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(RESEARCH ARTICLE)

PHYTOREMEDIATION POTENTIAL AND PHARMACOLOGICAL EFFICACY OF *TRIDAX PROCUMBENS* L.: A COMPARATIVE ANALYSIS OF POLLUTED AND UNPOLLUTED SAMPLES

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ABSTRACT

Medicinal plants serve as a rich reservoir of bioactive compounds with therapeutic potential, yet environmental pollution may influence their phytochemical composition and pharmacological efficacy. The present study evaluated and compared the phytochemical composition, antioxidant, anti-diabetic, antibacterial properties, and heavy metal accumulation in *Tridax procumbens* L. collected from polluted (roadside, Veli, Trivandrum) and unpolluted (Attingal) environments. Methanolic extracts were prepared using the cold maceration method and subjected to preliminary phytochemical screening, quantitative analysis of total phenolic content (TPC) and total flavonoid content (TFC), DPPH radical scavenging assay, α-amylase inhibition assay, and antibacterial testing against *Escherichia coli* (MTCC 56) and *Staphylococcus aureus* (MTCC 9760) using the agar well diffusion method. Heavy metal concentrations were determined using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Preliminary screening revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, glycosides, quinones, and starch in both samples, with saponins absent. The unpolluted sample exhibited higher TPC (28.48 mg/ml) compared to the polluted sample (24.4 mg/ml), while the polluted sample showed slightly higher TFC (7.70 mg/ml) than the unpolluted sample (7.03 mg/ml). The unpolluted extract demonstrated superior antioxidant activity (IC_{50} = 171.1 µg/ml) compared to the polluted extract (IC_{50} = 621.1 µg/ml) and greater anti-diabetic potential (IC_{50} = 250 µg/ml vs. 350 µg/ml). Antibacterial activity was more pronounced against *S. aureus* than *E. coli*, with the unpolluted sample showing a larger zone of inhibition against *E. coli* (20 mm) compared to the polluted sample (10 mm). ICP-MS analysis of the polluted sample revealed Chromium (~39.5 µg/g), Zinc (~17.2 µg/g), Nickel (~6.7 µg/g), Lead (~4.5 µg/g), and Cadmium (~0.48 µg/g), with Cadmium slightly exceeding WHO permissible limits for herbal raw materials. The findings confirm the phytoremediation potential of *T. procumbens* while emphasizing the importance of sourcing medicinal plants from uncontaminated environments to ensure safety and therapeutic efficacy. The study recommends targeted cultivation practices and regular quality control measures for the safe utilization of this medicinal plant.

Keywords: *Tridax procumbens*, Phytochemicals, Antioxidant, Anti-diabetic, Antibacterial, Heavy metals, Phytoremediation, ICP-MS

1 INTRODUCTION

Since the beginning of human existence, plants have been utilized for food and medicine, with early humans distinguishing medicinal plants for their healing properties. India, blessed with abundant medicinal flora, is termed the "medicinal garden of the world," with clinical applications documented in ancient texts (Shakya, 2016). Medicinal plants continue to play a dominant role in healthcare systems, particularly in developing countries, offering bioactive compounds categorized as small biomolecules, plant-derived biologics, and phytopharmaceuticals (Noohi et al., 2022).

Tridax procumbens L. (Asteraceae), commonly known as 'coat buttons,' is a resilient herbaceous plant native to Tropical America but widespread in tropical and subtropical regions. It grows up to 1m tall with creeping stems (20–75 cm), opposite pinnate leaves, capitulum inflorescences with white ray and yellow disc florets, and achene fruits (Beck et al., 2018). The plant produces numerous secondary metabolites with medicinal value and exhibits phytoremediation potential, accumulating lead and cadmium in roots, indicating phytostabilization mechanisms (Sharma and Rani, 2021). Heavy metals—cadmium, lead, mercury, arsenic, and thallium—are toxic at low concentrations, with anthropogenic activities increasing their environmental release, causing bioaccumulation and serious health issues (Richard and Gupta, 2024; Omar et al., 2023).

