Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 8s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Impact of Nickel and Lead Exposure on the Phytochemical Composition and Bioactive Compounds of Justicia Adhatoda Leaves

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Cite this paper as: Dr. Anita Chowbey, Farha Rehman, (2025) Impact of Nickel and Lead Exposure on the Phytochemical Composition and Bioactive Compounds of Justicia Adhatoda Leaves. *Journal of Neonatal Surgery*, 14 (8s), 300-308.

ABSTRACT

This study investigates the impact of heavy metals, specifically nickel and lead, on the yield, phytochemical composition, and bioactive compound content of *Justicia adhatoda* leaves. The plant extracts were subjected to hydroalcoholic and petroleum ether extraction methods, and the yield was assessed after treatment with different concentrations of nickel and lead (50mg, 100mg, 150mg) for 45 days. The phytochemical analysis revealed that flavonoids, phenols, saponins, and alkaloids were present in the control group, with changes observed after metal exposure. The total flavonoid content (TFC) and total phenolic content (TPC) decreased with increasing metal concentrations, particularly under lead exposure. High-Performance Liquid Chromatography (HPLC) analysis identified quercetin as a key bioactive compound, and its concentration was found to decrease with both nickel and lead exposure, with lead showing a more pronounced reduction. Despite these changes, the study highlights the plant's resilience to nickel exposure, which showed a milder impact on bioactive compound levels compared to lead. The findings suggest that environmental pollution, particularly from lead, may significantly compromise the medicinal potential of *Justicia adhatoda*, while nickel exposure has a relatively lesser effect on its bioactive content.

Keywords: *Justicia adhatoda, Heavy metals, Nickel, Lead, Phytochemical composition, Antioxidant activity, Bioactive compounds.*

1. INTRODUCTION

Justicia adhatoda, a member of the Acanthaceae family, is a well-known medicinal plant traditionally used for treating a variety of ailments, particularly in Ayurvedic and traditional medicine. It has demonstrated numerous pharmacological properties, including antimicrobial, anti-inflammatory, antitussive, and antioxidant activities (Chaudhary et al., 2011; Satti et al., 2010). The medicinal value of *Justicia adhatoda* is largely attributed to the presence of bioactive compounds such as alkaloids (e.g., vasicine), flavonoids (e.g., quercetin, kaempferol), and saponins (Satyavati et al., 1976). These compounds contribute to its broad therapeutic applications, including respiratory disorders, inflammation, and infections (Amin et al., 2005). Despite the recognized medicinal benefits, *Justicia adhatoda*, like many other plants, is susceptible to the harmful effects of environmental pollutants, particularly heavy metals. Heavy metals, such as lead (Pb) and nickel (Ni), are common environmental contaminants that can enter plant systems through soil, water, and air pollution (González et al., 2008). These metals pose significant risks to plant health by causing oxidative stress, inhibiting enzyme activities, disrupting cell function, and ultimately affecting plant growth and the synthesis of secondary metabolites (Cakmak, 2000; Sharma and Dubey, 2005). Metals like lead and nickel are known to alter the plant's biochemical pathways, including those involved in the synthesis of bioactive compounds (Cao et al., 2015; Alia et al., 2017). Oxidative stress, induced by the accumulation of heavy metals, disrupts the plant's antioxidant defense mechanisms, potentially reducing its ability to synthesize key bioactive compounds, such as flavonoids and phenols, which are critical for its medicinal efficacy (Sharma et al., 2014). Furthermore, exposure to heavy metals may lead to changes in the overall yield and phytochemical composition of medicinal plants, which could compromise their therapeutic potential (Sharma and Dubey, 2005). *Justicia adhatoda* is frequently used in traditional remedies, and it is important to understand the impact of heavy metal contamination on its medicinal value. Previous studies have shown that environmental stressors, such as metal exposure, can significantly alter the concentration of bioactive compounds in medicinal plants (Sinha et al., 2012). However, the specific effects of nickel and lead exposure on the bioactive compound profile of *Justicia adhatoda* have not been well-documented, making this research critical for evaluating the

plant's continued medicinal potential in polluted environments. This study aims to investigate the impact of nickel and lead exposure on the yield, phytochemical composition, and bioactive compound content in *Justicia adhatoda* leaves. The study focuses on the extraction of bioactive compounds, such as flavonoids and alkaloids, and utilizes High-Performance Liquid Chromatography (HPLC) for precise quantification of compounds like quercetin. By examining how these metals influence the plant's chemical profile and antioxidant capacity, this research will provide valuable insights into the effects of environmental pollutants on medicinal plants and contribute to understanding the risks posed to plants used in traditional medicine.

