



PHYSICOCHEMICAL ANALYSIS, PHYTOCHEMICAL & ANTIOXIDANT CHARACTERIZATION OF CASCABELA THEVETIA L. IN CONTAMINATED SOIL AND GARDEN SOIL

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Abstract

The poisonous plant Cascabela thevetia L., also known as Thevetia peruviana, is native to Mexico and Central America and is commonly grown as an ornamental plant. Around the world, T. peruviana frequently causes toxicological emergencies in tropical and subtropical regions use its huge seed, which resembles a "Chinese lucky nut," is encased in a deep red-black fruit. In India, its ability to withstand drought and high temperatures, it can be found in many Indian states with semi-arid climates, including Andhra Pradesh, Bihar, Delhi, Gujarat, Madhya Pradesh, Telangana, West Bengal, Rajasthan, Tamil Nadu, Uttar Pradesh, Odisha, and Assam. In India, its bright yellow blossoms are worshipped by Hindu followers for religious purposes. The aim of the present study was to determine the total phenol, tannin and flavonoid content in leaf, stem parts of Cascabela thevetia L. in methanol and distilled water extract by spectrophotometric method. Saponin, tannin, flavonoid, cardiac glycoside alkaloid and phenolic chemicals were detected by phytochemical analysis, using standard gallic acid, tannic acid and Quercetin as the marker point's, the total phenol, flavonoid, tannin and alkaloid content of the plant in various extracts was determined. The high concentration of phenol was found in methanolic stem extract collected from Fresh garden site, and Pirana dump site was (4.79±0.485) mg/GAE and (4.73±0.468) mg/GAE sample. The presence of such metabolites indicates therapeutic importance of plant. The anti-oxidant assay such as DPPH determines the reducing agent which is present in plant. The conservation and appropriate use of soil and water are two essential resources and life-sustaining elements for plant growth. Numerous physiochemical characteristics, such as pH, TDS test, Dissolved oxygen test, total hardness and calcium hardness of soil and water, affect how well plants absorb it.

Keywords: DPPH assay, Heavy metals, Dissolved oxygen, Total hardness.

1. INTRODUCTION

Native to Mexico and Central America, the toxic plant Cascabela thevetia L. Also called Thevetia peruviana, is frequently grown as an ornamental. It is frequently referred to as yellow oleander due to its resemblance to Nerium oleander. Toxicological situations caused by T. Peruviana are commonly grown in tropical and subtropical areas worldwide. "Cascabel," "cascavel," "Cascabela" are Spanish terms that describe a rattlesnake, a little bell, or a snake's rattle. In tropical and subtropical areas across the world, T. Peruviana commonly causes toxicological emergencies. Its huge seed, which resembles a "Chinese lucky nut," is enclosed in a deep red- black fruit. Cascabela thevetia is commonly known in India as Kaneir or Kaner in Hindi. Many Indian states with semi- arid climates, such as Andhra, Bihar, Delhi, Gujarat, Madhya Pradesh, Telangana, West Bengal, Rajasthan, Tamil Nadu, Uttar Pradesh, Odisha, and Assam, are home to it due to its resistance to drought and high temperatures. Hindu devotees in India revere its vivid yellow flowers for religious reasons. Its leaves are linear- lanceolate, glossy green, and resemble willows. As a typical of oleanders, they have a waxy coating to stop water loss. Its green stem turns silver or grey in age. Flowers flourish from summer to fall. Terminal clusters are long, funnel-shaped, and often aromatic, with few-flowered yellow (less often apricot, sometimes white) blossoms (M. Stephenhuik, 2021). The majority of vertebrates are at risk from the cardiac glycosides present in all parts of the C. Thevetia plant. Cascabela thevetia L., is a popular member of the Apocyanaceae family and a yellow oleander that is planted as a tree in garden and along roadsides. Terpenoids, flavonoids, steroids, and glycosides are among the pharmacological active components found in seeds. The seeds of Cascabela thevetia L. are very toxic to the germination of plant root cells. The cardenolides thevetin A and thevetin B are main toxins; ruvoside, neriifolin, and peruvoside are also poisons. As with digoxin from Digitalis purpurea, drying and cooking do not harm these cardenolides (Langford, S., et al., 1996). They have digestive and cardiotoxic effects. The Cascabela thevetia plant has been found to have a wide variety of secondary metabolites, such as alkaloids, flavonoids, steroids,

