (Tadi *et al.,* 2024). Nevertheless, there has not been much research done on the buildup of heavy metals in local medicinal plants. Due to the widespread usage of medicinal herbs such as *Citrullus colocynthis, Capparis decidua*, and *Calotropis procera*, thorough risk evaluation is essential for public health protection and policy development (Dahiya *et al.,* 2025).

Medicinal plants as antimicrobial agents: Plant-derived sources provide the best opportunities to find antimicrobial products with multiple mechanisms of action and diverse chemical structures due to their unique chemical richness (Sevindik *et al.,* 2025). Plant materials are different from traditional antibiotics because they are usually composed of many different types of compounds (plant extracts), which can work together to fight microbial pathogens, reducing the possibility of developing resistance due to the presence of multiple compounds. Furthermore, plant-based compounds have lower toxicity and are more biocompatible than synthetic drugs. South Asia, and especially Pakistan, is a region of rich plant diversity and has a long history of use of medicinal plants. Medicinal plants resistant to drought are common in arid and semi-arid regions like the Cholistan, Thal, and Salt Range rich in medicinal plant resources used in traditional medical systems (Midhat *et al.,* 2021). Some common examples of these plants are *Citrullus colocynthis*, *Capparis decidua* and *Calotropis procera* which are widely used as antibacterial and anti-inflammatory agents.

Historically, *Citrullus colocynthis* (bitter apple) has been used for treating parasitic illnesses, skin infections, and gastrointestinal disorders. The active compounds in *Citrullus colocynthis* include cucurbitacins, flavonoids, alkaloids, glycosides, and sterols. Of them, cucurbitacins are the most potent in their antibacterial activity as well (Cheng *et al.,* 2023; Ngozi, 2024). There has been some scientific evidence to suggest that extracts of cucurbitacin can be effective in inhibiting the growth of both Gram-positive and Gram-negative bacteria; however, the extent of this inhibition is influenced by the extraction method used to create the extract and the specific type of microorganism being treated (Ahmad Nejhad *et al.,* 2023).

Capparis decidua is commonly found throughout the world's arid regions and has traditionally been used to treat respiratory infections, fever, urinary disorders, and skin-related diseases. It also contains many phytochemical components, such as flavonoids, alkaloids, glucosinolates, and phenolic acids, which have antimicrobial and antioxidant activity. However, there are currently few studies that have evaluated and compared antimicrobial activity of *Capparis decidua* against different microbial pathogens (Ahmed *et al.,* 2012).

In traditional medicine, the drought-resistant shrub *Calotropis procera* is used to cure microbial infections, wounds, and inflammation. The therapeutic qualities of *Calotropis procera* are derived from a variety of bioactive substances, including cardenolides, flavonoids, terpenoids, tannins, and phenolics (Khanna *et al.,* 2025). Although *Calotropis procera* has been demonstrated to have an antibacterial effect, the antimicrobial activity of extracts varies greatly due to variations in extraction techniques as well as other aspects of study design and execution (Elbagory & Ekpo, 2025).

Numerous reports exist on the individual antimicrobial properties of both *Calotropis procera* and *Capparis decidua* (Kumari & Chaudhary, 2021). However, comparative studies on the antimicrobial activity of both plants conducted using similar experimental conditions or standardized extraction methods are still very limited (Ahmed *et al*., 2012). Principal factors limiting the direct comparison of results across studies regarding the antimicrobial activity of *Calotropis procera* and *Capparis decidua* include differences in extraction methods, use of different plant parts, and use of different microbial strains. As a result, there is an ongoing need for standardized studies using clinically relevant and environmentally pertinent pathogens to substantiate ethnobotanical claims and support the development of commercially viable pharmaceutical products using these plants (Ahmed Nejhad *et al.,* 2023).

The following goals guided the design of this investigation:

1. To measure the bioaccumulation levels of As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn in *Calotropis procera*, *Citrullus colocynthis*, and *Capparis decidua* that were collected from four environmentally varied districts in Punjab, Pakistan.
2. To use EDI, HRI, THQ, and HI methods to assess heavy metal concentrations and related non-carcinogenic health hazards.
3. To evaluate the antibacterial activity of certain extracts from medicinal plants against bacterial strains that are therapeutically relevant.

Table 1. Traditional medicinal uses and pharmacological properties of medicinal plants.

Medicinal plant	Major phytochemicals	Pharmacological properties	Traditional medicinal uses
Citrullus colocynthis	Flavonoids, phenolic compounds, alkaloids, terpenoids	Antidiabetic, anti-inflammatory, antimicrobial, antioxidant	Treatment of gastrointestinal disorders, rheumatism, skin diseases, and digestive problems
Capparis decidua	Phenolics, flavonoids, alkaloids, glycosides	Hepatoprotective, antidiabetic, antimicrobial, anti-arthritic	Used in folk medicine for cough, asthma, fever, liver disorders, and digestive ailments
Calotropis procera	Flavonoids, terpenoids, cardiac glycosides, phenolic compounds	Analgesic, anti-inflammatory, antimicrobial, antioxidant, anticancer	Traditionally used for wound healing, pain relief, treatment of skin diseases, and inflammatory disorders

Materials and Methods

Study area: This research was conducted in four biologically diverse districts in southern Punjab, Pakistan: Bahawalpur, Bahawalnagar, Rahim Yar Khan, and Lodhran. With high summer temperatures and relatively little annual precipitation, these districts have an arid to semi-arid climate. Agriculture is the main economic activity in the region, and irrigation systems using both ground and canal water are significant (Afzal *et al*., 2026). Agricultural intensification, insecticides, fertilizers, and anthropogenic activities have all played a part in environmental pollution through toxic heavy metals (Figs. 2a and 2b). As per Xie *et al*., (2024), such environmental factors make an ideal setting for studying the bioaccumulation of heavy metals in medicinal plants and any risks to human health resulting from their use.

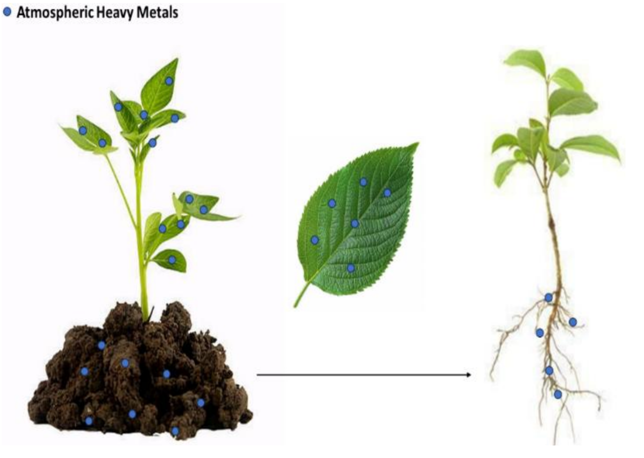

Fig. 1. Sources of heavy metals, foliar and root uptake of heavy metals in plants

Fig. 2a. Plant samples.