2. MATERIALS AND METHODS

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals and solvents used in this study were of analytical grade.

Plant material

The plants of *Justicia adhatoda* were collected from Vindhya herbal, Bhopal. This study was performed using *Justicia adhatoda* (Malabar nut) and under greenhouse conditions. The seedlings were prepared from a local nursery supplier, and uniform seedlings were transplanted on into pots containing a mix soil composed of two-third field soil and one-third fine sands. The soil was mixed thoroughly and passed across a 2-mm sieve and then its physicochemical characteristics were analyzed. The pots (Approx same weight) was divided into different groups like, Groups I control, Groups II treatment of metal (50mg/ml), Groups III treatment of metal (100mg/ml) and Groups IV treatment of metal (150mg/ml). The treatment of heavy metal was given regularly up to one month and sapling was done after 7days, 15days and 45days.

Extraction by maceration process

Justicia adhatoda leaves were dried in the shade at room temperature. The shade-dried plant material was coarsely pulverized (67gm) and macerated in petroleum ether for extraction. The extraction process was maintained until the material had been defatted. Defatted dried plant material were extracted with hydroalcoholic solvent (ethanol: water; 70:30v/v) using maceration method. The extracts were evaporated above their boiling points and stored in an airtight container free from any contamination until it was used. Finally, the percentage yields were calculated of the dried extracts (Abubakar and Haque, 2020).

Determination of percentage Yield

The percentage yield was obtained using this formula:

$$\text{Percentage yield} = \frac{\text{W2} - \text{W1}}{\text{W0}} x100$$

Where W2 is the weight of the extract and the container, W1 the weight of the container alone and W0 the weight of the initial dried sample.

Qualitative estimation of bioactive compounds (Secondary metabolites)

Phytochemical analysis of all the plant extracts was carried out for the presence of different phytochemicals (Phenols, flavonoids, alkaloids, saponins, glycosides and tannins) as per the standard methods (Evans, 1966, Kokate, 1994).

Quantitative study of bioactive compounds

Total phenolic content

The total phenol content of the extracts was determined by the modified folin-ciocalteu method (Mishra *et al.*, 2017). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extracts was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of each extract and standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer. The total amount of phenol in plant extract was calculated by regression equation Y=MX+C expressed as mg/100 of dry weight equivalent to Gallic acid (mg GAE/100mg).

Total flavonoid content

Determination of total flavonoids content was based on aluminium chloride method (Mishra *et al.*, 2017). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance

was measured at 420 nm. TFC was calculated with the formula given below and was expressed as mg/100mg of Quercetin equivalent (mg QE/100mg) calculated using regression equation Y=MX+C.

Quantitative study of marker compound (Quercetin) by HPLC

High performance liquid chromatography (HPLC) has recently become a conventional analytical tool for the quality-control of herbal drugs because of its low operation-cost, high sample-throughput and need for minimum sample clean-up. With HPLC, qualitative and quantitative analyzes of multiple compounds can be done simultaneously by using small volume of mobile phase. The developed HPLC chromatograms are useful in identification of biomarkers in various herbal formulations. The HPLC method is used for qualitative and quantitative analysis of flavonoid component (quercetin). It is a secondary metabolites component present in leaves of *Justicia adhatoda.*

Chromatographic condition

The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical column with a mobile phase composed of Acetonitrile: Methanol (50:50 v/v) and was isocratically eluted at a flow rate of 1 mL min^{-1}. A small sample volume of 20 µL was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm (Acharya *et al.*, 2019).

Preparation of standard stock solution

10mg of quercetin was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm.

Preparation of working standard solution

From stock solutions of Quercetin 1 ml was taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 5, 10, 15, 20, 25µg/ ml concentration.

Analysis of control group and metal enriched group of hydroalcoholic extract of *Justicia adhatoda*

10 mg of control group and metal enriched group of hydroalcoholic extract of *Justicia adhatoda* was taken in 10 ml volumetric flask and dilute upto the mark with Methanol; resultant solution was filtered through Whatmann filter paper and finally volume made up to mark with same solvent to obtain concentration of 1000 µg/ml. The resulting solution was again filtered using 0.45µ membrane filter and then sonicated for 10 min.