Traditionally, *T. procumbens* serves as an anticoagulant, antimicrobial, insect repellent, and wound-healing agent, used for treating boils, ulcers, skin diseases, liver disorders, gastritis, and diabetes (Ghosh et al., 2019). Phytochemicals include alkaloids, carotenoids, tannins, saponins, flavonoids (quercetin, luteolin, kaempferol), sterols, phenolic acids, and minerals (Ali et al., 2001). Pharmacologically, it exhibits antioxidant, antibacterial, anticancer, antidiabetic, hepatoprotective, antifungal, anti-leishmanial, analgesic, anti-inflammatory, anti-ulcer, and wound-healing activities (Prakash and Rao, 2019; Gupta and Roy, 2020). Methanolic extracts show antifungal activity against skin pathogens, while acetone extracts demonstrate potent anticancer activity (Vishnu Priya et al., 2011). Leaf extracts significantly affect diabetes-related metabolic disturbances (Subramanian et al., 2011) and protect against mucosal lesions (Rajalakshmi and Aruna, 2020). Antioxidant properties are attributed to flavonoids, phenolic acids, and carotenoids (Babu and Subramoniam, 2019).

Phytoremediation is an eco-friendly, cost-effective method using plants to clean contaminated soil, water, and air. *T. procumbens* is ideal due to its robust root system, rapid growth, and stress tolerance. In this present study, *T. procumbens* latex from polluted and unpolluted areas was used for phytochemical analysis to validate and standardize its effectiveness in treating metabolic, oxidative, and infectious conditions.

2 MATERIALS AND METHODS

2.1 Collection of Plant Material

T. procumbens plants were collected from two distinct sites: a polluted roadside area at Veli, Trivandrum, and an unpolluted site at Attingal. The selected sites represented contrasting environmental conditions to evaluate the influence of pollution on the plant's phytochemical and pharmacological properties.

2.2 Preparation of Plant Extract

Collected plant materials were washed, shade-dried, cut into small pieces, and ground into coarse powder. Methanol extraction was performed using the Soxhlet method with a sample-to-solvent ratio of 10:100 (g:ml) at room temperature for 24 hours. The extract was filtered through Whatman No. 1 filter paper, dried, and stored in Eppendorf tubes for further analysis (Azwanida, 2015).

2.3 Preliminary Phytochemical Analysis

Methanolic extracts were screened for alkaloids, flavonoids, glycosides, phenolics, quinones, saponins, starch, steroids, tannins, and terpenoids using standard methods (Harborne, 1998; Sofowora, 1993).

2.4 Quantitative Phytochemical Analysis

Total Phenolic Content (TPC): Determined using the Folin-Ciocalteu reagent method (Singleton and Rossi, 1965). An aliquot of 0.1 ml extract was treated with 0.5 ml Folin-Ciocalteu's reagent, mixed, and after 5 minutes, 0.4 ml of 7.5% sodium carbonate was added. The mixture was incubated at 45°C for 15 minutes, and absorbance was read at 765 nm. Gallic acid was used as the standard, and TPC was expressed as mg gallic acid equivalents (GAE)/g dry weight.

Total Flavonoid Content (TFC): Evaluated by the aluminium chloride colorimetric method (Woisky and Salatino, 1998). To 0.1 ml extract, 0.3 ml methanol, 0.02 ml of 10% $AlCl_3$, 0.02 ml of 1M potassium acetate, and 0.56 ml distilled water

were added. After 30 minutes at room temperature, absorbance was measured at 415 nm. Quercetin was used as the standard, and TFC was expressed as mg quercetin equivalents (QE)/g dry weight.

2.5 Antioxidant Assays

DPPH Free Radical Scavenging Assay: The radical scavenging property was measured according to Mensor et al. (2001) with minor modifications. A methanolic solution of 1 ml DPPH (0.1 mM) was added to 1 ml of different concentrations of the sample and allowed to react at room temperature for 30 minutes in the dark. Absorbance was measured at 517 nm. Methanol served as the blank, DPPH in methanol without sample was the control, and ascorbic acid was used as the reference standard. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capacity to scavenge the DPPH radical was calculated using the equation:

% radical scavenging activity = [(Abs Control – Abs Sample)/Abs Control] × 100.

A graph was plotted with percentage inhibition against concentration, and IC_{50} values (concentration required to scavenge 50% of free radicals) were calculated from the regression equation. A smaller IC_{50} indicated higher antioxidant activity of the sample (Mensor et al., 2001).