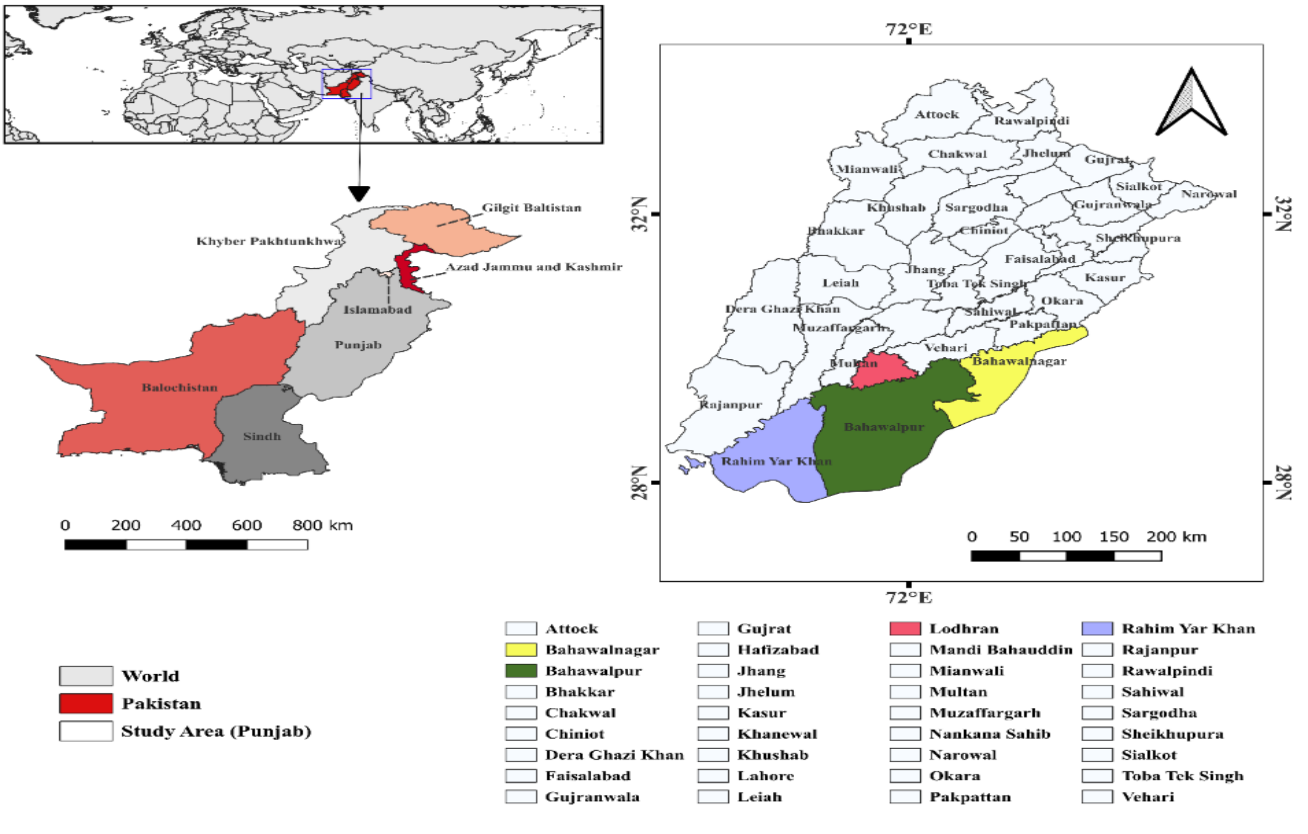

Fig. 2b. Map of study area.

Selection of medicinal plant species: The study examined three traditional medicinal plants used in ethnomedicine, *Citrullus colocynthis* (L.) Schrad., *Capparis decidua* (Forssk.) Edgew., and *Calotropis procera* (Aiton.) W.T. Aiton, with a long history of curing a range of ailments. They are routinely found in the arid and semiarid zones of Pakistan and have been used by traditional practitioners to treat a broad variety of ailments. The reported hypoglycemic, anti-inflammatory, and antibacterial properties of *Citrullus colocynthis* account for its widespread traditional use in Pakistan. *Capparis decidua* has been valued in traditional medicine for its antioxidant properties and its role in managing cardiovascular/metabolic disorders. *Calotropis procera* is a common treatment for wounds, respiratory illness, and digestive disorders and is an important part of many herbal formulations, making it essential to assess these three species for their potential contamination with heavy metals (Qaisar *et al.,* 2026).

Sample collection and preparation: Plant materials were collected as representative samples from naturally growing populations in selected districts during the peak of plant growth. Three independent samples from species of interest were chosen based on physical observations of healthy, mature plants from multiple collection sites that allowed for representative sampling. All plant samples were collected using sanitized stainless-steel scissors to eliminate any chance for cross-contamination of plants within a sample. Each plant collected from each location was labeled by species and location and then placed into sterile polyethylene bags immediately after collection. All samples (n = 9, total samples in triplicate) were then transported to a laboratory under controlled conditions to ensure that plants did not deteriorate or become cross-contaminated before analysis (Daoud *et al.,* 2025). A total of three plant species were studied, and samples were obtained for all the districts involved in this study, making a total of 12 initial plant samples (3 species × 4 districts). Three replicates per plant species in each location were made for accurate analysis (Perveen *et al.,* 2024). All plant tissue surfaces were aseptically cleaned with tap water to remove any foreign materials that had adhered to the plant surfaces, followed by distilled water. Each plant sample was allowed to air dry in a room under ambient climatic conditions for several days. After air drying, all the plant samples were positioned in a hot-air oven set at 60 °C until they reached a constant weight (dry weight). All samples were finely processed using stainless-steel electric grinders. Ground plant material (powder) was stored in a sealed polyethylene container in a desiccator until analysis (Khalid *et al.,* 2024; Ali *et al.,* 2025).

Acid digestion of plant samples: Standard acid digestion techniques were used to remove heavy metals. The powdered plant material was weighed (approximately 0.5 g) and then put into digestion flasks. A solution of hydrogen peroxide (H_2O_2) and strong nitric acid (HNO_3) was added to finish breaking down the organic matrix. A hot plate was utilized in the heating of the mixtures until they became clear and the complete digestion of the mixtures was evident (Liu *et al.,* 2023; Hyder *et al.,* 2025). The mixtures were digested and then allowed to cool before being diluted with deionized water to a final volume of 50 mL. Then, the diluted digested mixtures were filtered through Whatman No. 42 filter paper for 15 minutes. The resulting filtered mixture was analyzed for heavy metals via a sample-to-acid ratio of 1:10 (w/v).

Determination of heavy metal concentrations: Heavy metal concentrations were determined in all three collected species of medicinal plants using Atomic Absorption Spectrophotometry (AAS) (BS EN 1122:2001) for the determination of As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn. Prior to analysis, the device was calibrated, and standard calibration solutions were prepared for each metal. Every measurement was repeated three times (total 24 samples) to ensure accuracy and consistency. Quality Assurance and Quality Control (QA/QC) included reagent blanking, duplicate analysis, and the use of certified reference materials to ensure accurate measurements (Al-Keriawy *et al.,* 2023). The LOD values for arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), and zinc (Zn) were 0.08, 0.01, 0.08, 0.20, 0.50, 0.06, 0.01, and 0.50 µg/L, respectively (Elbagory & Ekpo, 2025).

Health risk assessment: To ascertain the possible non-carcinogenic effects of ingesting contaminated medicinal herbs, a Health Risk Assessment was carried out (Abebe *et al*., 2024). Metal concentrations, ingestion rate, frequency of exposure, duration, body weight, and average time were used to calculate the Estimated Daily Intake (EDI). The EDI values of each metal were compared to its oral reference dose (RfD) to determine the Health Risk Index (HRI). There may be health risks if the HRI value is higher than one; otherwise, no risk is expected. Additionally, the cumulative risk of exposure to multiple metals was estimated using the Total Hazard Index (HI) (Elbagory & Ekpo, 2025; Zafar *et al.,* 2026).

