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EVALUATION OF PHYTOCHEMICAL PROFILE AND PHYTO-REMEDIATION POTENTIAL OF SELECTED MEDICINAL PLANTS OF PUNJAB, PAKISTAN

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Abstract

Medicinal plants represent vital therapeutics and sources for the synthesis of drugs due to their bioactive phytochemicals, especially polyphenols. Such plants have antioxidant and antimicrobial activities, which make them beneficial against a variety of diseases. Recently, in the 21st century, traditional herbal remedies particularly from the developing world have attracted global interest. The application in nutraceuticals and cosmetics has increased the further demand for medicinal plants in developed countries, which demands international research collaboration focused on the identification of potent therapeutic species. Such plants may prove to be valuable alternative foods and assist in the discovery of new drugs of commercial importance. Moreover, they can act as a potential source of germplasm for the socio-economic uplift of local communities and the nation as a whole. Based on this, it is suggested that although the present work offered some baseline data, comprehensive phytochemical profiling and functional properties research is recommended. The current research has been considered with the aim to examine the phytochemical content, its HPLC profiles, and phytoremediation potential of three resistant medicinal plants, *Citrullus colocynthis* (L.) Schrad, *Capparis decidua* (Forssk.) Edgew., and *Calotropis procera* (Aiton.) W.T. Aiton, which were collected from four ecologically different districts of Punjab: Bahawalpur, Bahawalnagar, Rahimyar Khan, and Lodhran.

Key words: Phytochemicals; Phytoremediation; Medicinal Plants; Heavy Metals; *Citrullus colocynthis*; *Capparis decidua*; *Calotropis procera*; Punjab; Pakistan

Introduction

Medicinal plants have been used traditionally from prehistoric times and demonstrate therapeutic and pharmacological effects on the human or animal body (Namdeo, 2018). Over 70% of documented plant species are reportedly used as medicinal plants, particularly by indigenous tribes worldwide (Ulian *et al*., 2020; Borrell *et al*., 2020). But according to recent scientific research, traditional medicine is becoming less and less common, particularly in Asian and African nations (Oyebode *et al*., 2016). Medicinal plants include a wide range of bioactive compounds, including primary and secondary metabolites. Plant growth and development are significantly influenced by primary metabolites. More than 1,000,000 secondary metabolites have been identified in plant species due to their anti-inflammatory, anti-cancer, anti-microbial, antioxidant, and anti-competitive characteristics (Afendi *et al*., 2012). The prevention and treatment of many human diseases are significantly impacted by these metabolites. Additionally, a range of bioactive compounds present in plant species are used to make modern drugs that treat conditions including migraines and cancer (Castillo-Pérez *et al*., 2021).

Medicinal plants' phytochemistry: Treatments for skin infections and other dermatological diseases greatly benefit from the use of medicinal plant species (Saikia *et al*., 2006). Pakistan is endowed with 6000 species of varied flora, 400–600 of which are significant for medicinal purposes (Ali & Qaiser, 2009). To treat skin conditions, people in the Himalayan region primarily rely on folk remedies. Many common skin illnesses, including dermatitis, gonorrhea, boils, pimples, and pustules, can be healed using plant-based remedies. Simple phenolics, tannins, flavonoids, lignans, coumarins, stilbenes, chromones, and Xanthones are the seven additional categories into which polyphenolics are organized. All plant parts commonly contain phenolic acids, a type of simple phenolic, in their seeds, leaves, and fruit skins (Kumar & Goel, 2019). Substances like gallic acid possess astringent, antibacterial, anticarcinogenic, anticancer, anti-anaphylactic, and bronchodilatory qualities (Harborne *et al*., 1993). The antibacterial activity of all phenols is easily noticeable. Interestingly, phenol was the original antiseptic (Pelczar *et al*., 1988).

The leaves, bark, seeds, fruits of plants are rich in flavonoids, which provide these plant components with their color. According to Hernández-Rodríguez *et al*., (2019), they attract pollinators, protect plants from UV rays, bacterial and fungal phytopathogens, and defend against abiotic stresses. Plants contain around 9,000 flavonoids. Flavonoids have a broad range of biological activities that make them useful for medicine, such as anti-ulcerogenic, cardiovascular protective, analgesic, anti-infective, anti-HIV, anti-cancerous, anti-

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hepatotoxic, vasodilatory, and enzymatic activity-modulating effects (Hamad *et al*., 2018). The potential therapeutic effect of medicinal plants is determined by their phenolic and flavonoid components' ability to function as antioxidants (Baidez *et al*., 2007). Halliwell and Gutteridge (2015) define an antioxidant as "any substance that delays, prevents, or removes oxidative damage to the target molecule." According to Liebert and Jones (2006), reducing agents can minimize oxidative stress by activating transcription factors that favorably regulate the synthesis of antioxidant enzymes and inhibit oxidation by scavenging free radicals. Because of their high redox potential, which enables them to function as hydrogen donors, reducing agents, singlet oxygen quenchers, metal chelators, and more, flavonoids have shown exceptional antioxidant benefits among phytochemicals (Tsao & Yang, 2003).

Skin infections are treated with a variety of antibiotic medications, including dicloxacillin, mupirocin, anthralin, erythromycin, tetracycline, and dicloxacillin (antibacterial), as well as clotrimazole, ketoconazole, fluconazole, and itraconazole (antifungal) (Skopelja-Gardner *et al.,* 2021). Synthetic antibiotics have benefits, but they also come with drawbacks, including environmental harm, carcinogenicity, and antimicrobial resistance. The chemical structure of the different classes of polyphenols is demonstrated in Fig. 1. Moreover, the overuse of antibiotics is leading to an increase in the prevalence of resistant microbial strains (Hamad *et al*., 2018). Conversely, phytomedicines are safer, more reliable, and more affordable medications that offer both medical and financial advantages (Anand *et al*., 2019).

Heavy metals: Since heavy metal contamination directly impacts crop physiology and subsequently affects yield and production quality, it is currently regarded as the largest threat to the agricultural sector. According to Wang *et al*., (2018), plants subjected to toxic metal stress undergo a range of physiological changes, including leaf necrosis, chlorosis, stunted growth, decreased respiration and photosynthetic activities, reduced germination rate and percentage, and cell apoptosis, which ultimately leads to plant death. Heavy metal stress also disrupts plant homeostasis, as indicated by abnormalities in transpiration, water and nutrient absorption, and gated ion channels (Demidchik, 2017).