terpenoids, tannins, saponins, and cardiac glycosides. In addition to their alleged medicinal benefits, the plant's leaves are used in traditional medicine for their diuretic and cardiac tonic effects. The pharmacological activity of several plant components, including seeds, flowers, bark, fruits, and leaves, has been shown in numerous investigations. Numerous cardiac glycosides, such as neriifolin, oleandrin, thevetin A, and thevetin B, are present in this potentially hazardous plant. These are only a few instances of glycosides. After consuming oleander, certain symptoms may manifest, including diarrhoea, vomiting, nausea, hyperkalaemia, and abdominal pain. Oleander can also cause diarrhoea. The plant or its components can aid with a variety of human conditions, including diabetes, liver damage, microbial and fungal infections, inflammation, fever, and discomfort (Dixit et al., 2015). This plant may be used to treat a variety of illnesses, such as cancer, spermatogenic bugs, termites, fungi, bacteria, antioxidant, HIV, inflammation, and diarrhoea, according to Bhoyar (2021).

1.1 Scientific Classification:

Kingdom: Plantae
 Sub kingdom: Tracheobionata
 Division: Magnoliophyta
 Class: Magnoliopsida
 Subclass: Asteridae
 Order: Gentianales
 Family: Apocynaceae
 Genus: Cascabela
 Species: C. thevetia

2. MATERIAL AND METHODOLOGY:

2.1 Sample collection: Cascabela thevetia L. leaves and stems, which were mature and in good condition, were collected from the Pirana waste site in Chhipakuva, which is close to Chandola Lake. The same plant parts were also collected from a fresh garden site near Bapunagar, which is near General Hospital. Academicians of the department assisted in identifying the plant.

2.2 Qualitative Analysis: Qualitative phytochemical screening was carried out using the Sheikh and Patil, 2020 procedure for Cascabela thevetia L. The analysis of phytochemical screening was done to detect the presence of various phytochemicals such as alkaloid, flavonoid, phenolic compounds, saponins, tannin, proteins and cardiac glycosides.

1) Alkaloid:

Dragendroff's Test: 1 to 2 millilitres of Dragendroff's reagent were added to a small amount of plant sample. Alkaloids are present when a radish brown precipitate forms (Raaman, N., 2006).

2) Flavonoids:

Lead Acetate test: A few drops of lead Acetate reagent were added to 1 millilitre of sample. When flavonoids are present, a yellow precipitate will form (De silva, G., et al., 2017).

3) Phenolic compound:

Potassium dichromate test: A few drops of potassium dichromate solution were added to plant extract. The presence of phenolic compounds is shown by the formation of brown precipitate (Jagessar, R., 2017).

4) Saponins:

Olive oil test: 2 ml of plant extract were shaken with plant extract after being treated with a few drops of olive oil reagent. Saponin is indicating by the formation of stable emulsions (Foamy milky layer) (Dahanayake, J., et al., 2019).

5) Tannin:

Lead Acetate test: 1ml of 10% lead acetate reagent was added to 2ml of plant extract. The presence of tannin is shown by the formation of white precipitate (Sheel, R., et al., 2014).

6) Protein:

Millon's test: 1-2 ml of Millon's reagent were added to 2 ml of extract. When protein is present, a white precipitate will form (Chamakuri et al., 2021).

7) Cardiac- glycoside:

Kellar-Killani test: Take a small amount of extract to test. Add a little amount of ferric chloride and a few drops of glacial acetic acid. Along the side of the test tube, carefully add concentrated sulphuric acid. The two layers meet to form a bluish or blue-green ring. There are times when the uppermost layer turns green as well (Akinwumi et al., 2024).