FRAP (Ferric Reducing Antioxidant Power): Reducing power was evaluated according to Gow-Chin and Pin-Der (1994). Various concentrations of sample (500 µl) were mixed with 1.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes, followed by the addition of 5 ml of 10% trichloroacetic acid, which was then centrifuged at 6000 rpm for 5 minutes at 4°C. The upper layer of the solution (1.5 ml) was mixed with 1.5 ml of distilled water and 300 µl of ferric chloride (0.1%), and absorbance was read at 700 nm. Ascorbic acid was used as standard. Increased absorbance of the reaction mixture indicated increased reducing power. Concentration at 0.5 absorbance was marked as the reference to determine the scavenging potential of each sample (Uddin et al., 2014).

2.6 Anti-diabetic Activity

α-Amylase Inhibition Assay: The screening of extracts for α-amylase inhibition was carried out according to the method described by Ononamabu et al. (2020) with slight modifications. To 250 µl of each extract in a test tube (62.5, 125, 250, 500, 1000 µg/ml) in triplicates, the following were added sequentially: 250 µl of phosphate buffer (200 mM, pH 6.9, containing 6 mM sodium chloride), phosphate-buffered α-amylase (250 µl, 0.05 mg/ml), and starch (250 µl, 1% w/v). The reaction mixture was incubated for 15 minutes at 37°C. One molar HCl (20 µl) was added to stop the enzymatic reaction, followed by the addition of 100 µl of iodine reagent (5 mM I_2 and 5 mM KI). The colour change was noted, and absorbance was read at 625 nm. The control reaction representing 100% enzyme activity contained no test extract. Acarbose was used as the reference standard. Inhibition of enzyme activity was calculated as:

Inhibition (%) = (C − S)/C × 100, where S is the absorbance of the sample and C is the absorbance of control (Ononamabu et al., 2020).

α-Glucosidase Inhibition Assay: The effect of the sample on α-glucosidase activity was determined according to the method described by Shai et al. (2011) with slight modification. 400 µl of α-glucosidase (0.067 U/ml) was preincubated with different concentrations of the sample for 30 minutes. Then 200 µl of 3.0 mM pNPG (p-nitrophenyl-β-D-glucopyranoside) dissolved in 0.1 M sodium phosphate buffer (pH 6.9) was added to start the reaction. The reaction mixture was incubated at 37°C for 30 minutes and stopped by adding 2 ml of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow-coloured para-nitrophenol released from pNPG at 400 nm. The results were expressed as percentage inhibition. Acarbose (1 mg/mL stock) was used as standard.

Inhibitory activity (%) = (C − T)/C × 100,

where T is the absorbance in the presence of test substance and C is the absorbance of control (Shai et al., 2011).

2.7 Determination of Antibacterial Activity

Bacterial Strains: Escherichia coli (MTCC 56) and *Staphylococcus aureus* (MTCC 9760) were obtained from the Department of Biotechnology, University of Kerala (Tenaillon et al., 2010; Lowy, 1998).

Antibacterial Assay: Agar well diffusion method (Alzoreky and Nakahara, 2003) was used. Plant extract (250 mg/ml) was transferred to wells (6 mm diameter) on nutrient agar plates swabbed with bacterial broth. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured.

2.8 Determination of Heavy Metal Concentration using ICP-MS

Powdered samples (0.1 g) were analyzed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) in Kinetic Energy Discrimination (KED) mode. Elements analyzed included Chromium (^{50}Cr), Nickel (^{62}Ni), Zinc (^{67}Zn), Cadmium (^{112}Cd), and Lead (^{208}Pb). External calibration was performed using standard solutions (1–500 ppb), and concentrations were expressed in parts per billion (ppb) (USEPA, 2007).

3 RESULTS

3.1 Preparation of Plant Extract

Dried plant materials of *T. procumbens* (polluted and unpolluted) were extracted using the cold maceration method with methanol as the solvent. Precisely 25 grams of dried plant material was combined with 300 ml of methanol, sealed, and left at room temperature for 48 hours. After incubation, the extract was filtered, and the methanol was evaporated to obtain the crude extract, which was stored in Eppendorf tubes for further analysis.