Health effects of heavy metals: The absorption of heavy metals by plants, at high concentrations, may pose noteworthy health risks." The consumption of medicinal plants contaminated with heavy metals may be an important exposure pathway, through the food chain, for humans who consume them. Heavy metals become toxic when they persist in the body after metabolism and subsequently accumulate in the soft tissues (Singh, 2011). Humans who consume metals on a long-term basis may experience negative consequences, which may not show up for years. The specific gravity of cadmium (Cd), a heavy metal toxin, is 8.65 times that of water. The liver, placenta, kidneys, lungs, brain, and bones are all impacted by cadmium. Renal function and the pulmonary system (emphysema, bronchiolitis, and alveolitis) may be affected by sub chronic inhalation exposure to cadmium and its compounds. Anosmia, heart failure, several types of cancer, cerebrovascular infarction, emphysema, osteoporosis, proteinuria, and cataracts are among the clinical outcomes linked to exposure to Cd. Lead is a bluish-grey substance found in trace levels in the Earth's crust. Although lead is found naturally, human activities like burning fossil fuels, mining, and manufacturing cause Pb concentrations in the environment to rise to hazardous levels. Children may absorb more than 50% of the amount of lead absorbed by adults through drinking water. Pb poisoning may be caused by inhaling lead dust or fumes, smoking, ingestion of lead by consuming food or drinks tainted by lead, or a combination of these ways (Hopkins *et al*., 2000).

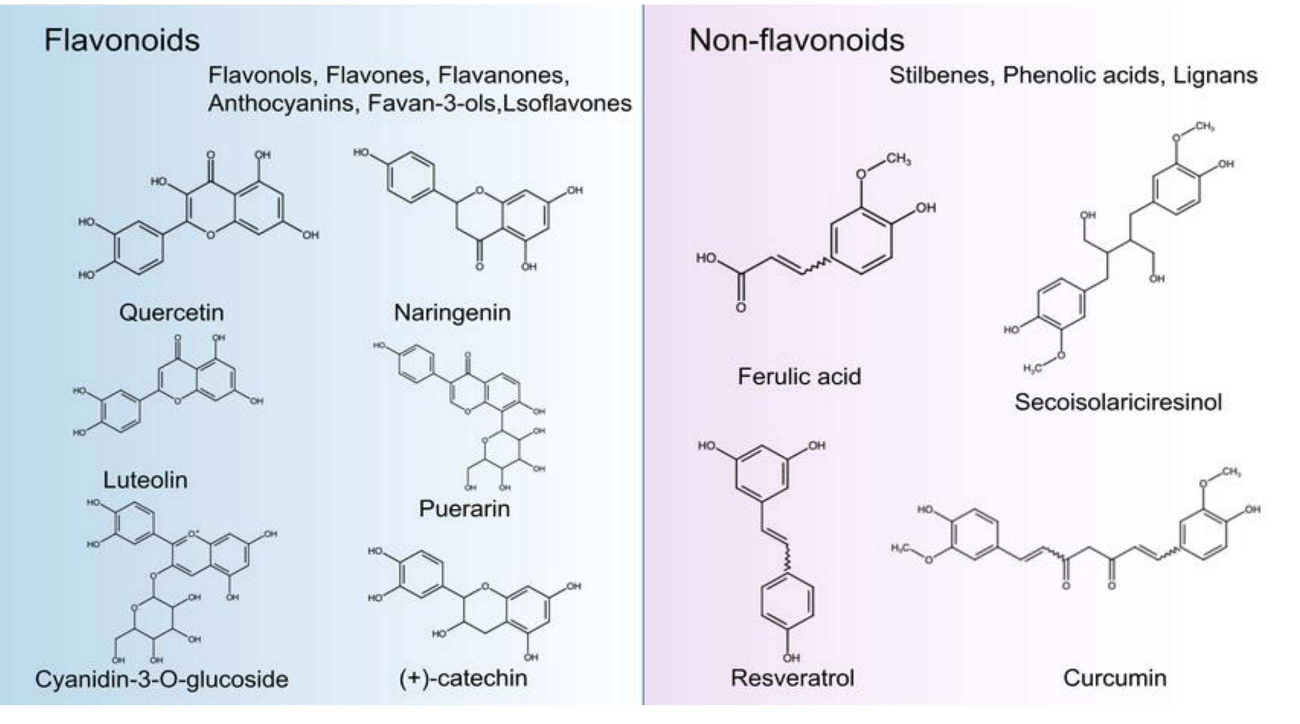

Fig. 1. Chemical structure of the different classes of polyphenols (https://pubmed.ncbi.nlm.nih.gov/37545494/).

According to Martin *et al*., (2006), zinc is the second most common metal in the human body after iron. The sources of Zn contamination in food are refineries, brass manufacturing, metal plating, plumbing, steel, and galvanizing. This metal is an essential micronutrient for children's growth, immune system performance, and resistance to infection. Zn deficiency increases children's risk of illness and death from infectious diseases in many low-income countries. In addition, the health effects of heavy metals on human health are exhibited in Fig. 2. In addition to being used to treat influenza, common cold illnesses, and respiratory infections, this metal lowers the incidence of acute lower respiratory infections and the incidence of malaria episodes in children (Roohani *et al*., 2013).

Heavy metals (HMs) phytoremediation: The end of the heavy metal phytoremediation process is known as producing the contaminated biomass that is created greatly each time after each harvest opportunity. The disposal of contaminated biomass using methods that have included chemical extraction, incineration, Phyto mining, and burning has occurred over time. Many research papers have studied the risks of using aromatic and medicinal plants as potential phytoremediation crops. Medicinal plants provide essential oil, which is the only derivative used in non-food products that include soaps, laundry detergents, insect sprays, and cosmetics. For this reason, these crops play an important role in mitigating food chain contamination (Kamle *et al*., 2022). Plants can remove toxic metals found in contaminated soils. The reliance on carefully studied technologies termed as phytoremediation can also help to recover alto soils that have been contaminated with toxic heavy metals. "The implementation of plant-controlled relationships to both organic and inorganic molecules and wastewater at contaminated sites to meet location-specific remediation targets" (Carlson *et al*., 2025). By utilizing techniques such as phytoextraction, phytostabilization, rhizofiltration, phytovolatilization, and phytodegradation, plants can absorb toxic metals from top soil and transfer them into less toxic structures that do not contaminate the food chain. This technique is both cost-effective and sustainable, as it preserves soil health and lowers pollution load while maintaining unaltered environmental conditions for a more environmentally friendly future. The limited entry of medicinal and aromatic plants into the general food chain is one of the main benefits of using them as phytoremediation plants. In addition, the essential oil composition is not affected, and there is no significant risk of metal pollution. Various HMs are naturally present in the environment or released to the environment due to human activities. Either by contamination of the air, soil, or precipitation, or by passing down the food chain. And the population of biota is being harmed by rising levels of such hazardous metals in their environment (Hajeb *et al*., 2016).

HPLC assessment: Particularly in traditional medical systems, medicinal plants are essential for treating skin infections and other dermatological conditions (Stoenescu *et al*., 2022). More than 600 species of medicinal plants with strong antibacterial, antioxidant, and anti-inflammatory properties are found in Pakistan. These plants are rich in phenolic and flavonoid chemicals (Vega *et al*., 2025). Plant-derived treatments are becoming safer and more environmentally friendly as antibiotic resistance and the negative consequences of manmade medications increase (Naquvi *et al*., 2025). High-performance liquid chromatography (HPLC) is used to precisely identify bioactive components including rutin, quercetin, and gallic acid, highlighting their pharmacological importance in modern medicine (Noba *et al*., 2024). The objectives of this study are,

- To quantify key phytochemicals and characterize the HPLC-based metabolites in *C. colocynthis, C. decidua,* and *C. procera.*
- To assess and compare the antioxidant activities of the three species by using standard radical-scavenging and reducing-power assays.
- To evaluate the phytoremediation potential of the plants by means of metal uptake, translocation efficiency, and detoxification enzyme responses.