2.3 Quantitative analysis:

A variety of quantitative techniques are used to estimate secondary metabolites assist. Adaptation and defence against certain stressful situations.

1) Total Flavonoid Content (TFC): The total flavonoid content was ascertained using a colorimetric assay. One hundred microlitres of extracts were combined with four millilitres of distilled water. After that, 0.3 millilitres of sodium nitrite at 5% were added. After five minutes, 0.3 mL of 10% aluminium chloride was added. After six minutes, two millilitres of 1 M sodium hydroxide were added to the mixture. The mixture was properly mixed and immediately diluted with 3.3 mL of distilled water. Absorbance was measured at 510 nm and compared to a blank (Zhishen et al., 1999). Quercetin served as the standard for the calibration curve. Per gram of material, the extract's total flavonoid content was expressed as mg/ mL Quercetin equivalents.

2) Determination of Total Phenolic Content (TPC): The total phenolic content was ascertained using the Follin-Ciocalteu method. Gallic acid was used as standard. Different concentrations of plant extract are ingested, including 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL. Add 0.5 mL of the folin-ciocalteu reagent and 2 mL of 20% Na₂CO₃ to the test tube. The total concentration in the test tube will be three millilitres. After that, the test tubes were prepared for a minute in a water bath. After the test tube had cooled to room temperature; the absorbance was measured at 650 nm (Somawathi et al., 2015).

3) Determination of Total Tannin Content (TTC): To ascertain the total tannin content of the plant extract, the Folin-Ciocalteu method was utilised. After dissolving roughly 0.1 mL of plant extract in 7.5 mL of distilled water, 0.5 mL of Folin reagent was added to the mixture. 10 millilitres of distilled water were used to dilute the 1 millilitre of 35% Na₂CO₃; the mixture was shaken thoroughly and then left to rest for 30 minutes at room temperature. A series of Gallic acid reference standard solutions with concentration of 0.2, 0.4, 0.6, 0.8, 1.0 mg/mL were made. The absorbance of the standard and remaining solutions was measured at 725 nm in proportion to the blank using a UV/visible spectrophotometer (Lahare et al., 2021). Milligram of GAE per gram of extract was the unit of measured for the tannin content. Using quercetin as the standard, a calibration curve is produced.

2.4 Measurement of Antioxidant activity: DPPH radical scavenging activity was performed with few changes; the procedure is mentioned as follows. The anti-radical qualities of various substances are ascertained using the DPPH radical scavenging test. A stable free radical called DPPH was used to gauge the antioxidant capability (Germano et al., 2002). Using one millilitre of extract per test tube and concentrations ranging from 20 to 100 mg/mL, the standard and plant extract series were prepared in triplets. Since DPPH is light-sensitive, 4 mg of powdered DPPH was dissolved in 100 mL of methanol to create a new solution, which was then kept in a dark location. Three millilitres of DPPH solution were added after a series of extracts with varying concentrations had been prepared, and the mixture was then incubated for twenty to thirty minutes. The purple solution produced by adding DPPH will turn light yellowish after some time of incubation, demonstrating the extract's capacity to scavenge free radicals. A spectrophotometer was used to measure the absorbance of these incubated colour-altered extracts at 517 nm, and the ascorbic acid standard curve was used to calculate from the results. The observed absorbance was used to calculate the radical scavenging activity using the following equation derived from this formula (Patani et al., 2023):

$$\% \text{ of Inhibition} = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right] \times 100$$

When A control = absorbance of the DPPH solution without extract; A sample = absorbance of the DPPH solution with plant extract.

2.5 Physicochemical Analysis:

The physical and chemical properties of soil and water are analysed in physicochemical analysis to assess their quality, appropriateness for different uses, and contamination potential. Important metrics are calcium hardness, total hardness, dissolved oxygen, pH, and TDS.