3.2 Preliminary Phytochemical Screening

The phytochemical constituents present in *T. procumbens* extracts are summarized in Table 1. In the polluted sample, alkaloids, flavonoids, and phenolic compounds were present at high levels, while tannins and terpenoids were detected at moderate levels. Glycosides, quinones, and starch were present in low quantities. In the unpolluted sample, high levels of flavonoids, alkaloids, phenolic compounds, tannins, and terpenoids were observed, whereas glycosides, starch, and quinones were present at moderate levels. Saponins were absent in both samples.

Table 1: Phytochemical analysis of *T. procumbens* extracts

Phytoconstituents	Polluted Sample	Unpolluted Sample
Flavonoids	+++	+++
Alkaloids	+++	+++
Glycosides	+	++
Phenolic compounds	+++	+++
Tannins	++	+++
Starch	+	++
Terpenoids	++	+++
Quinones	+	++
Saponins	–	–

Note: +++ = High, ++ = Moderate, + = Low, – = Absent

3.3 Quantitative Phytochemical Analysis

Total Phenolic Content (TPC): The total phenolic content was assessed using the Folin-Ciocalteu reagent with gallic acid as the standard. The calibration curve for gallic acid demonstrated linearity within the concentration range of 100–1000 µg/ml, with a correlation coefficient (R^2) of 0.9961 (Table 2). Based on the calibration curve, the phenolic content was estimated to be 24.4 mg/ml in the polluted sample and 28.48 mg/ml in the unpolluted sample.

Table 2: Absorbance of the standard Gallic acid

Concentration (mg/ml)	Absorbance (765 nm)
0.2	0.1981
0.4	0.4893
0.6	0.7013
0.8	1.0277
1.0	1.2423

Total Flavonoid Content (TFC): The total flavonoid content was determined using the aluminium chloride colorimetric method with quercetin as the standard. The calibration curve for quercetin exhibited linearity within the concentration range of 100–1000 µg/ml, with a correlation coefficient (R^2) of 0.9876 (Table 3). Based on the calibration curve, the flavonoid content was calculated to be 7.70 mg/ml in the polluted sample and 7.03 mg/ml in the unpolluted sample.

Table 3: Absorbance of the standard Quercetin

Concentration (mg/ml)	Absorbance (415 nm)
0.2	0.397
0.4	0.793
0.6	1.195
0.8	1.466
1.0	1.716

3.4 Antioxidant Activity (DPPH Assay)

Table 4: DPPH activity for Ascorbic acid standard

Concentration (µg/ml)	Absorbance (517 nm)	Percentage (%) of inhibition
6.25	0.0873	17.25
12.5	0.0774	26.64
25	0.0663	37.16
50	0.0542	48.63
100	0.0386	63.41

Table 5: DPPH scavenging activity of polluted sample

Concentration (µg/ml)	Absorbance (517 nm)	Percentage (%) of inhibition
62.5	0.0799	24.27
125	0.0703	33.36
250	0.0678	35.73
500	0.0557	47.20
1000	0.0452	57.16

Table 6: DPPH scavenging activity of unpolluted sample

Concentration (µg/ml)	Absorbance (517 nm)	Percentage (%) of inhibition
62.5	0.0753	28.63
125	0.0612	41.99
187.5	0.0534	49.38
250	0.0447	57.63
312.5	0.0351	66.73

The antioxidant activity of the plant extracts was assessed using the DPPH radical scavenging assay. The IC_{50} values for *T. procumbens* extracts were found to be 621.1 µg/ml for the polluted sample and 171.1 µg/ml for the unpolluted sample, compared to 49.85 µg/ml for the ascorbic acid standard (Tables 4, 5, and 6). The polluted sample demonstrated

24.27% inhibition at 62.5 µg/ml, increasing to 57.16% at 1000 µg/ml. In contrast, the unpolluted sample showed 28.63% inhibition at 62.5 µg/ml, which increased to 66.73% at 312.5 µg/ml. The lower IC_{50} value of the unpolluted sample indicates superior antioxidant activity compared to the polluted sample.