The present study will be conducted to ensure an in-depth investigation into the biochemical and environmental importance of three medicinal plants tolerant of abiotic stresses by profiling their phytochemical composition, determining their antioxidant capacities, and testing their remediation efficiency in contaminated soils. By integrating metabolite analysis, antioxidant performance, and phytoremediation assessment, the study will seek to identify species with high therapeutic value along with practical potential for the restoration of polluted environments.

Materials and Methods

Samples and samples area: The species of interest were *Citrullus colocynthis*, *Capparis decidua*, and *Calotropis procera* (fruits). Only mature plants in healthy condition were selected. Before being shade-dried for seven to ten days at room temperature, the plants were cleaned with distilled water and sampled early in the morning to allow for any diurnal variations. The processed samples were dried, then ground into a fine powder and kept in airtight containers in a dark, cool place until they could be analyzed (Nafiu *et al*., 2024). An ecological variation sampling method was used to sample ecological variation among four distinct ecological areas of Punjab, Pakistan (Bahawalpur, Bahawalnagar, Rahimyar Khan, and Lodhran) based on climate zone differences, including temperature and availability of moisture, soil type, and anthropogenic activities; thus, odic influences on the phytochemical and phytoremediation features of representative species are inferred. Figs. 3 and 4 represent the map of the sampling sites and fresh fruits of medicinal plants, respectively.

Sample processing

- Collected samples were rinsed with tap water, spread over paper sheets, and dried in the shade for 10-15 days (Vasco *et al*., 2014). Dried plant samples were pulverized into fine powders and kept at room temperature in plastic bottles for further analysis. This research was conducted in The Islamia University of Bahawalpur and Beijing Forestry University China.

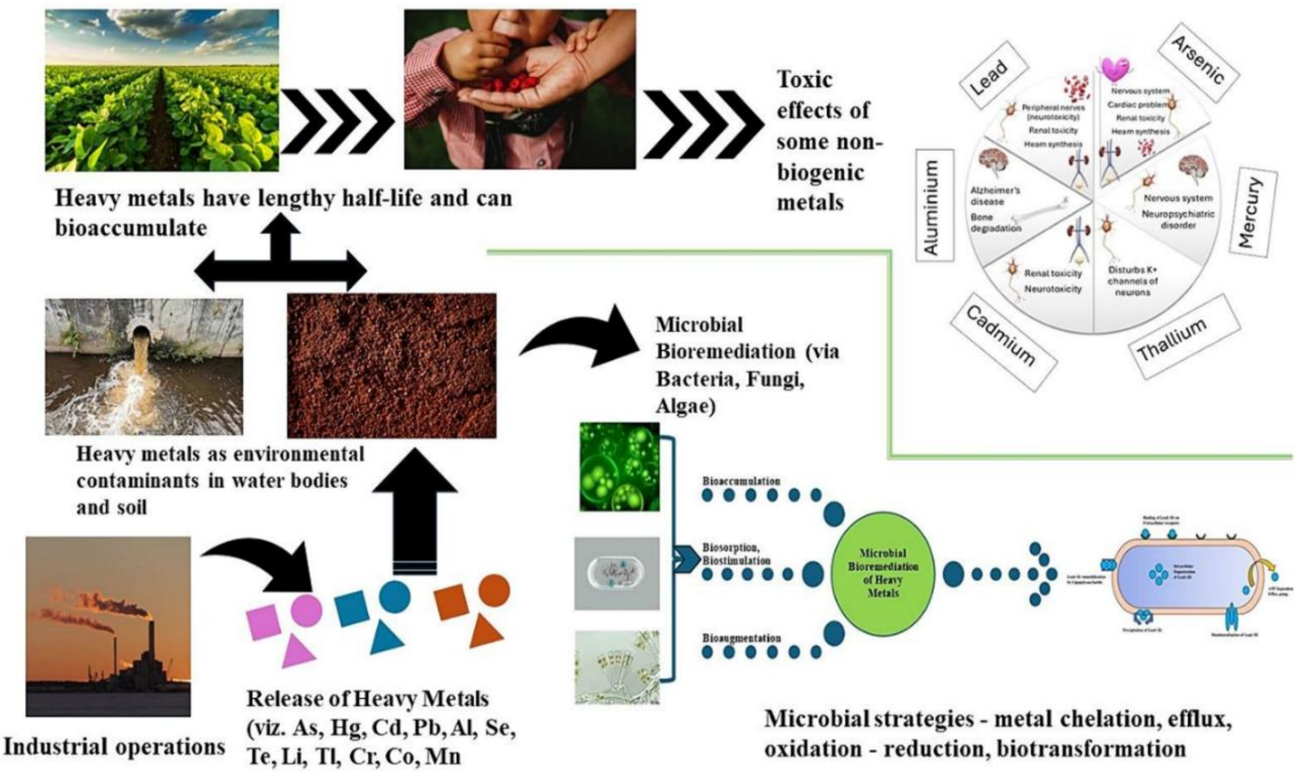

Fig. 2. Health effects of heavy metals on human health (https://www.sciencedirect.com/science/article/abs/pii/S0020169324001580).