2.5.1 Soil sample extraction: Soil samples were taken from the locations of the Pirana dump site and the fresh garden site. By digging the earth to a depth of 20 to 25 cm, soil samples were collected. Before being sent to the lab for additional examination, the soil samples were stored in clean, airtight plastic containers. Following that, soil sample were allowed to air dry in the lab for three days at room temperature. In order to remove visible leaf remnants and other waste materials, the soil was manually cleaned, mixed and sieved through a 2 mm sieve for additional analysis. A 1:5 w/v soil extract was created in order to analyse the soil. Twenty grams of the collected soil sample were combined with 100 millilitres of distilled water. After 12 hours at room temperature in a mechanical shaker, this solution was filtered through Whatman no 1 filter paper. Numerous metal ion concentrations were measured using the filtrate, sometimes referred to as soil extract, using tests for dissolved oxygen, total hardness, calcium hardness, pH, and TDS. A pH meter was used to measure the pH.

2.5.2 Water sample collection: Water samples were collected from the Pirana dump and fresh garden sites. Clean, sealed plastic bottles were used to store the water samples.

2.5.3 Total Hardness:

Procedure: Fill the flask with 10 millilitres of the sample. Put 2 to 3 drops of Erichrome Black-T indicator in the flask (become red in the presence of hardness). Fill the flask with 1-2 millilitres of ammonium buffer. After adding the EDTA to the burette, titrate it. The colour changed from red to blue (Indalkar, P., et al., 2023).

Formula of Total hardness: $\left[\frac{\text{Volume of EDTA (ml)} \times \text{Molarity of EDTA} \times 1000}{\text{volume of water/soil sample}} \right] \times 100$

2.5.4 Calcium Hardness:

Procedure: Fill a flask with 10 mL of the sample. Add two drops of Erichrome Black-T indicator and two millilitres of ammonia buffer solution. After adding the EDTA to the burette, titrate it. (Indalkar, P., et al., 2023). Formula of Ca^{+} hardness: $[\text{Volume of EDTA used (ml)} \times \text{Molarity of EDTA} / \text{Volume of sample (Water/soil)}] \times 100$

2.5.5 pH: Take the beaker and add some sample then measure the pH of water and soil sample with pH meter.

2.5.6 TDS: Take the beaker and add some sample then measure the TDS of water and soil sample with TDS Meter.

2.5.7 Dissolved Oxygen (Wrinkled test):

Procedure: Fill the BOD bottle with the sample. To the water sample, add 1 millilitre of manganese sulphate solution. This deoxidises manganese precipitate when it combines with oxygen. One millilitre of the alkaline-iodide solution is added, Add one millilitre of concentrated sulphuric acid. Titrate turns pale yellow when it comes in contact with sodium thiosulphate. A few drops of starch solution are then added, and the colour blue forms and then titrate till the colour blue vanishes (Bruckner, M., 2011).

DO = Volume of titration No of sodium thiosulphate \times 8/ Volume of Sample (water / soil)

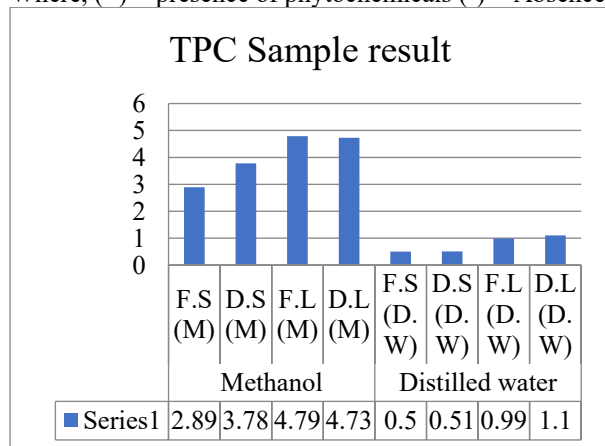
3. RESULTS AND DISCUSSION:

Qualitative and quantitative analysis of phytochemicals and antioxidant activity was performed to analyse the presence of certain phytochemical constituents in leaves and stem of *Cascabela thevetia* L. on Pirana dumpsite and fresh garden site.