Estimated Daily Intake (EDI): IRd is the daily ingestion rate of vegetables, which is 0.345 kg. Body weight (BW) was 65 kg. The daily ingestion rate was expressed as µg/kg BW/day. It can be calculated by the following formula (Griboff *et al*., 2017).

$$EDI = (C \times IRd) / BW \qquad \text{Eq. 1}$$

Where C is heavy metals concentration in test samples (mg/kg dry weight), IRd = average ingestion rate per day, BW = average body weight in kg.

Target hazard quotient (THQ): The THQ is determined to estimate non-carcinogenic risk associated with toxic metal intake (Anon., 2006). If the THQ value is < 1, it indicates no significant health risk, while ≥1 indicates a probable health hazard to consumers. THQ of heavy metals in test samples was calculated by following the equation.

$$THQ = (C \times 1 \times 10^{-3} \times EFr \times ED) / (RfD \times BW \times ATn) \qquad \text{Eq. 2}$$

Where "C" is metal concentration (mg/L) in test samples, "I" represents food ingestion rate per day per person, EFr represents exposure frequency (365 days/year), ED represents exposure duration (30 years), "BW" represents body weight (kg), and "ATn" represents average time (non-carcinogenic exposure).

Health index (HI): HI is the summation of heavy metal hazard proportions (HQ) for each metal and was calculated using the formula as reported by the Anon., (2006).

$$HI = THQ_1 + THQ_2 + \; + THQ_n \quad \text{Eq. 3}$$

Where THQ_1, THQ_2, …, and THQ_n represent the Target Hazard Quotients of the individual heavy metals.

HPLC analysis: The extraction of plant powders for HPLC analysis was done by sonication in 80% methanol and followed by filtering the extracts through a 0.45 µm membrane filter before injection into the HPLC system. Chromatographic separations were performed using a reversed-phase C18 column with a gradient of acetonitrile and water (with 0.1% formic acid), flowing at 1.0 mL/min. Detection was done by utilizing the UV-Vis detector in the wavelength range of 254-280 nm according to the absorption maxima of phenols. Identification was accomplished by comparison of retention times of the analyte against those of analytical standards run under the same conditions. The quantification of the compounds was achieved via the calibration curve of external standards in different concentrations (Pathania *et al*., 2020).

Antimicrobial assay: Antimicrobial activity of *Citrullus colocynthis, Capparis decidua*, and *Calotropis procera* was determined against *E. coli, B. subtilis*, and *S. aureus* using the agar well diffusion method. Methanolic plant extracts were prepared and filtered, and the filtrates were concentrated under reduced pressure (Bushra *et al.,* 2025). The bacterial cultures were grown on nutrient broth and standardized to 0.5 McFarland turbidity before they were inoculated into the Mueller-Hinton agar plates. Wells of 6 mm diameter were aseptically prepared on the agar plates, and each well was loaded with 50 µL of plant extract. After overnight incubation at 37°C, zones of inhibition in millimeters were determined. Controls were included appropriately to validate antimicrobial activity. The analysis of antimicrobial activities in medicinal plants was demonstrated in Figure 3. Negative control consisted of either distilled water or solvent (methanol), whereas the positive control was an appropriate antibiotic. Microdilution was employed to determine the minimum inhibitory concentrations (MIC) of the plant extracts using the broth microdilution technique. Serial two-fold dilutions of plant extracts were prepared in nutrient broth and inoculated with standardized bacterial suspension (Ahmed *et al.,* 2012; Shafiq *et al.,* 2024). The minimum inhibitory concentration was the lowest extract concentration at which no bacterial growth occurred after 24 h of incubation at 37°C.

Statistical analysis

Data were presented as mean ± SD from measurements performed in triplicate. SPSS software was used to perform statistical analysis (version 25). Significant variations in heavy metal concentrations between plant species and sampling locations were determined using ANOVA ($p<0.05$). Correlations between various heavy metals detected in plant samples were also evaluated using Pearson correlation analysis. Every experiment was conducted in triplicate for antimicrobial activity, and the mean inhibition zones were calculated. Data were statistically analyzed using one-way ANOVA and Tukey's post-hoc test to find significant differences between plant extracts.

Results and Discussion

Medicinal plants description: *Citrullus colocynthis* (Cucurbitaceae) is a herbaceous perennial that grows best in hotter, drier areas. It can climb or creep along the ground. It is also known as desert gourd or bitter apple. The plant produces bioactive substances with antibacterial, anti-inflammatory, and antioxidant properties, such as flavonoids and cucurbitacins. The plant shows signs of possible phytoremediation potential and abiotic stress tolerance and thrives in sandy, dry soil.

Capparis decidua (Capparidaceae), often referred to as caper bush or karir, is a drought-tolerant spiny shrub native to areas experiencing drought. It is leafless, and its close relatives are found in desert or semi-desert climates. The plant has a robust root system and produces bioactive substances (alkaloids, flavonoids, and phenolic compounds) and is traditionally used for the treatment of digestive issues and inflammation. Caper bush's ability to thrive in desert or semi-desert environments makes it a candidate for use in phytoremediation programs. The resilience of *C. decidua* to stresses in desert ecosystems with low nutrients further supports its suitability as a candidate plant for phytoremediation, especially in areas affected by metal contamination (Liu *et al.,* 2023). A summary of the medicinal plants selected in this study is provided in Table 1.

Calotropis procera (Apocynaceae), commonly known as ak (or madar), is a vigorous perennial shrub that demonstrates tolerance to various environmental extremes and produces a substantial amount of milky latex. *Calotropis procera* has been reported to produce multiple secondary metabolites (e.g., cardiac glycosides, flavonoids, and terpenoids) and has a long history of use in the treatment of skin infections and respiratory diseases (Sindhu *et al.*, 2012; Brima, 2017; Sharma *et al.,* 2024).

Heavy metals concentration: Whole plants of *Citrullus colocynthis*, *Capparis decidua*, and *Calotropis procera* collected from four ecologically distinct locations of Punjab have varying concentrations of heavy metals (As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn). Cadmium (1.344 mg/kg), lead (3.666 mg/kg), nickel (5.424 mg/kg), and mercury (6.566 mg/kg) were found to be more concentrated in *Capparis decidua* than in the other two species. With the exception of copper (24.64 mg/kg) and arsenic (2.445 mg/kg), which were both greater than in the other species, *Calotropis procera* exhibited the lowest concentrations of non-essential metals, but *Citrullus colocynthis* displayed a moderate accumulation of all eight elements. *Calotropis procera* had the highest content of zinc (17.64 mg/kg), whereas *Capparis decidua* had the lowest value (43.23 mg/kg). Additionally, *Calotropis procera* had higher levels of copper than either *Citrullus colocynthis* or *Capparis decidua*, suggesting that the plant is capable of physiologically absorbing significant amounts of vital nutrients. In terms of chromium, all three species had low levels, with the greatest concentration being found in *Capparis decidua* at 0.244 mg/kg, indicating that limited contamination existed in the environment from chromium. The amounts of As, Cd, Cu, and Hg were notably higher than WHO-permitted limits in several cases, indicating possible environmental pollution and related health hazards. All species had significant amounts of zinc; *Capparis decidua* had the greatest quantity (43.23 mg/kg), but it was still within permissible limits. All species had consistently low chromium levels, with *Capparis decidua* having the highest result of 0.244 mg/kg, indicating little chromium-related pollution.

Non-carcinogenic risks that might result from consumption of the whole plants were assessed by determining the health risk index using HRI calculations. Assessment of HRI values indicated that the levels of arsenic and mercury exceeded the acceptable threshold of 1 in both *Capparis decidua* and *Citrullus colocynthis*. Therefore, it can be concluded that mercury has the greatest potential health risk, followed by arsenic and cadmium (Meng *et al.,* 2022). Long-term exposure to any of these plants could potentially result in neurotoxicity, renal dysfunction, and other types of health issues. Regarding essential metals (i.e., zinc, copper, nickel, and chromium), the HRI values were well below 1, indicating that consuming these metals at the levels measured does not pose significant risks to consumers. Metal accumulation in plants varies by species and may be attributed to various plant physiological responses to metal uptake through root and stem structures, metal translocation, and other environmental conditions (i.e., soil, irrigation water, and human activity causing environmental degradation).