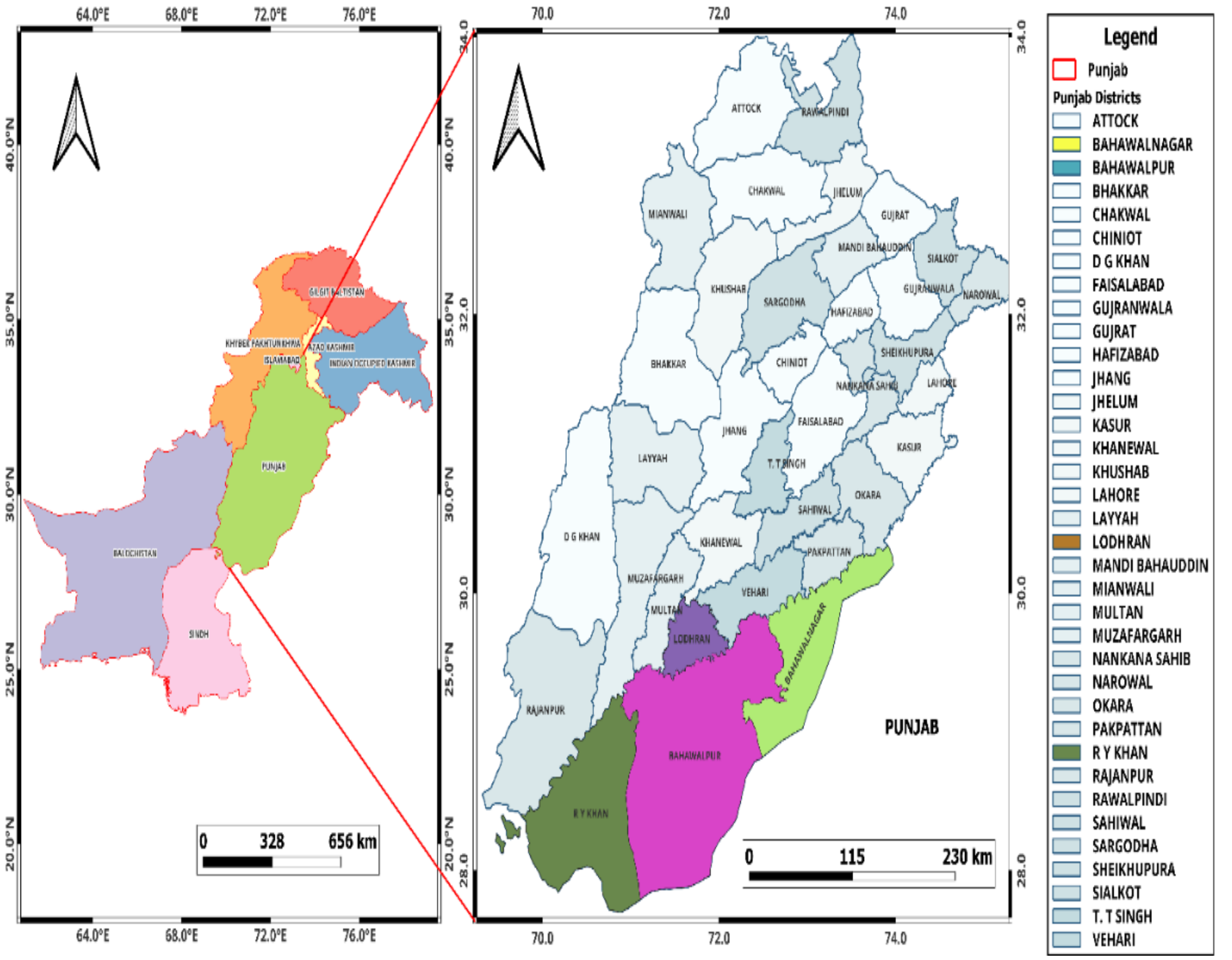

Fig. 3. Map of sampling sites.

Fig. 4. Selected fresh fruits of medicinal plants.

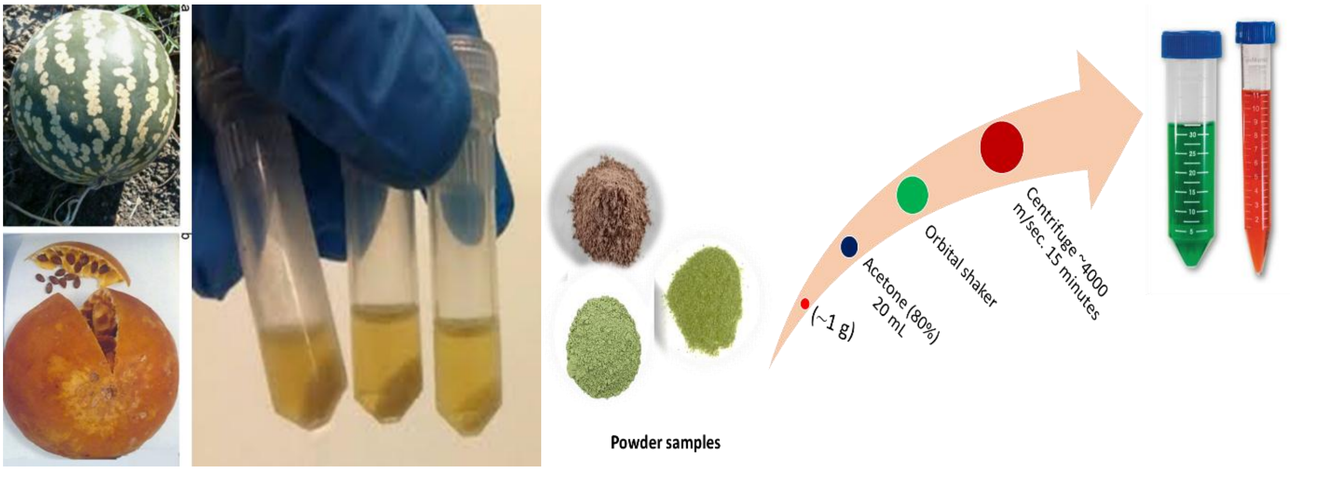

Fig. 5. Extraction of dried medicinal plant species.

Extraction: Plant sample methanolic extract was made using the previously mentioned maceration procedure (Jo *et al*., 2012). In short, 30 milliliters of methanol were used to soak one gram of dried plant material, then left over night in a shaking incubator (Wise Cube WIS-20). The extract was centrifuged using a Jouan Thermo BR4i multifunction centrifuge for 10 minutes at 4000 rpm the following day. After collecting the supernatant in a different container (Fig. 5), the extraction procedure was done three times. The final crude extract was kept at 4°C until it could be examined further.

Phytochemical analysis of medicinal plants

Estimation of total phenolic contents (TPC): Total phenolic content (TPC) was measured using a slightly modified Folin-Ciocalteu calorimetric technique. To summarize, 4 mL of 7% Na_2CO_3, 1 mL of the plant sample and 2 mL of 15-fold diluted FC reagent were added and kept in the dark for six minutes. A UV-Vis spectrophotometer (UV-1100) was used to measure absorbance at 760 nm after the mixture was incubated at 30°C in the dark for 60 minutes. Gallic acid equals (GAE)/100 g dried weight (DW) was used to express TPC (Lin *et al*., 2011).

Estimation of total flavonoid contents (TFC): The TFC assay was carried out to measure the total flavonoid concentration. To put it briefly, 0.5 mL of the sample, 2.5 mL of distilled water, and 0.075 mL of 7% $NaNO_2$ were combined, incubated for 6 minutes at 25°C, and then 0.3 mL of 10% $AlCl_3$ was added. Two mL of 1 M NaOH were used to halt the reaction after five minutes, and 0.55 mL of distilled water was used to dilute it. The absorbance was measured by using the UV-Vis spectrophotometer. The outcomes were exhibited as mg of quercetin dihydrate equivalents (QDE) per 100 g dry weight (Lin *et al*., 2011).