3.5 Anti-diabetic Activity (α-Amylase Inhibition Assay)

The anti-diabetic potential of *T. procumbens* was assessed using the alpha-amylase inhibition assay. Acarbose was used as the reference standard with an IC_{50} value of 192 µg/ml (Table 7). The IC_{50} values for *T. procumbens* extracts were 350 µg/ml for the polluted sample and 250 µg/ml for the unpolluted sample. At a concentration of 200 µg/ml, the polluted extract showed 45.9% inhibition, which increased to 57.8% at 400 µg/ml. Across all tested concentrations, the unpolluted extract consistently exhibited higher percentage inhibition, indicating superior anti-diabetic potential.

Table 7: Alpha-amylase activity of Acarbose Standard

Concentration (µg/ml)	Absorbance (625 nm)	Percentage (%) of inhibition
200	0.48	52.00
400	0.35	65.00
600	0.26	74.00
800	0.18	82.00
1000	0.12	88.00

3.6 Antibacterial Activity

Table 8: Antibacterial activity of *T. procumbens* extract

Sample	Zone of inhibition (mm) *S. aureus*	Zone of inhibition (mm) *E. coli*
Positive control (Streptomycin)	35	30
Negative control	NA	NA
Polluted sample	15	10
Unpolluted sample	16	20

NA = No activity

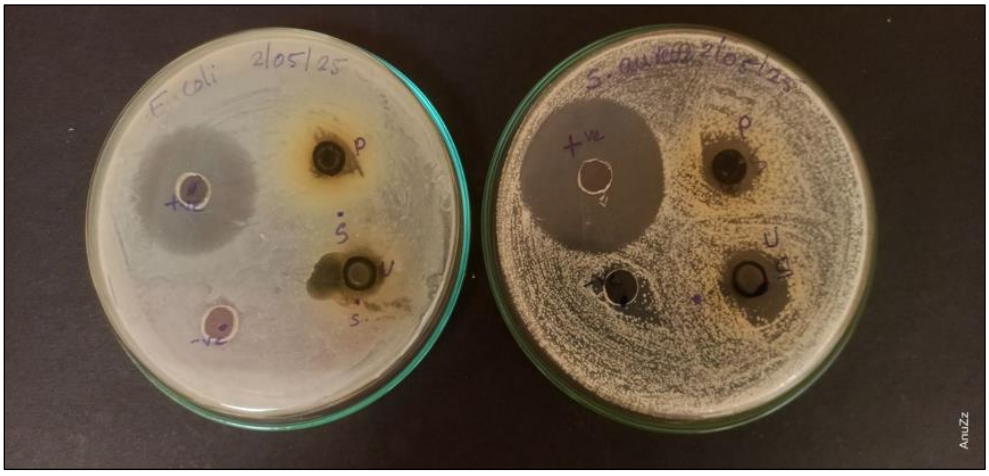


Figure 1: Zones of inhibition revealed by *T. procumbens* against *E. coli S.aureus*

P-Polluted sample, UP-Unpolluted sample, +ve control- streptomycin

The antibacterial activity of *T. procumbens* was assessed using the agar well diffusion method against *Staphylococcus aureus* (MTCC 9760) and *Escherichia coli* (MTCC 56). Both polluted and unpolluted extracts were examined and compared with streptomycin as the positive control and sterile water as the negative control (Table 8, Fig 1). The findings indicate that *T. procumbens* extracts demonstrated greater inhibitory activity against *S. aureus* compared to *E. coli*. For *S. aureus*, the positive control showed a zone of inhibition of 35 mm, while the polluted and unpolluted samples

showed 15 mm and 16 mm, respectively. For *E. coli*, the positive control showed 30 mm, while the polluted and unpolluted samples showed 10 mm and 20 mm, respectively.

3.7 Heavy Metal Analysis using ICP-MS

The ICP-MS analysis revealed the presence of Chromium (~39.5 µg/g), Nickel (~6.7 µg/g), Zinc (~17.2 µg/g), Cadmium (~0.48 µg/g), and Lead (~4.5 µg/g) in the *T. procumbens* powder sample collected from the polluted area (Fig 2). Chromium showed the highest concentration, warranting speciation to determine if the toxic Cr (VI) form is present. Nickel and Zinc levels were within acceptable ranges, with Zinc contributing as a beneficial micronutrient. Cadmium slightly exceeded WHO limits for herbal raw materials, while Lead was within limits but close to the threshold for finished products.