In addition to their high accumulation of toxic metals, *Capparis decidua* and *Citrullus colocynthis* may pose a risk to public health, although these plants are also believed to possess a high degree of medicinal value. The use of such plants should be regularly monitored for their safety, and appropriate quality assurance procedures implemented, whereas the use of essential minerals in these plants is likely to provide nutritional benefits that carry little risk.

Each segment of the stacked bar chart represents a different metal (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn) and shows the total heavy metal concentrations (mg/kg) in the whole plants of the three medicinal species. Despite being found in comparatively modest concentrations in all species, arsenic (As) and cadmium (Cd) nevertheless add to the total hazardous metal burden. *Capparis decidua* presents a greater potential health risk because of elevated levels of several hazardous metals, as this image clearly illustrates species-specific accumulation patterns.

The results reveal clearly interspecific differences in heavy metal accumulation among the plant species studied. *Capparis decidua* demonstrates the highest overall concentration, characterized by notable enrichment of Zn, Pb, Hg, and Ni, indicating a strong capacity for multi-metal uptake (Fig. 4).

In comparison, *Citrullus colocynthis* shows elevated levels primarily of Zn, Hg, and Pb, reflecting a moderate but selective accumulation pattern. Meanwhile, *Calotropis procera* is distinguished by higher concentrations of Cd, Cr, Zn, and As, suggesting a different metal affinity profile relative to the other species. Statistical comparison using post-hoc grouping exposed substantial intergroup variation ($p<0.05$), as indicated by distinct letter assignments.

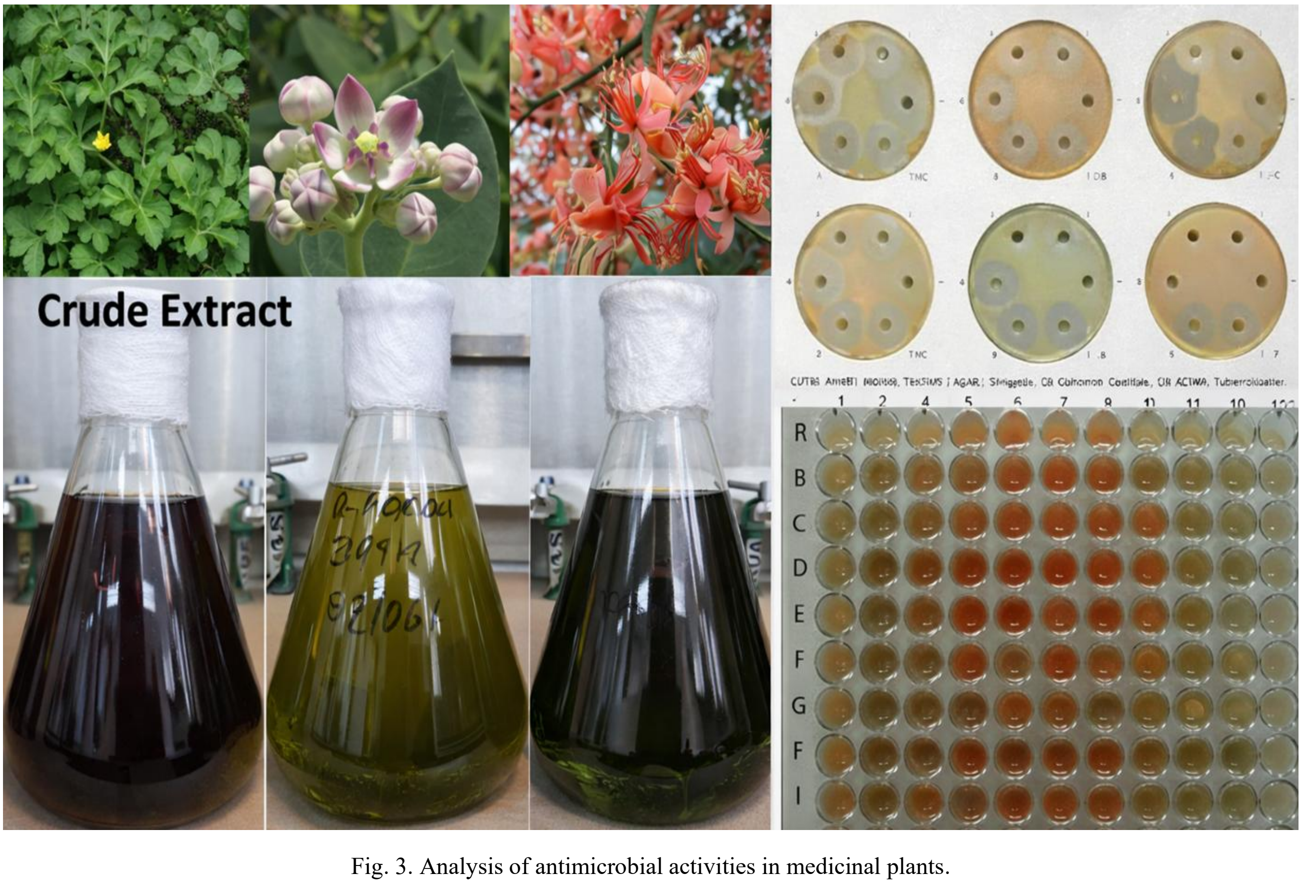

Fig. 3. Analysis of antimicrobial activities in medicinal plants.

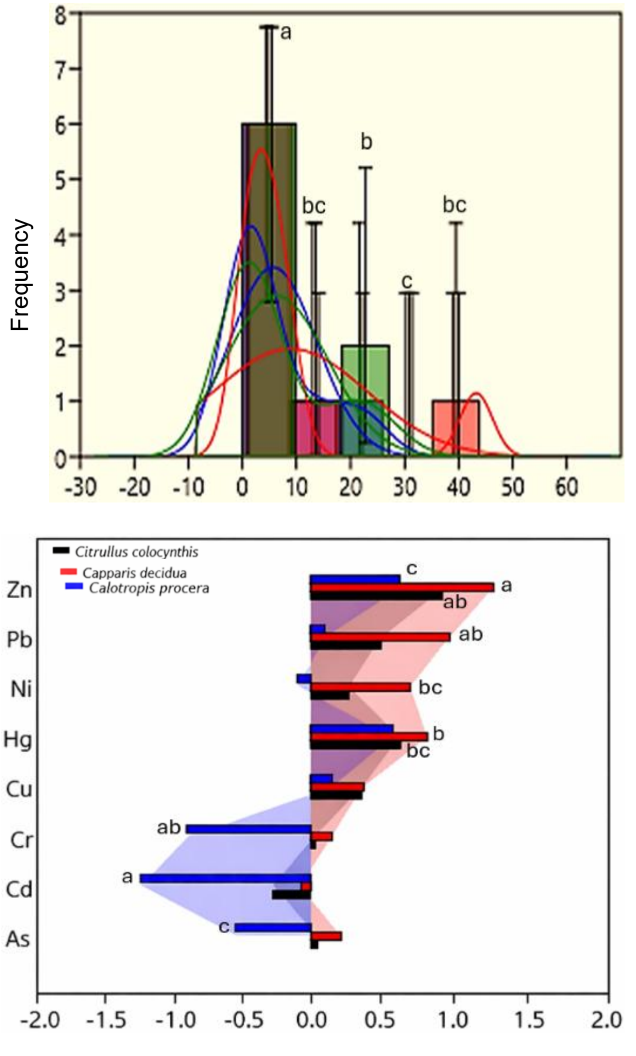

Fig. 4. Frequency distribution, variability, and comparative accumulation of heavy metals in medicinal plants.