DPPH scavenging assay: The DPPH assay was assessed. To put it briefly, two mL of sample extract and two mL of 0.2 mM DPPH solution were mixed (in duplicate) and vigorously shaken. To assess the mixture's absorbance at 517 nm in relation to a blank, after 30 minutes of dark incubation at room temperature by using the spectrophotometer (Wenli *et al*., 2004). The percentage inhibition (%) was calculated using the following formula:

$$\text{DPPH (\%)} = \frac{\text{Absorbance blank} - \text{Absorbance sample}}{\text{Absorbance blank}} \times 100 \qquad \text{Eq. 1}$$

Hydroxyl radical (OH) inhibition assay: According to Wenli *et al*., (2004), The Fenton reaction was used to assess hydroxyl radical-scavenging activity. In summary, 40 µL of 0.02 M $FeSO_4$, 2 mL of 0.2 M phosphate buffer (pH 7.2), 1 mL of 0.04 M 1,10-phenanthroline, and 2 mL of plant extract were combined. One ml of 7 mM H_2O_2 stock solution was added, then left at room temperature for five minutes. A UV-Vis spectrophotometer was used to detect the absorbance at 560 nm. The extract was substituted with distilled water to create a blank. Every measurement was taken three times (mean ± SD). The hydroxyl radical-inhibition activity (%) was computed using the following formula:

$$\text{OH (\%)} = \frac{\text{Ablank} - \text{Asample}}{\text{Ablank}} \times 100 \qquad \text{Eq. 2}$$

FRAP Assay: With a few small adjustments, ferric reducing antioxidant power (FRAP) was assessed in compliance (Hazra *et al*., 2008). In short, two mL of extract, 2mL of stock buffer solution of phosphate with (pH 6.6), and 2mL of 0.1% potassium Ferri-cyanide were combined and incubated for twenty minutes at 50°C. Two mL of 10% trichloroacetic acid (TCA) were added to stop the process. After mixing two ml of the supernatant with two ml of 0.01% $FeCl_3$, the absorbance was measured to calculate the reducing power. The positive control was gallic acid. Gallic acid equivalents per 100 g dry weight (µM GAE/100 g DW) were used to express the data.

Phosphomolybdenum complex assay (PMCA): According to Prieto *et al*., (1999), The Phosphomolybdenum (TAC) test was used to determine total antioxidant capacity. In summary, 6.6 ml of reagent (in triplicate) comprising 0.6 M sulfuric acid, sodium phosphate of 28mM soln. And ammonium molybdate of 4mM was combined with 2 mL of extract. Absorbance was measured at 695 nm following the mixture's cooling to ambient temperature and 90-minute incubation at 95°C. Ascorbic acid, as a standard solution per 100 g dried weight, or µM AAE/100 g DW, was used to measure TAC.

HPLC profiling

Extraction for HPLC: Phytochemical extracts were prepared via cold maceration (1:10) for 72 hours at 100 rpm with 80% methanol (v/v). The extracts were then filtered using Whatman No. 1 filter paper and dried to yield a crude extract under reduced pressure using by rotary evaporator.

HPLC conditions: An Agilent HPLC-1260 Infinity system with a C18 reverse-phase column (4.6 × 250 mm) was used for high-performance liquid chromatography (HPLC) profiling. Methanol, water, and acetic acid were used as the mobile phase at a flow rate of 1.00 mL/min in a ratio of 70:28:2. The detecting wavelength was set at 280 nm, and a 20 µl injection volume was created (Arabaci & Akduman, 2024). As previously mentioned, all samples were homogenized and then filtered using a 0.45 µm syringe filter. Gallic acid, quercetin, rutin, catechin, and ferulic acid were the reference compounds used to characterize phytochemicals in order to identify the components. In order to identify and quantify the phytoconstituents, the samples of peak areas and retention periods were also compared to standards.

Digestion and instrumentation: Dried plant and sediment samples were analyzed for heavy metals after being dried in the oven for 48 hours at 80ºC. The dried pulverized samples were then treated hydro metallurgically by acid digestion with a 0.5 g sample mass. Plant samples were stripped using a 2:1 solution of nitric and perchloric acid, while soil samples were carefully scraped. Dilutions from the algal powder digest were used to measure the amounts of Zn, Cu, and Pb, Ismail *et al*., (2017). The bioconcentration factor (BCF), translocation factor (TF), and enrichment factor (EF) of the measured mass of Zn, Cu, and Pb in the harvest were determined using the Atomic Absorption Spectrophotometer Model Analyst 400.

Chemicals, Reagents, and Instruments: Every chemical and reagent that was utilized was analytically pure. Potassium ferricyanide, ethanol, sodium carbonate, hydrogen peroxide, 1,10-phenanthroline, DPPH, gallic acid, quercetin, ascorbic acid, HNO_3, H_2SO_4, and $AlCl_3$. VMR International (USA) provided perchloric acid, ammonium molybdate, NaOH, ferric chloride, sodium phosphate, ferrous sulphate, and phosphate buffer. Oxford and Merck (Germany) provided the TCA and Folin-Ciocalteu reagent, respectively. For calibration, stock solutions containing 1000 mg/L of heavy metals were created. An orbital shaker, centrifuge, digital balance, rotary evaporator, spectrophotometer (UV-1100), flame atomic absorption spectrophotometer (PerkinElmer, USA), HPLC (Japan) with UV detector, and micropipettes were among the equipment. Additionally, common laboratory tools such as a refrigerator, oven, analytical balance, and glassware were utilized.

Results and Discussion

Medicinal plants description: *Citrullus colocynthis* (Cucurbitaceae), commonly known as bitter apple or desert gourd, is a creeping or climbing herbaceous perennial more commonly found in hotter and drier regions of the world. Bioactive compounds produced by the plant, such as flavonoids and cucurbitacins, possess antibacterial, anti-inflammatory, and antioxidant properties. The plant grows in sandy, dry soils and also has indications for potential phytoremediation and abiotic stress responses (Mondal *et al*., 2020).

Capparis decidua (Capparidaceae), commonly known as karir or caper bush, is a spiny shrub with drought tolerance, is leafless, and is closely related to desert and semi-desert climates. The plant has deep roots generating bioactive compounds such as alkaloids, flavonoids, and phenolics, and in some regions of the world parts of the plant are used traditionally as medicine for digestive issues and inflammatory conditions. The adaptation of the plant to desert and semi-desert environments makes it a candidate for phytoremediation (Bhasin *et al*., 2020). Table 1 is the brief explanation of all selected medicinal plants.

Calotropis procera (Apocynaceae) or ak or madar is a perennial shrub with a high degree of robustness, milky latex and environmental extremes. The plant produces numerous secondary metabolites including cardiac glycosides, flavonoids, and terpenoids. The plant has a history of disproportionate use as a wound healer, or in skin infections or/and respiratory diseases (Iqbal *et al*., 2023).

Table 1. Brief medicinal uses of fruit part (Azhar *et al*., 2020).