CLIF, UNIVERSITY OF KERALA
ICP-MS (iCAP Q) Analysis

Date: 06-05-2025

Name: Biovent Innovations Pvt.Ltd

No of Samples: 1

Type of Sample: Powder

Sample analysis date: 06-05-2025

Sample weight/volume: 0.1g

Instrument: iCAP Q

Elements analyzed:

50Cr (KED)	62Ni (KED)	67Zn (KED)	112Cd (KED)	208Pb (KED)
Y (ppb)	Y (ppb)	Y (ppb)	Y (ppb)	Y (ppb)

SampleList	SampleList	ExtCal.Average	ExtCal.Average	ExtCal.Average	ExtCal.Average	ExtCal.Average
SampleId	SampleLine	0	0	0	0	0
		50Cr (KED)	**62Ni (KED)**	**67Zn (KED)**	**112Cd (KED)**	**208Pb (KED)**
		Y (ppb)	**Y (ppb)**	**Y (ppb)**	**Y (ppb)**	**Y (ppb)**
1	STD 1 (1ppb)	1.366682225	1.300395294	1.754978294	0.90193261	0.831925907
2	STD 2 (5ppb)	5.902385358	5.88678184	6.131401598	4.387970181	4.060100124
3	STD 3 (10ppb)	11.28560462	11.60134538	12.06400195	8.593549376	8.053495307
4	STD 4 (50ppb)	55.72089967	55.64799811	52.43350274	42.51682013	52.67080374
5	STD 5 (100ppb)	106.4582342	108.3974859	105.7772875	83.79840016	103.5580374
6	STD 6 (250ppb)	259.6514225	261.0960786	261.8769872	262.3393124	257.850096
7	STD 7 (500ppb)	493.2750826	492.1661682	492.5865946	497.8534272	495.1449294
8	**BVOSCF 160**	**3948.993646**	**668.5527035**	**1721.094282**	**47.67998036**	**454.2608374**

Figure 2: ICP-MS Analysis of *Tridax procumbens* collected from polluted area

The elevated levels of heavy metals in *T. procumbens* suggest its strong capability to absorb and tolerate such metals, pointing to its phytoremediation potential. The plant appears capable of accumulating metals like Chromium and Zinc in its tissues, tolerating toxic metals like Cadmium and Lead possibly via detoxification mechanisms, and being utilized in the remediation of contaminated environments such as industrial or mining-affected soils. While this trait is beneficial for environmental purposes, it also emphasizes the importance of cultivating the plant in uncontaminated environments if it is to be used for medicinal applications. *T. procumbens* shows both medicinal potential and phytoremediation capacity. Its ability to accumulate heavy metals necessitates careful sourcing for health applications while also presenting opportunities for environmental cleanup.

4 DISCUSSION

The present study evaluated the phytochemical composition, antioxidant, anti-diabetic, antibacterial properties, and heavy metal accumulation in *Tridax procumbens* collected from polluted and unpolluted environments. The findings revealed notable differences between the two samples, with the unpolluted sample generally exhibiting superior phytochemical and pharmacological properties, while the polluted sample demonstrated significant heavy metal accumulation with implications for both phytoremediation and medicinal safety.

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, glycosides, quinones, and starch in both samples, with saponins absent. The unpolluted sample showed higher levels of tannins, terpenoids, glycosides, starch, and quinones compared to the polluted sample. These findings are consistent with previous reports documenting the rich phytochemical profile of *T. procumbens*, which includes flavonoids, alkaloids, saponins, tannins, phenolic acids, terpenoids, and sterols (Harpreet et al., 2026). The presence of these bioactive compounds supports the traditional medicinal uses of the plant, including wound healing, anti-inflammatory, and antimicrobial applications (Dasari et al., 2024). A comprehensive review by Harpreet et al. (2026) confirmed the presence of alkaloids, tannins, flavonoids, phenols, and amino acids in *T. procumbens*, which are associated with various biological activities including wound healing, antioxidant, and antibacterial effects.