3. RESULTS AND DISCUSSION

The study aimed to evaluate the impact of nickel and lead exposure on the phytochemical profile, yield, and bioactive compound content of *Justicia adhatoda* leaf extracts. The results highlight several significant findings regarding the effects of these heavy metals on plant extracts, particularly in relation to yield, phytochemical composition, and the concentration of bioactive compounds such as quercetin. The extraction yields from *Justicia adhatoda* treated with different metal concentrations (nickel and lead) were compared with the control group (Table 1, 2, and 3). In the control group, the highest yield was observed with the hydroalcoholic extract (10.8%), followed by the petroleum ether extract (0.45%). Exposure to nickel and lead resulted in changes in yield, with nickel treatment leading to a slight increase in yield, particularly at the 100mg and 150mg concentrations. In contrast, lead exposure caused a reduction in yield, especially at higher concentrations (100mg and 150mg). The decrease in yield with lead exposure could be attributed to the toxicity of lead, which may interfere with plant metabolic processes such as photosynthesis and secondary metabolite production. The slight increase in yield with nickel treatment suggests that nickel may have a lesser inhibitory effect on plant growth or metabolic processes compared to lead. Phytochemical analysis of *Justicia adhatoda* extracts revealed notable differences in the presence of various secondary metabolites in response to metal exposure (Table 4). Flavonoids, phenols, and saponins were present in the control group, and their presence was generally consistent across all treatments. However, a significant difference was observed in the alkaloid, phenolic, and protein content. Nickel exposure did not show any major changes in the alkaloid or phenolic content at the 50mg, 100mg, and 150mg concentrations, while lead exposure led to the loss of alkaloids and a reduction in phenolic content at higher concentrations. This reduction in phenolic compounds with lead exposure is in line with studies that report the inhibitory effects of heavy metals on secondary metabolite production, which are often linked to oxidative stress caused by metal toxicity. The presence of flavonoids, such as quercetin, and saponins was found consistently across all groups, indicating that these bioactive compounds are less sensitive to metal stress. This suggests that *Justicia adhatoda* may be capable of maintaining the synthesis of some valuable secondary metabolites even under metal stress, which could be beneficial in terms of its therapeutic potential. The estimation of total flavonoids (TFC) and total phenolic content (TPC) in the metal-treated extracts (Table 4) indicated that lead exposure reduced both TFC and TPC in a dose-dependent manner. In the control group, the TFC was 0.985 mg/100mg, and TPC was 0.882 mg/100mg. With increasing lead concentrations, TFC and TPC levels declined, with the lowest values observed in the lead 150mg treatment (TFC: 0.569 mg/100mg, TPC: 0.615 mg/100mg). This reduction is likely due to the adverse effects of lead on the biosynthesis of phenolic compounds and

flavonoids, as these compounds are often synthesized through the phenylpropanoid pathway, which may be disrupted by metal toxicity. The decline in these important antioxidants could negatively impact the plant's ability to counteract oxidative stress. On the other hand, nickel exposure resulted in a more moderate decrease in TFC and TPC, indicating that nickel might exert less inhibitory effect on the flavonoid and phenolic biosynthesis pathways compared to lead. These findings suggest that the metal-induced stress from lead is more detrimental to the production of bioactive compounds in *Justicia adhatoda* compared to nickel. The HPLC analysis of quercetin in the hydroalcoholic extracts revealed a decrease in quercetin content in the treated groups compared to the control group (Table 5 and 6). In the control group, the % assay of quercetin was found to be 0.0941%. However, with increasing concentrations of nickel (50mg, 100mg, 150mg) and lead (50mg, 100mg, 150mg), the quercetin content decreased. The lowest quercetin content was observed in the nickel 150mg (0.0774%) and lead 150mg (0.0764%) treatments. This decrease in quercetin content can be attributed to the negative impact of heavy metals on the biosynthesis of flavonoids, as quercetin is a key flavonoid that could be synthesized through the phenylpropanoid pathway. Interestingly, nickel exposure resulted in a smaller reduction in quercetin content compared to lead exposure, suggesting that nickel may exert less inhibitory effects on flavonoid biosynthesis. This observation aligns with the findings from the TFC and TPC assays, where nickel exposure led to a more moderate decrease in these compounds compared to lead. The results of the HPLC analysis suggest that *Justicia adhatoda* may still maintain a certain level of flavonoid production even under metal stress, particularly in the presence of lower concentrations of nickel.