Plant name	Ailment/Disease	Therapeutic use of fruit
Citrullus colocynthis	Constipation	Dried pulp of fruit is used as a strong purgative (taken orally in very small doses)
	Diabetes	Powdered dried fruit is combined with water and consumed orally
	Joint pain	Fruit extract is sometimes used in formulations applied externally
	Skin diseases	Fruit pulp mixed with oil and applied externally to infected areas
	Fever	Fruit powder used in combination with other herbs
	Edema	Bitter fruit extract is used orally in traditional medicine
	Parasitic worms	Dried fruit pulp used as an anti-helminthic (caution: toxic in large doses)
	Indigestion	A very small quantity of fruit powder was ingested
Capparis decidua	Pains	Dried fruit can be used alone or mixed with other medicinal plants orally
	Joint pain	Oral use of fruit in combination with other parts (leaves, fruits and roots)
	Appetizer	Unripe/fresh fruits consumed or pickled
	Stomach pain	Dry or fresh fruits taken orally with powdered root bark
	Hemorrhoids	Dry fruit powder taken orally
	Health tonic	Dry fruits are consumed with flowers and fruits as a general tonic
	Anemia	Fresh fruits are eaten directly
	Worms	Dried fruits are made into tablets and taken orally
	Spleen enlargement	Dried fruit is powdered and taken orally with leaves
Calotropis procera	Constipation	Ripe fruits are used in powdered form for a mild laxative effect
	Skin infections	Latex from immature fruit applied externally
	Indigestion	Small quantities of dried fruit pulp are used in traditional formulations
	Rheumatism	Fruit pulp/lotion is applied externally to swollen joints
	Toothache	Fruit ash mixed with other herbs and applied to the gums
	Snakebite (traditional)	Crushed fruit used externally as an emergency traditional remedy (not scientifically validated)

Phytochemical and antioxidant profiling

Total phenolic content (TPC): The TPC values ranged between 38.9 ± 0.9 mg GAE/g in *Capparis decidua* and 45.3 ± 1.2 mg GAE/g in *Citrullus colocynthis*, indicating that *C. colocynthis* had the highest phenolic concentration. Phenolic compounds are known for their ability to donate hydrogen atoms and act as potent antioxidants, suggesting that *C. colocynthis* may have superior radical scavenging potential among the studied species.

Total flavonoid content (TFC): TFC values were observed in the range of 29.7 ± 1.3 mg QE/g (*C. decidua*) to 33.1 ± 2.0 mg QE/g (*C. colocynthis*). Flavonoids contribute significantly to the antioxidant defense mechanism by modulating cell signaling pathways and preventing oxidative stress. Again, *Citrullus colocynthis* mounted as the peak flavonoid content.

Phosphomolybdenum complex assay (PMCA): The highest PMCA values were found in *Citrullus colocynthis*, at 88.4 ± 1.5 mg AAE/g, followed by *Calotropis procera*, with 85.6 ± 1.4 mg AAE/g, and *Capparis decidua*, with 81.2 ± 1.9 mg AAE/g. This assay measures the total antioxidant activity based on the reduction of molybdate ions (VI) to Mo(V), and the results support the potent antioxidant properties of *Citrullus colocynthis*.

FRAP/ ferric reducing antioxidant power: FRAP assay showed that all three species exhibited strong electron-donating capacity, and again the highest value was manifested by *Citrullus colocynthis* (110.5 ± 2.3 µmol Fe^{2+}/g). The values of *Calotropis procera* and *Capparis decidua* were marginally lower, with 104.1 ± 1.8 and 98.3 ± 2.0 µmol Fe^{2+}/g, respectively. FRAP values also relate to TPC and TFC values, indicating a direct relationship between the contents of phenolic/flavonoid compounds and antioxidant potential.

DPPH free radical scavenging activity: DPPH-scavenging abilities were between 68.5 ± 1.7% in the case of *C. decidua* and 72.2 ± 1.8% in the case of *C. colocynthis*, indicating high activities regarding neutralizing free radicals. The high DPPH inhibition percentage of *C. colocynthis* corresponds to its higher content of phenolics and flavonoids, thus confirming the relationship between phytochemical composition and radical scavenging activity.

Hydroxyl radical scavenging activity (OH): In this study, hydroxyl radical inhibition was highest in *Citrullus colocynthis* at 65.4 ± 2.1%, followed by *Calotropis procera* at 62.8 ± 2.2% and *Capparis decidua* at 60.2 ± 2.5%. The hydroxyl radical is among the most reactive ROS; therefore, this assay indicates the potential of the plant to protect biomolecules from oxidative damage.

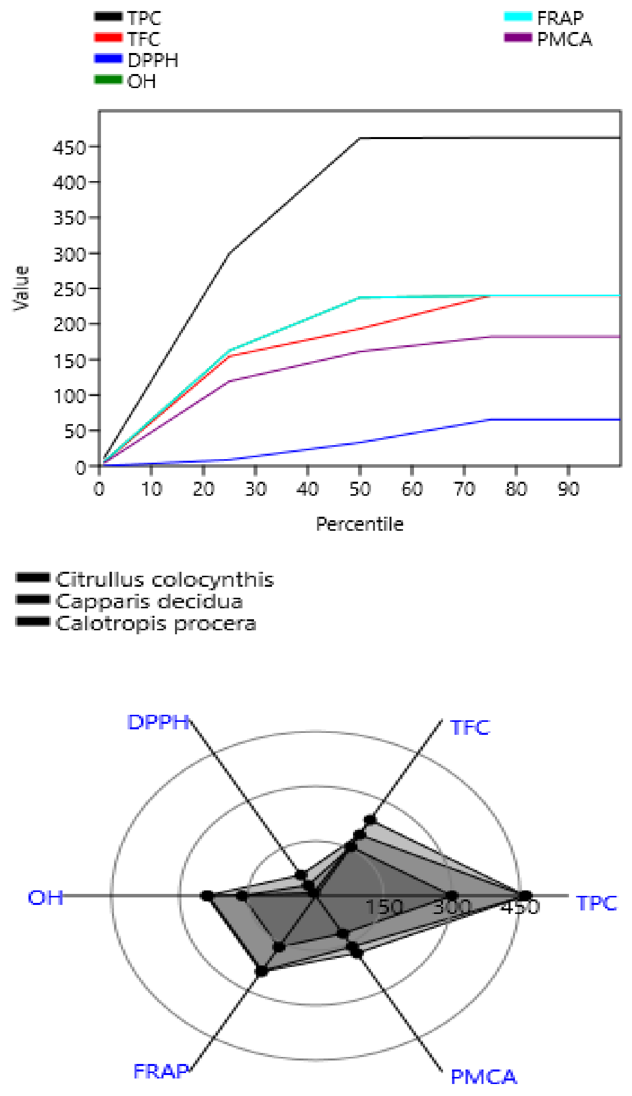

Fig. 6. Percentile and radar histograms of phytochemicals and ion-reducing activities.

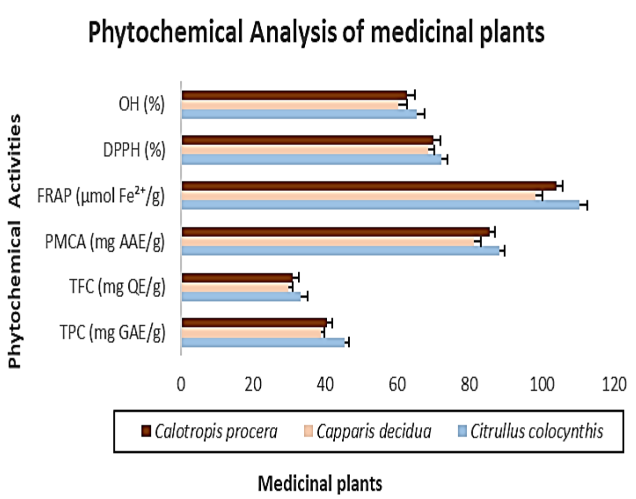

Fig. 7. Phytochemical and antioxidant analysis of medicinal plants.