The quantitative analysis revealed that the unpolluted sample had higher total phenolic content (28.48 mg/ml) compared to the polluted sample (24.4 mg/ml). However, the polluted sample exhibited slightly higher total flavonoid content (7.70 mg/ml) compared to the unpolluted sample (7.03 mg/ml). Phenolic compounds and flavonoids are well-documented for their antioxidant properties, and variations in their concentrations may be attributed to environmental stress factors. Plants grown under polluted conditions often produce elevated levels of secondary metabolites as a defense mechanism against oxidative stress caused by heavy metals (Asiminicesei et al., 2024). This may explain the slightly higher flavonoid content in the polluted sample despite its lower phenolic content.

The DPPH radical scavenging assay demonstrated that the unpolluted sample possessed significantly stronger antioxidant activity, with an IC_{50} value of 171.1 µg/ml compared to 621.1 µg/ml for the polluted sample. The unpolluted extract achieved 66.73% inhibition at 312.5 µg/ml, while the polluted extract required 1000 µg/ml to achieve only 57.16% inhibition. These results align with previous studies reporting that *T. procumbens* extracts exhibit concentration-dependent DPPH scavenging activity. Sagheer et al. (2024) reported that extracts and fractions of *T. procumbens* scavenge free radicals with IC_{50} values ranging from 14.70 to 93.80 µg/ml. A systematic review by Syed et al. (2021) documented that ethanol extracts of *T. procumbens* showed good antioxidant activity (61.52%–82.5%) at 200 µg/mL, attributed to the presence of phenolic compounds. The superior antioxidant activity of the unpolluted sample correlates with its higher phenolic content, as phenolic compounds are known to donate electrons to neutralize free radicals (Nair et al., 2016).

The reduced antioxidant capacity of the polluted sample may be attributed to the presence of heavy metals that could interfere with antioxidant mechanisms or induce oxidative stress that overwhelms the plant's defensive systems. Heavy metals such as cadmium and lead are known to generate reactive oxygen species and disrupt cellular redox balance (Asiminicesei et al., 2024). This suggests that while *T. procumbens* can tolerate and accumulate heavy metals, its antioxidant defense system may be compromised under such conditions.

The α-amylase inhibition assay revealed that the unpolluted sample exhibited superior anti-diabetic potential with an IC_{50} value of 250 µg/ml compared to 350 µg/ml for the polluted sample. Acarbose, the reference standard, showed an IC_{50} of 192 µg/ml. The unpolluted extract consistently demonstrated higher percentage inhibition across all tested concentrations. These findings are consistent with previous research demonstrating that *T. procumbens* extracts possess significant α-amylase inhibitory activity. Nair et al. (2016) reported that hydro-methanolic extract of *T. procumbens* has an IC_{50} value of 19.07 µg/ml for α-amylase inhibition, with the authors noting that the extract acts as a competitive inhibitor by binding to the active sites of the enzyme, thereby reducing its capacity to bind with starch substrate (Nair et al., 2016). The superior activity of the unpolluted sample may be attributed to its richer phytochemical profile, particularly the higher levels of tannins and terpenoids, which have been implicated in enzyme inhibition.

The antibacterial assay demonstrated that *T. procumbens* extracts exhibited greater inhibitory activity against *Staphylococcus aureus* (Gram-positive) compared to *Escherichia coli* (Gram-negative). The unpolluted sample showed a larger zone of inhibition against *E. coli* (20 mm) compared to the polluted sample (10 mm), while both samples showed comparable activity against *S. aureus* (15–16 mm). These results are consistent with previous studies reporting that *T. procumbens* exhibits antimicrobial activity against both Gram-positive and Gram-negative bacteria (Harpreet et al., 2026). A study by Singh et al. (2024) demonstrated that *T. procumbens* extracts exhibited antimicrobial activity against *Enterobacter aerogenes*, *Escherichia coli*, *Staphylococcus aureus*, and other bacterial strains, with phytochemical analysis confirming the presence of flavonoids, alkaloids, glycosides, saponins, terpenoids, and tannins. The differential

susceptibility of Gram-positive and Gram-negative bacteria can be attributed to structural differences in their cell walls. Gram-negative bacteria possess an outer membrane that acts as a permeability barrier, making them less susceptible to many antimicrobial agents (Dasari et al., 2024). The enhanced activity of the unpolluted sample against *E. coli* may reflect its superior phytochemical profile, particularly the higher content of phenolic compounds and flavonoids, which are known to disrupt bacterial cell membranes and inhibit nucleic acid synthesis.