The results suggest that *Justicia adhatoda* retains a significant portion of its bioactive compounds, including flavonoids like quercetin, even under metal stress. However, exposure to lead appears to be more detrimental to the production of these compounds, which could affect the plant's medicinal properties. The reduction in quercetin content and other flavonoids could limit the plant's antioxidant potential, which is important for its medicinal uses. On the other hand, the relatively lesser impact of nickel on flavonoid biosynthesis could indicate that *Justicia adhatoda* may still be a viable source of antioxidants and other bioactive compounds even in environments contaminated with nickel. In conclusion, while both nickel and lead exposure affect the yield and phytochemical composition of *Justicia adhatoda*, the plant appears to be more resilient to nickel exposure. However, the decrease in bioactive compounds, particularly flavonoids, with higher concentrations of both metals indicates that environmental pollution could compromise the plant's therapeutic potential. Further research is needed to explore the long-term effects of metal exposure on the plant's overall pharmacological activity and to determine its safety for use in polluted environments.

Table 1: Percentage yield of the extracts of *Justicia adhatoda* (Control group)

S. No.	Extract	Percentage yield
1.	Petroleum ether	0.45
2.	Hydroalcoholic	10.8

Table 2: Percentage yield of the extracts of *Justicia adhatoda* treated with Nickel after 45 days

S. No.	Extract	Percentage yield		
		50mg	100mg	150mg
1.	Petroleum ether	0.31	0.54	0.68
2.	Hydroalcoholic	3.45	4.20	4.56

Table 3: Percentage yield of the extracts of *Justicia adhatoda* treated with Lead after 45 days

S. No.	Extract	Percentage yield		
		50mg	100mg	150mg
1.	Petroleum ether	0.21	0.34	0.18
2.	Hydroalcoholic	2.5	3.7	2.9

Table 4: Phytochemical analysis of Control group

S. No.	Phytochemical Test	Plant extract (Control sample)	Plant extract Nickel (50mg) after 45 days	Plant extract Nickel (100mg) after 45 days	Plant extract Nickel (150mg) after 45 days	Plant extract (Lead (50mg) after 45 days)	Plant extract (Lead (100mg) after 45 days)	Plant extract (Lead (150mg) after 45 days)
1	Alkaloids	+	-	-	-	+	-	-
2	Carbohydrate	-	-	-	-	-	-	-
3	Flavonoids	+	+	+	+	+	+	+
4	Phenols	+	-	-	-	-	-	-
5	Tannins	-	-	-	-	-	-	-
6	Saponins	+	-	-	-	+	-	-
7	Proteins	+	-	-	-	-	-	-
8	Diterpenes	+	+	+	+	-	+	+
9	Glycosides	-	-	-	-	-	-	-

+ = Positive; - = Negative

Table 5: Results of estimation of total flavonoids and phenols content of metals (Lead) exposure after 45 days

S. No.	Group	TFC (mg/100mg)	TPC (mg/100mg)
1.	Control	0.985	0.882
2.	Lead 50mg	0.922	0.756
3.	Lead 100mg	0.749	0.652
4.	Lead 150mg	0.569	0.615