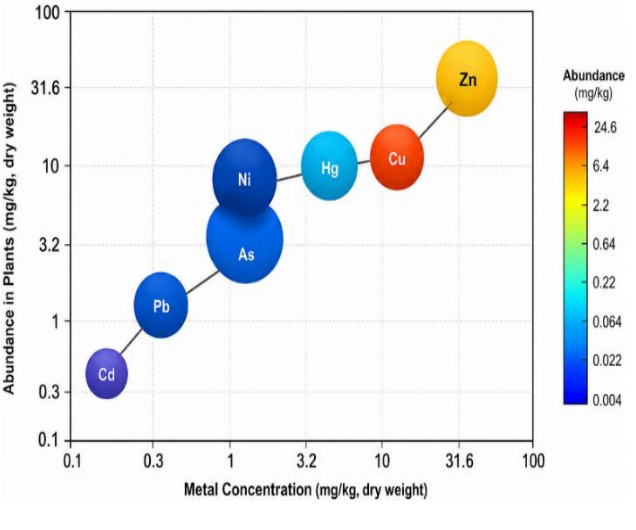

Fig. 5. Abundance and concentration of heavy metals in the examined medicinal plants as represented in the bubble chart.

From the bubble chart shown (Fig. 5), there exists an evident relationship between the concentration and abundance of the various metals found in the selected medicinal herbs in the logarithmic scale format. It is clearly evident that there is a very high positive relationship between metal concentration and abundance. The zinc (Zn) metal is the most dominant among the other selected metals due to its high concentration (31.6 mg/kg) and abundance (35 mg/kg). This is followed by copper (Cu) and mercury (Hg), whose concentrations and abundance are also relatively high. On the other hand, nickel (Ni) and arsenic (As) metals have relatively medium concentrations and abundance. Lead (Pb) and cadmium (Cd) metals have the least concentration and abundance. There exists a positive relationship between metal concentration and abundance, as seen from the increasing distribution of the data points from the lower left to the upper right part of the graph.

The locations and concentrations of eight distinct heavy metals - arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), and zinc (Zn) - in the whole plant samples of three different medicinal plants are shown in a heatmap. Concentration of heavy metals in each sample is shown by the heatmap through different colours, where dark colours mean high concentration while light colours mean low concentration of heavy metals in the respective samples. According to the heatmap, it was clear that there were differences between various plant species concerning heavy metal accumulation. One of the three plant species under study, *Capparis decidua*, showed high levels of selected heavy metals such as zinc (43.23 mg/kg), mercury (6.57 mg/kg), nickel (5.42 mg/kg), and lead (3.67 mg/kg). From the heatmap, it is evident that *Capparis decidua* has a high bioaccumulation capacity of the metals. Zinc accumulation in plants shows that they have absorbed enough essential micronutrients from the soil. High mercury and lead concentrations in the plant are due to contamination or the chemical composition of the soil where the samples were collected. Apart from essential metals, *Capparis decidua* had cadmium (1.34 mg/kg), which shows the ability of the plant to absorb toxic and non-essential metals. Moderately high concentrations of some metals in *Citrullus colocynthis* include 22.34 mg/kg of zinc (the highest), 13.73 mg/kg of copper (the second highest), while arsenic (1.76 mg/kg), mercury (4.62 mg/kg), and nickel (1.25 mg/kg).

These lower concentrations of cadmium and chromium suggest a similar pattern to most heavy metals observed across this species. A moderate to light color variation of colors seen with most heavy metals within this species may indicate some sort of balance of essential and non-essential element uptake, which may reflect differences in absorption via roots and plant physiological methods.

Calotropis procera exhibited a completely different accumulation pattern, primarily displaying the highest amount of copper (24.64 mg/kg) among the three species in this analysis, as shown by a dark shade in the heat map. Copper is an essential nutrient required for many enzymatic and physiological processes in plants; therefore, high levels of copper uptake in this species could indicate efficient nutrient uptake. In addition, *Calotropis procera* contained comparatively low levels of other toxic heavy metals: cadmium (0.004 mg/kg), chromium (0.024 mg/kg), nickel (0.45 mg/kg), and lead (0.25 mg/kg), with zinc (17.64 mg/kg) being moderate relative to the other species analyzed (Fig. 6).

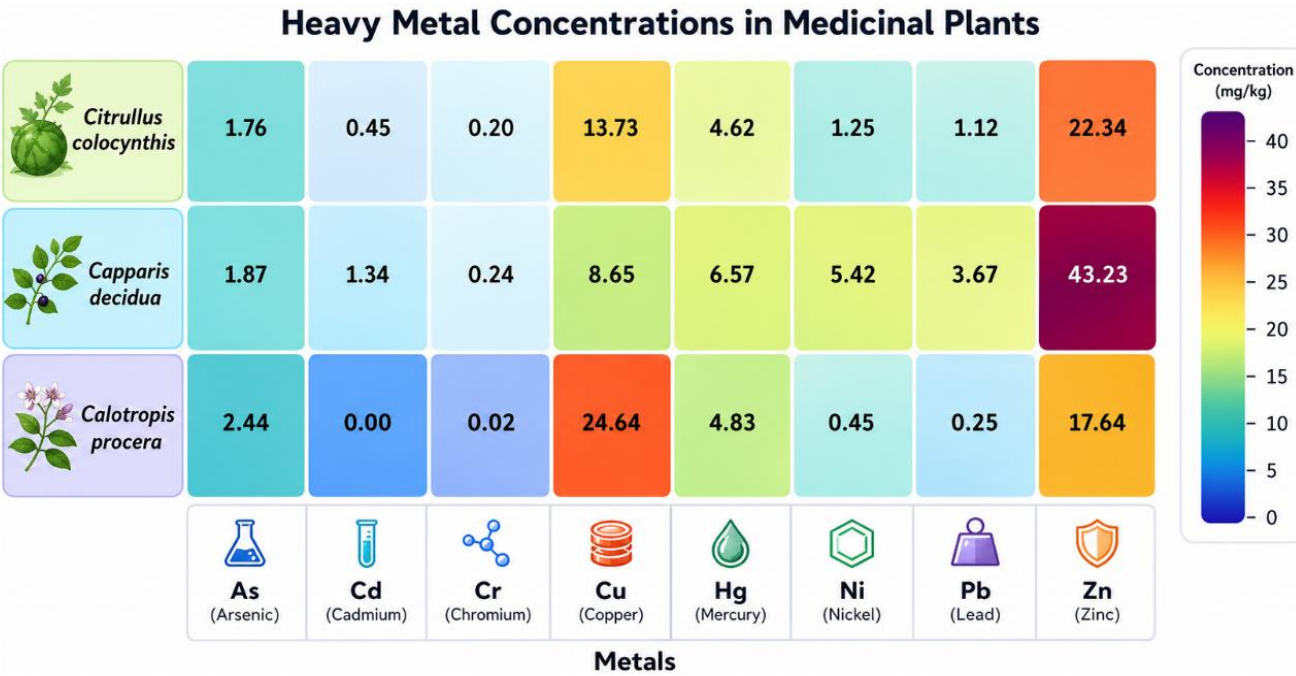

Fig. 6. Heatmap of heavy metal concentrations (mg kg^{-1}, dry weight) in medicinal plants (*Citrullus colocynthis*, *Capparis decidua*, and *Calotropis procera*) showing the distribution pattern.