The ICP-MS analysis revealed significant accumulation of heavy metals in the polluted sample, with Chromium (~39.5 µg/g) showing the highest concentration, followed by Zinc (~17.2 µg/g), Nickel (~6.7 µg/g), Lead (~4.5 µg/g), and Cadmium (~0.48 µg/g). These findings are consistent with the known phytoremediation capabilities of *T. procumbens*. A study published in ACS Omega (2025) reported that *T. procumbens* accumulated mercury in its tissues with bioconcentration factors exceeding 1, confirming its potential for phytoremediation of contaminated soils (Oliveira et al., 2025). The authors noted that *T. procumbens* can be considered a metallophyte due to its high accumulation and tolerance to mercury, contributing to the bioindication and phytoremediation of contaminated soils (Oliveira et al., 2025). The elevated Chromium levels warrant particular attention, as this metal can exist in different oxidation states with varying toxicity. While Cr(III) is an essential micronutrient, Cr(VI) is highly toxic and carcinogenic.

The Cadmium concentration (0.48 µg/g) slightly exceeded WHO limits for herbal raw materials, which recommend not more than 0.3 ppm for cadmium in crude herbal drugs (WHO, 2024). Lead was within limits but close to the threshold for finished products. According to WHO guidelines, the upper limits for lead, arsenic, cadmium, and mercury in herbal medicines are 10, 3, 0.3, and 0.3 ppm, respectively (WHO, 2024). These findings raise important concerns regarding the safety of consuming medicinal plants harvested from contaminated environments. The WHO has emphasized the importance of establishing uniform upper limits for elemental impurities in herbal medicines to ensure their safety (WHO, 2024).

The elevated levels of heavy metals in *T. procumbens* suggest its strong capability to absorb and tolerate such metals, pointing to its phytoremediation potential. The plant appears capable of accumulating metals like Chromium and Zinc in its tissues, tolerating toxic metals like Cadmium and Lead possibly via detoxification mechanisms, and being utilized in the remediation of contaminated environments such as industrial or mining-affected soils (Oliveira et al., 2025). While this trait is beneficial for environmental purposes, it also emphasizes the importance of cultivating the plant in uncontaminated environments if it is to be used for medicinal applications. *T. procumbens* shows both medicinal potential and phytoremediation capacity. Its ability to accumulate heavy metals necessitates careful sourcing for health applications while also presenting opportunities for environmental cleanup. As noted by Asiminicesei et al. (2024), heavy metal stress on medicinal plants disrupts biosynthetic pathways and alters concentrations of secondary metabolites, which may compromise the overall quality and efficacy of medicinal plants. Therefore, targeted cultivation practices and quality control measures are essential to ensure the safety and efficacy of *T. procumbens* for therapeutic applications.

5 CONCLUSION

The present study evaluated and compared the phytochemical composition, antioxidant, anti-diabetic, antibacterial properties, and heavy metal accumulation in *Tridax procumbens* collected from polluted and unpolluted environments. The results demonstrated that the unpolluted sample exhibited superior phytochemical and pharmacological properties, with higher total phenolic content, stronger antioxidant activity (IC_{50} = 171.1 µg/ml), and greater anti-diabetic potential (IC_{50} = 250 µg/ml) compared to the polluted sample. The unpolluted extract also showed enhanced antibacterial activity against *Escherichia coli* (20 mm zone of inhibition) while both samples exhibited comparable activity against *Staphylococcus aureus*. The polluted sample, however, demonstrated significant heavy metal accumulation, with Chromium (~39.5 µg/g), Zinc (~17.2 µg/g), Nickel (~6.7 µg/g), Lead (~4.5 µg/g), and Cadmium (~0.48 µg/g), with Cadmium slightly exceeding WHO permissible limits for herbal raw materials. These findings confirm the phytoremediation potential of *T. procumbens* while highlighting the importance of sourcing medicinal plants from uncontaminated environments to ensure safety and efficacy. The study underscores the need for targeted cultivation practices, regular quality control measures, and heavy metal monitoring to harness the therapeutic benefits of *T. procumbens* without compromising human health. Future research should focus on identifying and characterizing the specific bioactive compounds responsible for the observed pharmacological activities and investigating the mechanisms underlying heavy metal tolerance and detoxification in this plant species.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

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