Association between phytochemical potential: The hierarchical clustering heatmap permits a comparative interpretation of the antioxidant and phytochemical properties of *Citrullus colocynthis, Capparis decidua,* and *Calotropis procera.* The indicators of TFC, TPC, OH, DPPH, FRAP, and PMCA show strong correlations amongst the experimental response variables (Figs. 6 and 7). Within the heatmap, TFC and TPC are related, since flavonoids and phenols are substantially related biochemically. Similarly, OH and DPPH formed the same consortium. FRAP and PMCA formed another consortium, indicating that they share the same response characteristic based on the reducing power of the antioxidant indicator. From the plants tested, *Capparis decidua* and *Calotropis procera* were more similar, indicating their antioxidant characteristics were chemically related. *Citrullus colocynthis* was distinctly different and provided better efficiencies in the reducing power-based assays of FRAP and PMCA, as represented by blue color gradients. *Citrullus colocynthis* has the potential to have the greatest total antioxidant volume, so it could be useful for further research exploration of therapeutic capacity. The clustering of the response variables suggests the propensity of phytochemical assays to group based on natural antioxidant characteristics (Fig. 8).

Phytoremediation Potential of *Citrullus colocynthis*, *Capparis decidua*, and *Calotropis procera*: The phytoremediation capacities of *Citrullus colocynthis*, *Capparis decidua*, and *Calotropis procera* were assessed based on their bioaccumulation efficiency, growth response under stress, and pollutant specificity.

1. *Citrullus colocynthis*: *Citrullus colocynthis* exhibited exceptionally high phytoextraction and Phyto stabilization capabilities, particularly for heavy elements like chromium (Cr), nickel (Ni), zinc (Zn), lead (Pb), and cadmium (Cd). Biomass assessment demonstrated high concentrations of metals in aboveground parts of plants, with TF>1 for both Cd and Zn under controlled contamination ($p \leq 0.05$). Due to its high root strength, fast growth rate, and drought tolerance, this plant has proven to be a good candidate for further remediation applications even in arid and semi-arid regions. The ability of absorption and transportation processes in the species was reflected through BCF for Cd and Zn, fluctuating between 1.2 to 2.3. This corroborates previous literature on possible restoration of desertified regions using high-biomass halophytes.

2. *Capparis decidua*: *Capparis decidua* showed excellent Phyto stabilization and rhizofiltration capabilities. Most of the metal accumulation happened in the root system. Translocation was minimal for As, Cu, and Pb with TF < 1, indicating a retentive root-barrier function, which restricted substantial movement into the aerial tissues. High salinity trials have shown that the plant could grow despite NaCl concentrations greater than 200mM, hence its potential use for the remediation of saline-alkaline environments. The deep-rooting system functions to provide subsurface stabilization, therefore causing more reduction in leachate, exceeding that from shallow systems, with the added benefit of increased soil structure. About 150 mg / kg for Pb was recorded during elemental analysis of root metal concentration, while shoot concentrations were consistently <40 mg / kg, demonstrating its high potential for non-invasive soil recovery.

3. *Calotropis procera*: *Calotropis procera* demonstrated a multi-pathway remediation potential, stimulating phytoextraction and phytodegradation mechanisms. The plant successfully accumulated Pb and Cd with TF values >1.5, while also showing enzymatic activity involved in the degradation of hydrocarbons, such as additional peroxidase and laccase activities in root and shoot tissues upon exposure to petroleum hydrocarbons. The phytoremediation potential of selected medicinal plants is determined in Table 2. The latex-rich anatomy might further promote the supply of carbon at the soil-organism interface for sequestration or transformation of hydrophobic organic pollutants. *C. procera* also expressed stress tolerance in hydrocarbon-polluted soils through biomass loss of less than 15%, hence indicating that *C. procera* is highly suitable for a placement in hydrocarbon-polluted soils. Results indicate that *C. procera* can operate at a site with both mixed contaminants, particularly within a post-industrial context.

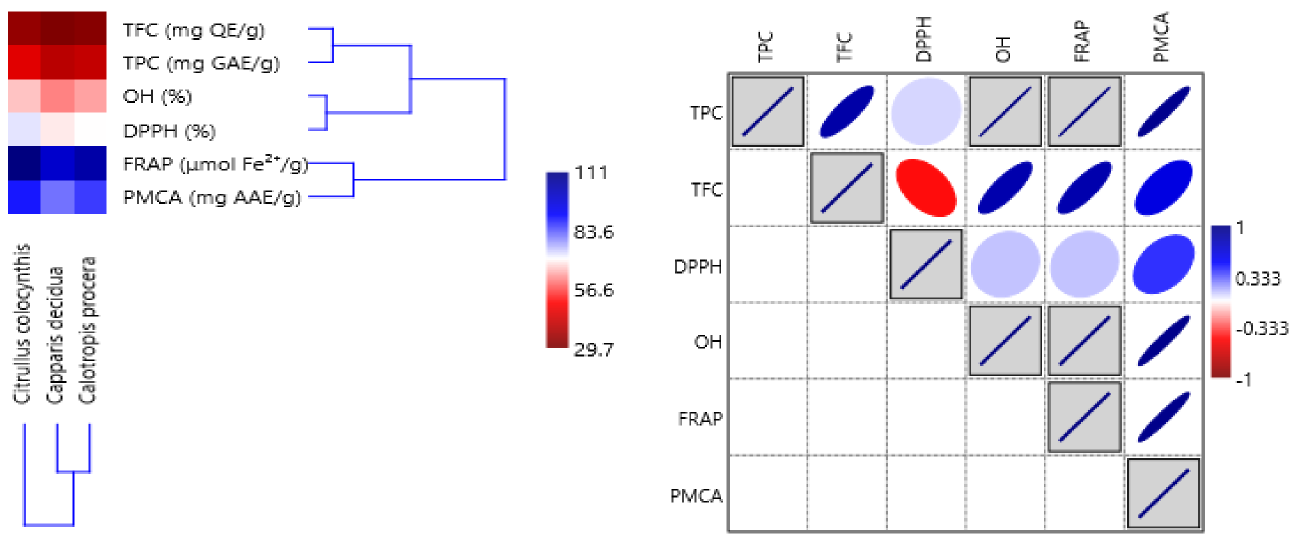

Fig. 8. Correlation and cluster analysis of *Citrullus colocynthis*, *Capparis decidua*, and *Calotropis procera*.

Table 2. Phytoremediation potential of selected medicinal plants.

Plant name	Phytoremediation mechanism(s)	Target pollutants	Notable features
Citrullus colocynthis	Phytoextraction, Phytostabilization	Heavy metals (Cd, Pb, Ni, Zn, Cr)	High biomass, drought-tolerant, rapid growth
Capparis decidua	Phytostabilization, Rhizofiltration	Salts, heavy metals (As, Cu, Pb)	Flourishes in saline soils, deep-seated
Calotropis procera	Phytoextraction, Phytodegradation	Pb, Cd, petroleum hydrocarbons	Latex-rich plant, hyperaccumulator tendencies

Comparative phytoremediation indexing: In order to determine success of remediation using phytoremediation techniques, we developed a Phytoremediation Potential Index (PPI), which integrates weighted values for biomass, metal accumulation, stress-resistance, and mechanism diversity (Fig. 9). The highest PPI value for phytoextraction was for *C. colocynthis* (8.7). The best PPI for in situ stabilization was for *C. decidua* (7.9). *C. procera* was ranked equally well with a PPI of 8.2 (it uses a combination of both remediation techniques). These species, histories and mechanism of remediation indicate that they will work particularly well for locations with a burden of metals and organic compounds (Table 3).