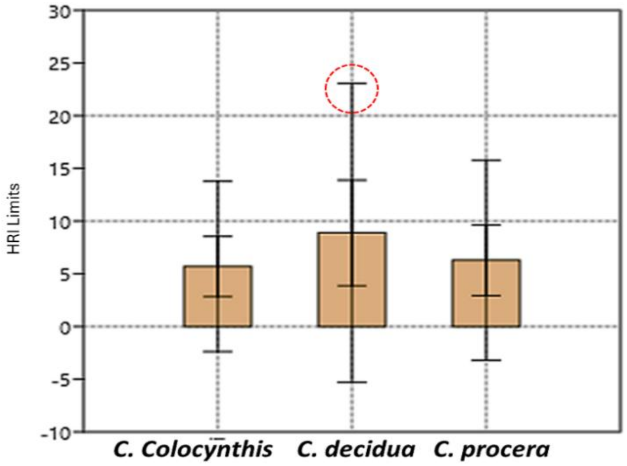

Fig. 7. HRI of heavy metals.

The health risk index (HRI) analysis showed significant differences in heavy metal accumulation among the examined medicinal plants, specifically *Citrullus colocynthis, Capparis decidua*, and *Calotropis procera*. At 14.27%, arsenic was the highest contributor to the heavy metal monitoring index (HRI) in *C. procera*, followed by *C. decidua* (8.33%) and *C. colocynthis* (11.07%), making these plants important species for arsenic accumulation. Cadmium (Cd) varied widely in the three species tested, with *C. decidua* having the highest concentration of 1.80%, compared to 0.85% in *C. colocynthis* and 0.007% in *C. procera*. Similarly, *C. decidua* also had the highest concentration of lead (Pb) at 1.23%, while concentrations in *C. colocynthis* and *C. procera* were much lower at 0.53% and 0.11%, respectively. Copper was the most abundant metal found in *C. procera* (1.08%), with *C. colocynthis* and *C. decidua* having lower concentrations (0.65% and 0.29%, respectively). Nickel and zinc were only detected in *C. decidua* at concentrations of 0.36% and 0.19%, respectively, and no other metals were analyzed in the other plant species. Based on this data, it appears that *C. decidua* accumulates a higher level of multiple toxic metals, while *C. procera* is more contaminated with arsenic and copper. The need for ongoing monitoring of heavy metals in medicinal plants is highlighted by the detected mercury levels in all plant species examined (more than 84%), which suggests that regular consumption of these plants may pose a serious health risk to consumers (Fig. 7).

Target hazard quotient (THQ): The Target Hazard Quotient (THQ) was used to quantify the potential non-carcinogenic risk of eating heavy metal-contaminated *Citrullus colocynthis, Capparis decidua*, and *Calotropis procera*. A THQ number greater than 1 indicates a potential health risk. The analysis reveals that THQ values for metals vary among plant species. All three plant species had the highest THQ values for mercury (Hg), supporting the HRI's assessment of the severe health concerns. Among the assessed metals, the highest THQ for mercury was 0.46 for *C. colocynthis*, 0.65 for *C. decidua*, and 0.48 for *C. procera*, suggesting a comparatively larger risk. Furthermore, *C. procera* (0.81) and *C. decidua* (0.62) were significantly at risk for arsenic, but *C. colocynthis* exhibited lower but still significant values (0.58).

Cadmium THQ levels differed amongst species. *C. decidua* contained the highest cadmium THQ (0.134), indicating the most risk; *C. colocynthis* had lower values (0.045); and *C. procera* had extremely low values (0.041). Lead showed moderate to high danger, especially in *C. decidua* (0.91), *C. colocynthis* (0.48), and *C. procera* (0.21). While *C. decidua* accumulated slightly greater THQ values for Ni (0.12) and Zn (0.14), *C. procera* had elevated levels of copper (0.61), indicating a moderate risk of exposure compared to other critical metals (nickel, chromium, and zinc) that had lower THQ values across all species (Fig. 8).

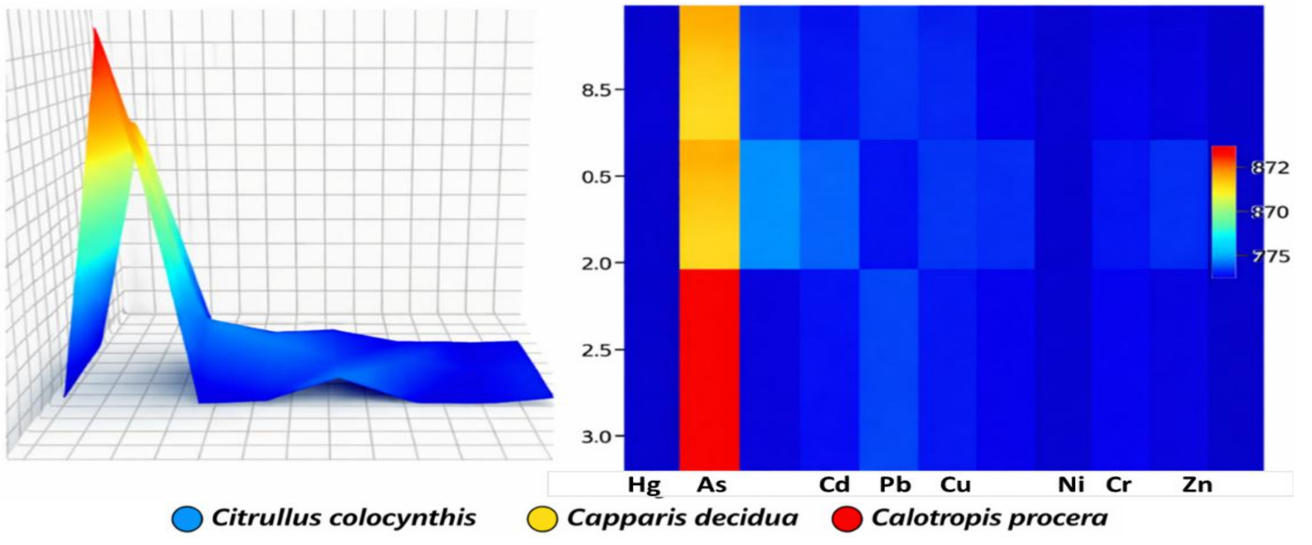

Fig. 8. Target Hazard Quotient (THQ) of *Citrullus colocynthis*, *Capparis decidua*, and *Calotropis procera*.