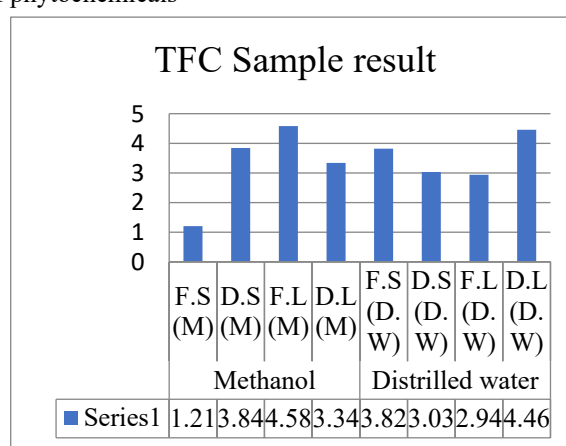
Table 1: Preliminary phytochemical analysis of stem and leaves in different solvent from fresh garden site and Pirana dump site

Phytochemical	Test	Extract type	Pirana dump site		Fresh garden site	
			Leaf	Stem	Leaf	Stem
Alkaloids	Dragondroff's test	Methanol	+	+	+	+
		Distilled water	+	+	+	+
Flavonoids	Lead Acetate test	Methanol	+	-	+	+
		Distilled Water	-	-	+	+
Phenol	Potassium Dichromate test	Methanol	+	+	+	-
		Distilled Water	+	+	+	+
Tannin	Lead Acetate test	Methanol	+	+	-	+
		Distilled Water	+	+	+	+
Protein	Millon's test	Methanol	+	+	+	+
		Distilled Water	+	-	+	+
Cardiac glycoside	Keller -Killani Test	Methanol	+	+	+	+
		Distilled Water	-	-	-	-
Saponin	Olive oil	Methanol	+	+	+	+
		Distilled Water	+	+	+	+

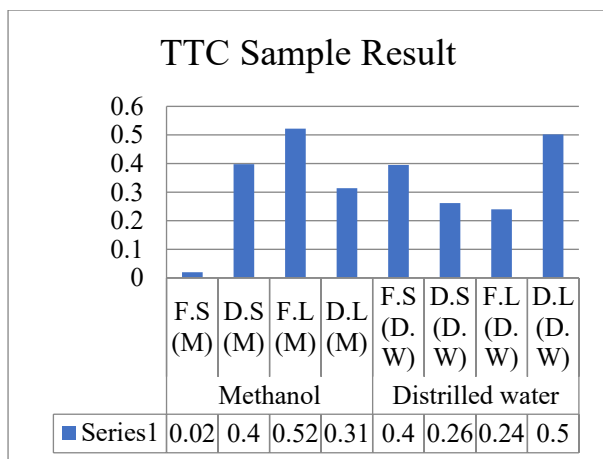
Where, (+) = presence of phytochemicals (-) = Absence of phytochemicals



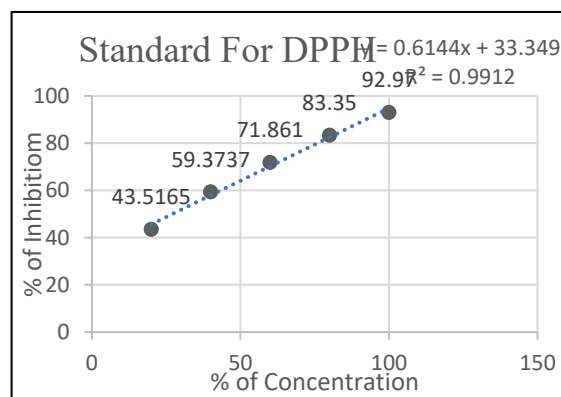
Graph 1: Comparison of Total Phenolic Content in Methanolic and distilled water extracts of stem and leaf from dump site and fresh site