Species	Mechanisms(s)	Target pollutants	PPI score
Citrullus colocynthis	Phytoextraction Phytostabilization	Cd, Pb, Ni, Zn, Cr	8.7
Capparis decidua	Phytostabilization Rhizofiltration	As, Cu, Pb, Salts	7.9
Calotropis procera	Phytoextraction Phytodegradation	Pb, Cd, Petroleum Hydrocarbons	8.2

Fig. 9. Phytoremediation efficiency and pollutant preference index (PPI) of selected plant species.

Comparative analysis and implications: Overall, all three species displayed as multipurpose phytoremediation dimensions with separate assets:

- *C. colocynthis* was more effective in extracting and translocating transition metals,
- *C. decidua* showed superior retention and immobilization of contaminants in saline surroundings.
- *C. procera* appeared as a dual-action plant for both metal acceptance and biological contaminant dilapidation.

Mechanism of tolerance: The capacity of a plant to withstand a specific metal is governed by an elaborate network of physiological and molecular processes, mostly studied in view of the development of plants for phytoremediation of contaminated sites. Singh *et al.*, (2003) reported that plants may exhibit an apparent tolerance to increasing levels of toxic elements due to a combination of toxic element exclusion or organisms' metabolic tolerance to the elements.

Identified compounds and quantification (µg/g DW): Seven major phytochemical compounds, for high-performance liquid chromatography (HPLC) analysis are gallic acid, caffeic acid, p-coumaric acid, ferulic acid, quercetin, kaempferol, and rutin, were identified and quantified in *Citrullus colocynthis*, *Capparis decidua*, and *Calotropis procera*.

The results are expressed as µg/g dry weight (DW) and presented in Table 4.

Table 3. HPLC conditions.

Parameter	Value/Details
Column	C18 reverse-phase (250 mm × 4.6 mm, 5 µm)
Mobile phase	0.1% formic acid Acetonitrile
Flow rate	1.0 mL/min
Detection	UV-Vis at 280 nm (for phenolics), 330 nm (flavonoids)
Injection volume	20 µL
Runing time	40 minutes

Table 4. HPLC analysis of polyphenols.

Compound	*C. colocynthis*	*C. decidua*	*C. procera*
Gallic acid	85.2 ± 2.5	92.7 ± 3.1	78.5 ± 2.0
Caffeic acid	34.1 ± 1.2	47.3 ± 1.8	29.4 ± 1.0
p-Coumaric acid	28.6 ± 0.9	35.4 ± 1.0	26.7 ± 0.8
Ferulic acid	22.7 ± 0.7	30.1 ± 1.1	20.9 ± 0.6
Quercetin	63.8 ± 2.3	71.5 ± 2.5	58.2 ± 1.9
Kaempferol	41.9 ± 1.4	49.2 ± 1.7	39.8 ± 1.3
Rutin	59.6 ± 2.0	66.1 ± 2.2	54.4 ± 1.6

Values are presented as mean ± SD (n = 3)

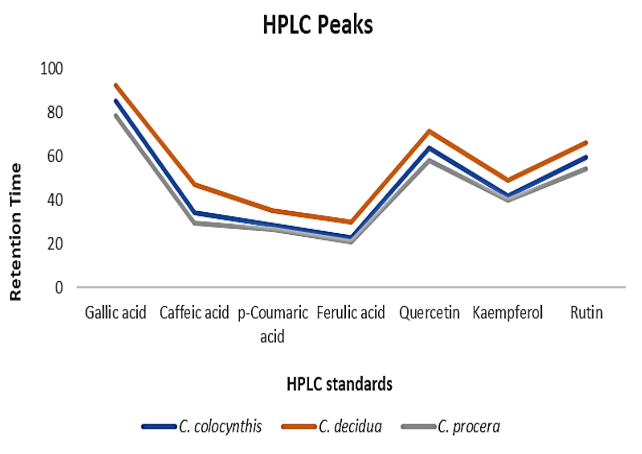

Fig. 10. Simulated HPLC chromatogram representing the phytochemical analysis of TCME (Total Crude Methanolic Extract) for *Citrullus colocynthis* (L.) Schrad., *Capparis decidua* (Forssk.) Edgew., *and Calotropis procera* (Aiton) W.T. Aiton.. Each peak corresponds to a specific compound, marked along the retention time axis.

Most abundant compounds

1. *Capparis decidua* contained the highest concentrations of gallic acid, quercetin and rutin, which are known to have potent antioxidant, anti-inflammatory and antimicrobial activity. The abundance of these flavonoids and phenolic acids supports their ethnopharmacological and therapeutic potential in use.

2. *Citrullus colocynthis* had especially high concentrations of kaempferol and caffeic acid, which are noted to have potent anticancer and anti-inflammatory activity, indicating that *Citrullus colocynthis* can be a valuable bio-resource for drug discovery and development.

3. *Calotropis procera* had comparatively moderate concentrations of all targeted compounds; however, the still measurable concentrations of the primary phenolics would indicate this plant has potential therapeutically as a multifunctional medicinal plant, but not as potent as the previously mentioned two plants (Fig. 10).

Chemotaxonomic Significances

The fact that phenolic acids (gallic, caffeic, and p-coumaric) were found in all three species further confirms their ability to maintain biosynthetic potential and support their historical ethnobotanical uses. Furthermore, the classifications of these compounds correlated positively with antioxidant capacity as determined by FRAP, DPPH, and OH radical scavenging assays.

Statistical Analysis

Replicates were conducted by means of an MS spreadsheet, and represented as mean ± SD (standard deviation; n = 3). Associations were analyzed using SPSS (version 13.0). Data presentation was conducted by Sigma software (version 12.0).

Conclusion and Recommendations

The noteworthy potential in phytochemical, antioxidant, and phytoremediation of *Citrullus colocynthis*, *Capparis decidua*, and *Calotropis procera* is demonstrated in this study. *C. colocynthis* had the uppermost levels of phenolics and flavonoids and displayed strong antioxidant capacity and metal accumulation ability. *C. colocynthis* shows great potential to be used as a phytopharmaceutical or environmental application. *C. decidua and C. procera* had similar remediation traits and stress tolerance that could also be useful for restoration plants.

- Field trials should be completed to examine performance in natural, contaminated conditions.
- Integrated phytoremediation models that include these species could provide better remediation results.
- Further pharmacological studies should be done with *C. colocynthis* due to its strong antioxidant capacity.
- Molecular studies that provide more information about mechanisms of stress tolerance and pollutant uptake should be performed.

Declaration of Competing Interest: The authors affirm that the work described in this publication was not influenced by any known competing financial interests or personal ties.

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