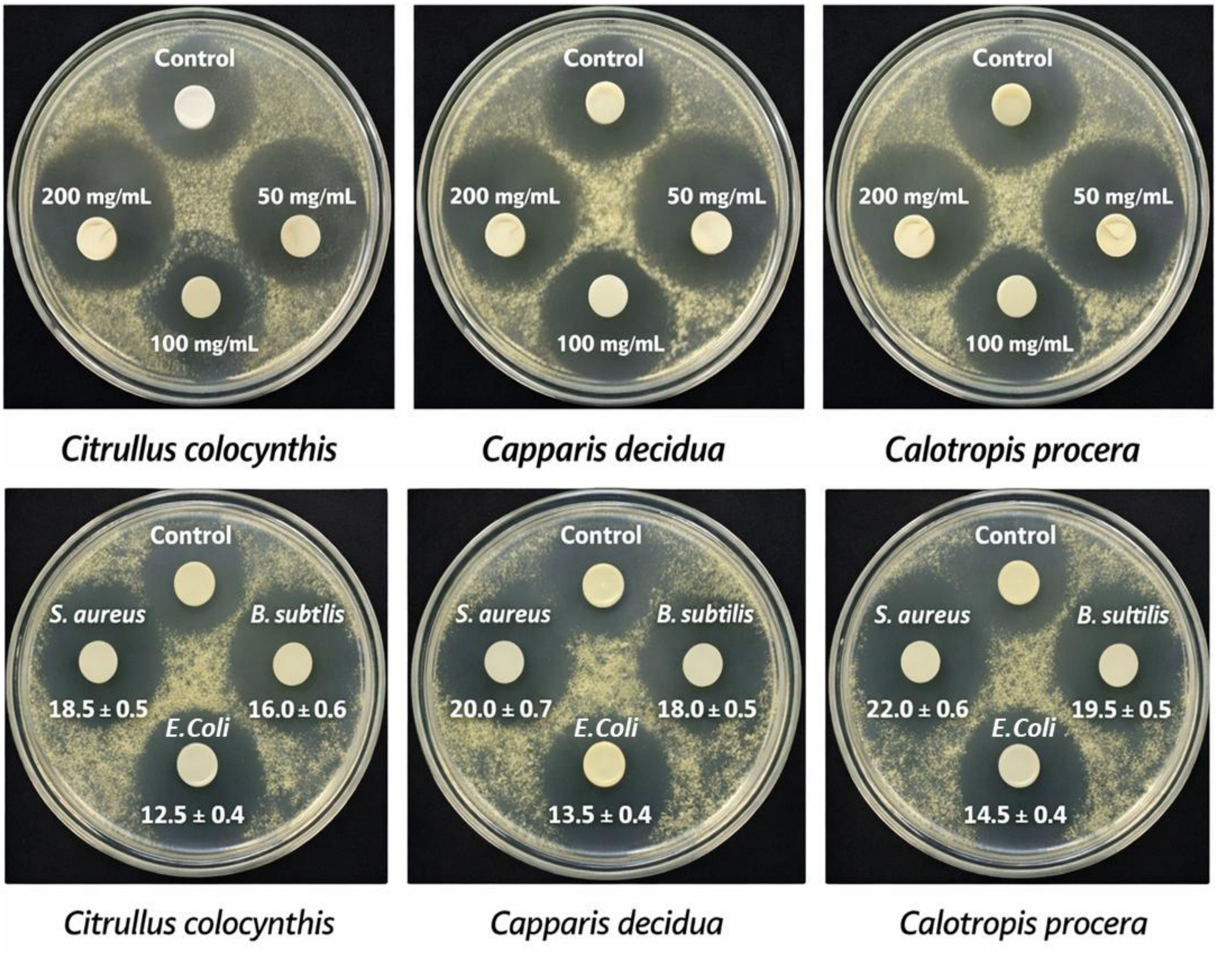

Fig. 9. Comparative antimicrobial activity against selected pathogenic bacteria (*Staphylococcus aureus, Bacillus subtilis,* and *Escherichia coli*) in agar well diffusion assay.

Hazard index (HI): The sum of the individual THQs was used to calculate the Hazard Index (HI), which represents the cumulative risk from all metals in each plant species. Potential non-carcinogenic health concerns from combined exposure are indicated by an HI score greater than 1.

The findings show that all species had higher HI values, which is indicative of the combined harmful impacts of multiple metals:

- *Citrullus colocynthis*: HI ≈ 0.66
- *Capparis decidua:* HI ≈ 0.79
- *Calotropis procera:* HI ≈ 0.58

These findings demonstrate that *C. decidua* has the highest cumulative risk due to its substantial accumulation of mercury, cadmium, lead, and nickel, whereas *C. procera* presents elevated HI values largely driven by arsenic and copper. Significant health risks are also associated with *C. colocynthis*, mostly because of mercury and arsenic exposure.

Antimicrobial activity: *Staphylococcus aureus, Bacillus subtilis,* and *Escherichia coli* were among the pathogenic microorganisms against which the antibacterial activity of *Citrullus colocynthis, Capparis decidua*, and *Calotropis procera* was assessed. In the agar well diffusion assay, all three plant extracts demonstrated detectable inhibitory effects; the extent of inhibition varied according to the plant species, extract solvent, and microbial strain (Table 2).

Citrullus colocynthis showed strong antibacterial activity when tested against Gram-positive microorganisms (approximately 18.5 ± 0.5 mm and 16.00 ± 0.60 mm, respectively, for *Staphylococcus aureus* and *Bacillus subtilis*). A moderate level of antibacterial activity was noted for Gram-negative bacteria, including *E. coli*, which had an inhibition zone of approximately 12.5 ± 0.4 mm. *Capparis decidua* exhibited broad-spectrum antibacterial activity (20.0 ± 0.7 mm for *S. aureus*); its demonstrated efficacy towards Gram-positive strains has been attributed to the high levels of flavonoids and phenolic compounds it contains, which are known to disrupt the structural integrity of bacterial cell walls. *Calotropis procera* demonstrated the highest level of antimicrobial activity overall (22.0 ± 0.60 mm for *S. aureus*; 19.5 ± 0.50 mm for *B. subtilis*), with substantial inhibition of Gram-negative bacteria (14.5 ± 0.40 mm for *E. coli*). The presence of alkaloids, glycosides, and terpenoids likely increased the broad-spectrum antibacterial activities of the extracts. These findings support the use of all three plants as antimicrobial agents and demonstrate that *Calotropis procera* is the most potent of the three, suggesting it may serve as a viable source of bioactive compounds for developing plant-derived antimicrobial agents.

Table 2. Inhibition zones (mm) of plant extracts against selected pathogenic bacteria.

Plant species	*S. aureus* (mm)	*B. subtilis* (mm)	*E. coli* (mm)
Citrullus colocynthis	18.5 ± 0.5	16.0 ± 0.6	12.5 ± 0.4
Capparis decidua	20.0 ± 0.7	18.0 ± 0.5	13.5 ± 0.4
Calotropis procera	22.0 ± 0.6	19.5 ± 0.5	14.5 ± 0.4

Calotropis procera showed strong antibacterial action against a variety of pathogens, particularly the Gram-positive bacteria *S. aureus* and *B. subtilis*. Its bioactive components, such as alkaloids, cardiac glycosides, flavonoids, and terpenoids, which are known to disrupt bacterial cellular membranes, inhibit bacterial enzymes, and interfere with bacterial metabolic pathways, were responsible for the high level of inhibition observed with these organisms (22.0 ± 0.6 mm for *S. aureus* and 19.5 ± 0.5 mm for *B. subtilis*). The fact that *C. procera* was able to significantly inhibit *E. coli* further supports the idea that this plant may be used for its broad-spectrum antibacterial action.

Citrullus colocynthis also showed good levels of activity against the mentioned Gram-positive species, with inhibition zones of 18.5 ± 0.5 mm for *S. aureus* and 16.0 ± 0.6 mm for *B. subtilis*. This decreased level of action against Gram-negative species is likely due to the outer lipopolysaccharide membrane of many Gram-negative bacteria, which acts as a permeability barrier to many plant-derived bioactive compounds. Despite the lower level of inhibition, *C. colocynthis* contains phytochemicals, including phenolics and cucurbitacins that strengthened its antibacterial activity (Fig. 9).

Capparis decidua showed consistent and broad-spectrum antibacterial activity against all strains tested, with inhibition zones ranging from 15.0 ± 0.5 mm to 20.0 ± 0.7 mm. Its somewhat balanced activity against both Gram-positive and Gram-negative bacteria may be caused by its high concentration of flavonoids, tannins, and phenolic acids, which are known to exert antimicrobial effects through protein denaturation and disruption of cell wall integrity.