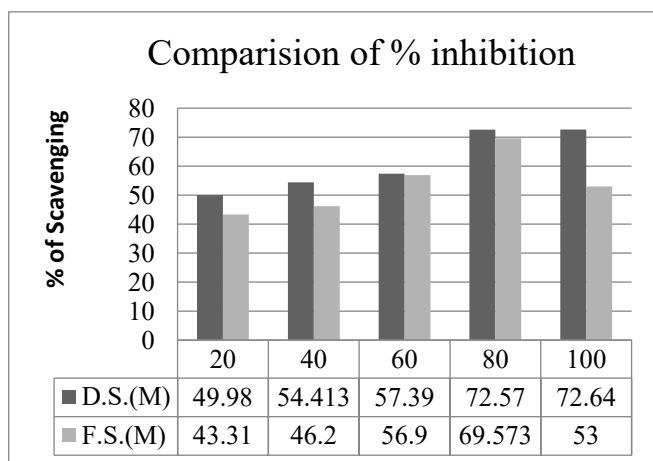
Graph 2: Comparison of Total Flavonoid Content in Methanolic and distilled water extracts of stem and leaf from Pirana dump site and fresh site.



Graph 3: Comparison of Total Tannin Content in Methanolic and distilled water Extracts of stem and leaf from dump site and fresh site



Graph 4: Standard Graph for DPPH



Graph 5: Comparison of % inhibition of the selected samples in the DPPH method

Where,

D.S (M) =Methanolic Extracts of stem from Pirana dump site
D.S (D.W.) = Distilled water Extracts of stem from Pirana dump site
F.S (M) = Methanolic Extracts of stem from Fresh Garden site.
D.S (M)= Methanolic Extracts of stem from Pirana dump site.
D.L (M) = Methanolic Extracts of Leaf from Pirana dump site
D.L (D.W) = Distilled water Extracts of leaf from Pirana dump site
F.L (M) = Methanolic Extracts of leaf from Fresh Garden site
F.L (D.W) = Distilled water Extracts of leaf from Pirana dump site

Physicochemical analysis of Soil and Water samples: The present study aims to compare the physico-chemical properties of water and soil samples collected from two different locations: Pirana dump Site and Fresh Garden site. The Unit of physicochemical parameter is mg/mL or ppm.

Table 2: Physicochemical analysis of soil / water

Parameter	Unit	Pirana dump site		Fresh garden site	
		Soil	Water	Soil	Water
pH	---	7.9	8.88 to 9.00	6.9 to 7.00	8.4
TDS (Total Dissolved Solids)	mg/mL	322	215	185	118
Calcium Hardness	mg/mL	0.46	0.76	0.36	0.7
Total Hardness	mg/mL	0.56	0.84	0.4	0.76
DO (Dissolved Oxygen)	mg/mL	0.0008	0.00126	0.00144	0.00128

4. DISCUSSION

Phytochemical research revealed the presence of secondary metabolites of biological importance. Since plants have been used for medicinal purposes since ancient times, it is crucial to screen plants for their primary active components. Therefore, it is essential to evaluate the phytochemical analysis of the *Cascabela thevetia* L. both quantitatively and qualitatively. Several authors have described the phytochemical analysis of *Cascabela thevetia* L., including Seetharaman et al., (2017). The present study discovered that cardiac glycoside was detected in the methanol extracts but lacking in the distilled water extracts. Cardiac glycosides are thought to be significant compounds that cause toxicity. The amount of total phenol, flavonoid and tannin in different plant extracts have been measured quantitatively. Gallic acid was used as a standard to measure the concentration of total phenol. Methanolic leaf extract from the fresh garden site and Pirana landfill site had the highest phenol content, with concentrations of 4.79 ± 0.485 mg/GAE and 4.73 ± 0.468 mg/GAE, respectively. The distilled water extract contained the least quantity of phenol.

Naturally occurring antioxidants called flavonoids support plant's defensive systems. The total flavonoid content was measured using standard quercetin, and the results showed that the methanolic stem extracts from the fresh garden site had lowest concentration (1.206 ± 0.102) mg/QAE, while the methanolic leaf and stem extracts from the fresh garden site and the Pirana dump site had the highest concentration (4.58 ± 0.024 mg/QAE and 3.84 ± 0.025 mg/QAE), respectively. Tannins can be found in a wide variety of plant parts in nature, including in the bark, leaves, seeds, and fruits. Tannin reduces oxidative stress and protects cells by scavenging free radicals. The total phenol content was estimated using the standard gallic acid. The plant *Cascabela thevetia* L. from the fresh garden site and Pirana landfill site exhibited the highest tannin content in both methanol and distilled water leaf extract, with concentrations of (0.552 ± 0.0639) mg/TAE and (0.502 ± 0.0414) mg/TAE, respectively.

Thus, there has been a lot of interest in employing natural antioxidants to protect against damage caused by free radicals. The DPPH test has been used extensively to evaluate antioxidant activity because it can process a large number of samples rapidly and is sensitive enough to detect active ingredients at low concentrations. When DPPH radicals encounter a proton-donating substance, such as an antioxidant, they are scavenged and their absorbance is reduced. Consequently, DPPH radicals were widely used to test the scavenging properties of different natural materials. In contrast to the methanolic extracts of plants from the fresh site, which displayed a lower IC₅₀ value (20.11), the methanolic extract from the Pirana dump site showed highest IC₅₀ value (24.13).

In addition to being essential for plant growth and development, water is needed in relatively large amounts and for various sorts of ecosystems, therefore its importance for both plants cannot be understated. To evaluate the quality, suitability for various applications, and potential for contamination, physicochemical analysis examines the physical and chemical characteristics of soil and water. Total hardness, dissolved oxygen, pH and TDS are all significant factors. The present study found that the physico-chemical parameters of soil and water samples collected from fresh and dump sites differed significantly: the soil and water samples from the dump site had higher values of pH (8.88 & 7.99), total hardness (0.84 & 0.56) mg/L, calcium hardness (0.76 & 0.46) mg/L, and TDS (215 & 322) than the fresh site samples, which had lower values of pH (8.04 & 6.09), total hardness (0.76 & 0.4) mg/mL, calcium hardness (0.7 & 0.36) mg/mL, and TDS (188 & 185) ppm.

5. CONCLUSION:

The study's main goal is to identify the numerous metabolites, their beneficial groups, and the antioxidant activity of plant extracts from both contaminated and fresh garden areas. The presence of secondary metabolites indicated potential therapeutic uses. It shows strong phenolic and flavonoid content from both sides in methanolic extracts. *Cascabela thevetia* L. stem extracts show DPPH scavenging action. Flavonoids have antioxidant qualities like ROS and RNS scavenging because of the functional groups that are best suited to finish the job. Because flavonoids can scavenge free radicals and stop enzymes like glutathione S-transferase, mitochondrial succinoxidase, microsomal monooxygenase, and NADH oxidase from producing ROS, they have antioxidant qualities. According to the physico-chemical analysis of the soil and water from the location where the *Cascabela thevetia* L. plant sample was taken, the pH, total dissolved solids, and total hardness value were higher in contaminated soil and lower in fresh soil, but the dissolve oxygen value was higher in the fresh garden site. As contaminated soil has a higher availability of heavy metals, the fresh site exhibited higher levels of dissolved

oxygen than the contaminated site. The morphological characteristics of *Cascabela thevetia* L. under metal stress conditions along Chhipakuva main road are in line with this downward tendency. Because they hinder the plant's capacity to absorb nutrients, the metals restrict the uptake of nutrients like K, Ca, and Fe that are essential for growth and development.

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7. REFERENCES:

- Angulo-Bejarano, P. I., Puente-Rivera, J., & Cruz-Ortega, R. (2021). Metal and metalloid toxicity in plants: An overview on molecular aspects. *Plants*, 10(4), 635.
- Atteh, J. O., Ibiyemi, S. A., & Ojo, A. O. (1995). Response of broilers to dietary levels of *Thevetia* cake. *The Journal of Agricultural Science*, 125(2), 307-310.
- Balandrin, B., Mert, T., & Tansel, H. (2006). Antimicrobial and cytotoxic activities of *Caratonia siliqua* L. extracts. *Turk. J. Biol*, 26, 197-200.
- Basumatary, B., Nath, B., Kalita, P., Das, B., & Basumatary, S. (2020). Yellow oleander (*Thevetia peruviana*) seed as a potential bioresource for industrial applications. *Mini-Reviews in Organic Chemistry*, 17(7), 855-871.
- Bhoyar, S. (2021). Physicochemical analysis and antibacterial activity of *cascabela thevetia* (l) lippold seeds. *World Journal of Pharmaceutical Research*, 10(6), 905-912.
- Dixit, A. N. U. P. M. A., Singh, H. E. M. L. A. T. A., Sharma, R. A., & Sharma, A. R. C. H. A. N. A. (2015). Estimation of antioxidant and antibacterial activity of crude extracts of *Thevetia peruviana* (Pers.) K. Schum. *Int J Pharm Pharm Sci*, 7(2), 55-9.
- Germano, M. P., De Pasquale, R., D'angelo, V., Catania, S., Silvari, V., & Costa, C. (2002). Evaluation of extracts and isolated fraction from *Capparis spinosa* L. buds as an antioxidant source. *Journal of agricultural and food chemistry*, 50(5), 1168-1171.
- GHORBANLI, M. L., BAKHSHI, K. G. R., & Bakand, Z. (2008). Air pollution effects on fresh and dry weight, amount of proline, number of stomata, trichome and epi-dermal cells in *Nerium oleander* and *Robinia pseudoacacia* in Tehran city.
- Lahare, R. P., Yadav, H. S., Bisen, Y. K., & Dashahre, A. K. (2021). Estimation of total phenol, flavonoid, tannin and alkaloid content in different extracts of *Catharanthus roseus* from Durg district, Chhattisgarh, India. *Scholars Bulletin*, 7(1), 1-6.
- Oderinde, R. A., & Oladimeji, G. R. (1990). Oil characteristics of *Thevetia peruviana* (yellow oleander) and *Plumeria alba* (white frangipani).
- Patani, A., Balram, D., Yadav, V. K., Lian, K. Y., Patel, A., & Sahoo, D. K. (2023). Harnessing the power of nutritional antioxidants against adrenal hormone imbalance-associated oxidative stress. *Frontiers in Endocrinology*, 14, 1271521.
- Procházková, D., Boušová, I., & Wilhelmová, N. (2011). Antioxidant and prooxidant properties of flavonoids. *Fitoterapia*, 82(4), 513-523.
- Rajhans, S., Pandya, J., Mankad, A. U., & Pandya, H. A. (2019). *Thevetia peruviana*-A Review on Its Characteristic Features and Toxic Constituents. *International Journal of Scientific Research and Review*, 8(3), 1391-1395.
- Riar, J. K., Bhanot, R., & Hundal, S. S. (2021). Assessment of heavy metals in samples of soil, water, vegetables, and vital organs of rat (*Bandicota bengalensis*) collected from adjoining areas of polluted water body. *Water, Air, & Soil Pollution*, 232(7), 251.
- Seetharaman, S., Indra, V., Sundar, N., & Geetha, S. (2017). Phytochemical profiling, antibacterial activity and antioxidant potential of *Cascabela thevetia* (L.) whole plant extracts. *Journal of Pharmacognosy and Phytochemistry*, 6(3), 93-97.
- Somawathi, K. M., Rizliya, V., Wijesinghe, D. G. N. G., & Madhujith, W. M. T. (2015). Antioxidant activity and total phenolic content of different skin coloured brinjal (*Solanum melongena*). *Tropical Agricultural Research*, 26(1).
- Soni, P., Mahendru, N., Dimri, R., Hamdy, R., Jena, N., & Kumar, S. (2024). Qualitative phytochemical analysis of *Cascabela thevetia* fruits. *Biological Archives*, 1.
- Yadav, P., Kumar, A., Mahour, K., & Vihan, V. S. (2010). Phytochemical analysis of some indigenous plants potent against endoparasite. *Journal of Advanced Laboratory Research in Biology*, 1(1), 56-59.
- Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food chemistry*, 64(4), 555-559.