Phytochemical constituents of *Citrullus colocynthis*: The HPLC chromatogram of *Citrullus colocynthis* extracts showed peaks for flavonoids, phenolic acids, and cucurbitacin derivatives. Quercetin, gallic acid, and cucurbitacin-type triterpenoids were reported to be the most abundant constituents of the extracts, with strong antioxidant and antimicrobial properties. Flavonoids were the main fraction of the extract and may therefore be responsible for the biological activity observed in the antimicrobial tests. In addition, phenolic acids may also be responsible for the high redox potential and the radical scavenging activity of this species.

Phytochemicals of *Capparis decidua*: HPLC analysis of *Capparis decidua* indicated the presence of several classes of alkaloids, flavonoids, and phenolic compounds. The major peaks were due to rutin, catechin, and chlorogenic acid derivatives, all of which have been documented as having anti-inflammatory and antimicrobial properties. Among the screened plant species, *Capparis decidua* showed the highest peak area, suggesting that high phytochemical density correlates with greater bioaccumulation capacity and tolerance to stressful conditions, resulting in the enrichment of secondary metabolites such as phenolic compounds and flavonoids.

Phytochemical content of *Calotropis procera*: The HPLC chromatogram of *Calotropis procera* showed the presence of large amounts of cardiac glycosides, flavonoids, and terpenoid derivatives, while important compounds included calotropin-like glycosides, quercetin derivatives, and ursolic acid-type triterpenes. Although *Calotropis procera* had fewer peaks than the others, it contained certain bioactive compounds at much higher intensities, indicating a more specialized and strong phytochemical profile likely contributing to the superior antimicrobial activity of *C. procera* in bioassays. Compared to the other two species, *Calotropis procera* exhibited fewer peaks but significantly higher peak intensities for specific bioactive compounds, indicating a more specialized but highly potent phytochemical profile. This concentrated composition may explain its superior antimicrobial activity observed in bioassays. The HPLC results strongly correlate with the observed antimicrobial activity. HPLC-UV provides useful analysis (Table 3) of phenolic constituents; however, LC–MS/MS is recommended for structural confirmation. The high flavonoid and phenolic content in *Capparis decidua* and *Citrullus colocynthis* support their moderate to strong antibacterial effects, while the presence of concentrated glycosides and terpenoids in *Calotropis procera* explains its superior antimicrobial performance.

Comparative analysis: *Citrullus colocynthis, Capparis decidua*, and *Calotropis procera* exhibit significant species-dependent differences in heavy metal levels, antimicrobial activity, and phytochemical content. These findings are consistent with earlier research on xerophytic medicinal plants as bioresource and bioindicator species. The significantly elevated levels of toxic metals (Hg, Cd, Pb, and Ni) in *Capparis decidua* strongly support previously published literature regarding the high capacity of desert-adapted shrubs to sequester heavy metals due to an extensive root system, efficient translocation mechanisms, and high stress metabolism. However, the selective uptake of Cu and As in *Calotropis procera* is indicative of a regulated micronutrient uptake mechanism, which is consistent with previously published literature on the adaptive physiological responses of the species to abiotic stress. The levels of mercury (Hg) and arsenic (As) in all species were above the permissible limits set by the WHO, indicating significant environmental contamination. This is consistent with previous literature on industrial and semi-arid zones of the region, where the main sources of elevated heavy metals in medicinal flora include irrigation with polluted water, vehicular emissions, and geochemical soil enrichment. The elevated THQ and HI values indicate the high toxicity of Hg and As, consistent with previous literature reporting the non-carcinogenic risk potential of these elements in herbal flora.

From the pharmacological point of view, all species showed positive antimicrobial activity despite the high levels of toxic metals. However, the highest antimicrobial activity was recorded in the species *Calotropis procera*. This observation is strongly supported by previous phytopharmacological studies, which attribute the potent antimicrobial activity of this species to synergistic effects of cardiac glycosides, terpenoids, and flavonoid derivatives. The low antimicrobial activity of *Citrullus colocynthis* against Gram-negative bacteria is supported by established evidence that the outer lipopolysaccharide membrane acts as a permeability barrier. The antimicrobial activity of *Capparis decidua* was found to be moderate and balanced. This observation is supported by previous studies showing that the phenolic profile of this species is responsible for its moderate antimicrobial activity. HPLC profiling substantiates the biological activities by clearly showing chemotaxonomic differentiation among the species. The high levels of flavonoids and phenolic acids in *Capparis decidua* and *Citrullus colocynthis* are supported by previous studies indicating that these compounds are responsible for antimicrobial activity. The high-intensity terpenoids and glycosidic compounds in *Calotropis procera* explain its potent antimicrobial activity despite low phytochemical diversity. This observation is supported by previous studies showing that phytochemical intensity is inversely related to diversity.

Table 3. Putatively identified phenolic compounds in plant extracts based on HPLC-UV analysis.

Compound (Tentative Identification)	Retention time (min)	*Citrullus colocynthis* (%)	*Capparis decidua* (%)	*Calotropis procera* (%)
Gallic acid	3.2	6.4	8.9	4.1
Catechin	6.1	5.9	12.4	3.8
Caffeic acid	7.3	6.1	9.2	4.5
Rutin	8.6	8.5	14.8	6.2
Quercetin	10.2	9.7	11.3	8.9
Ferulic acid	13.8	5.6	7.8	4.9

Conclusion and Recommendation

The study validates the traditional ethnomedicinal use of *Citrullus colocynthis*, *Capparis decidua*, and *Calotropis procera* by demonstrating their strong antibacterial activity. *Calotropis procera* showed the strongest inhibitory effects against both Gram-positive and Gram-negative bacteria, indicating that it may be a potential source of bioactive antimicrobial compounds. Heavy metal accumulation analysis revealed species-specific patterns: *Calotropis procera* accumulated significant amounts of arsenic and copper; *Citrullus colocynthis* showed moderate accumulation, while *Capparis decidua* exhibited the highest levels of hazardous metals (Hg, Cd, Pb, and Ni). Long-term use of these plants may pose serious non-carcinogenic health risks, according to health risk assessment indices (HRI, THQ, HI), particularly due to exposure to mercury, arsenic, cadmium, and lead.

1. Implement routine screening of medicinal plants for toxic metals before commercial use, with strict adherence to WHO/FAO safety limits.
2. Use uncontaminated soil and controlled environmental conditions to grow the plants used for herbal medicines to reduce the uptake of heavy metals.
3. Increase the awareness of traditional medicine practitioners, vendors of herbal medicines, and consumers about the heavy metal contamination that could occur in herbal medicines.
4. Create and enforce a national standard for permissible levels of heavy metals in raw and processed herbal products.
5. Investigate the possibility of growing specific species in a controlled manner and use phytoremediation techniques to reduce heavy metal accumulation.

Conflict of Interest: The authors declare no conflict of interest.

Author's Contributions: Conceptualization, Q.L. and M.K.A.; Data Curation, M.K.A., A.S., and H.A.; Formal Analysis, M.K.A. and A.S.; Investigation, M.K.A. and A.S.; Methodology, M.K.A. and A.S.; Software, M.K.A. and A.S.; Supervision, Q.L.; Project Administration, Q.L.; Visualization, M.K.A., A.S., and H.A.; Resources, Q.L.; Funding Acquisition, Q.L., H.A., M.K.A., and A.S.; Writing — original draft, M.K.A.; Writing — reviewing & editing, M.K.A., A.S., H.A., Q.L.; Validation, Q.L.

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