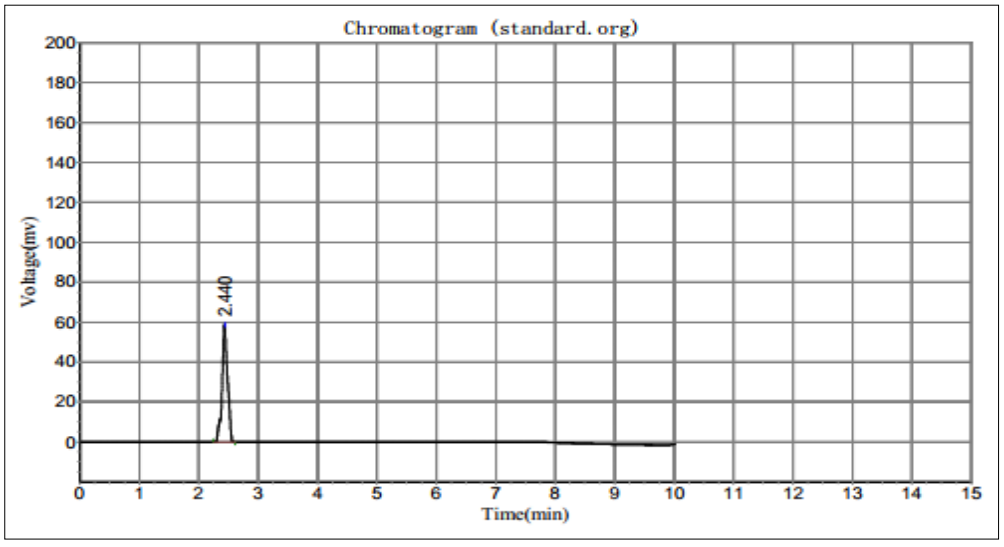

Figure 1: Chromatogram of standard Quercetin

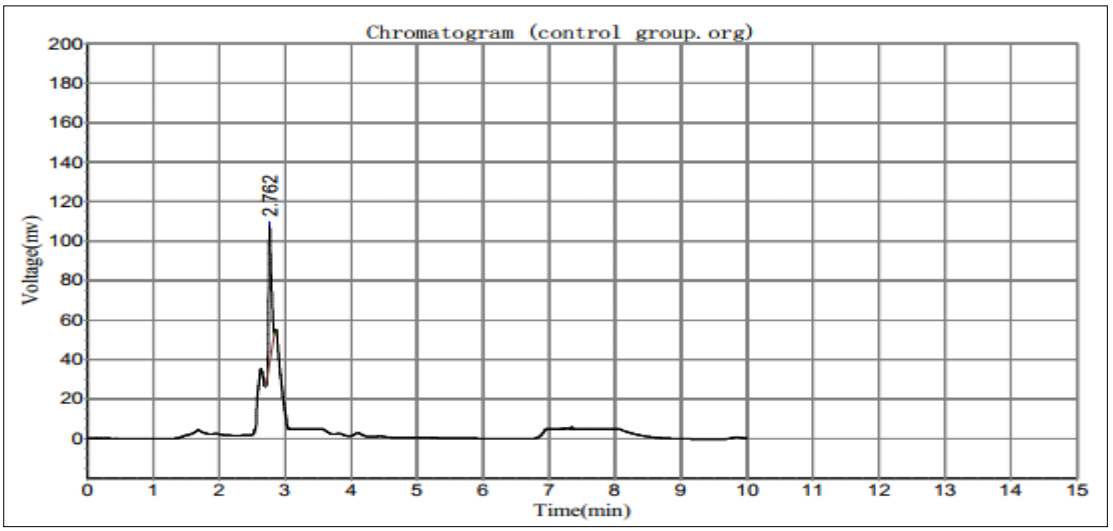

Figure 2: Chromatogram of hydroalcoholic extract of Control group

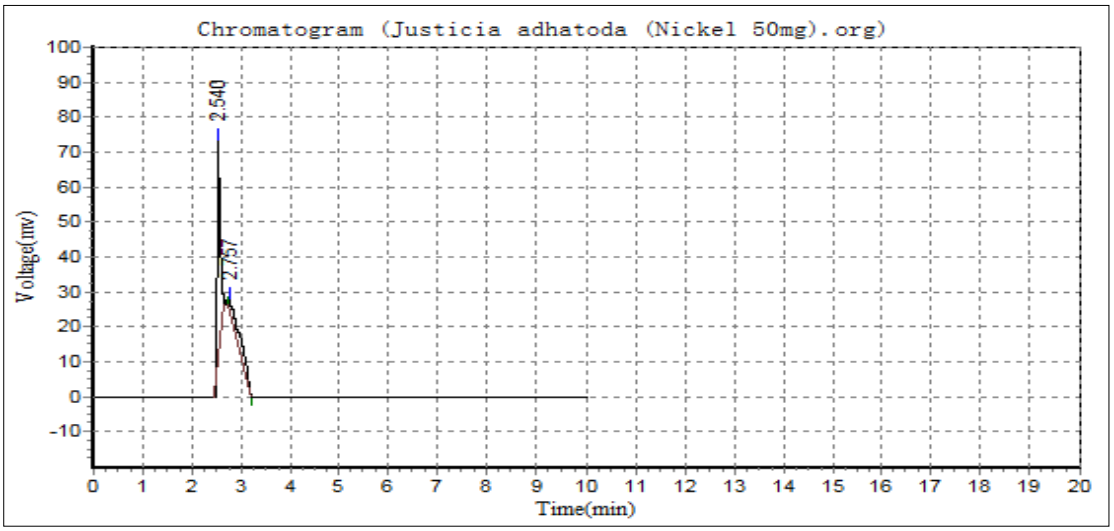

Figure 3: Chromatogram of hydroalcoholic extract (Nickel 50mg)

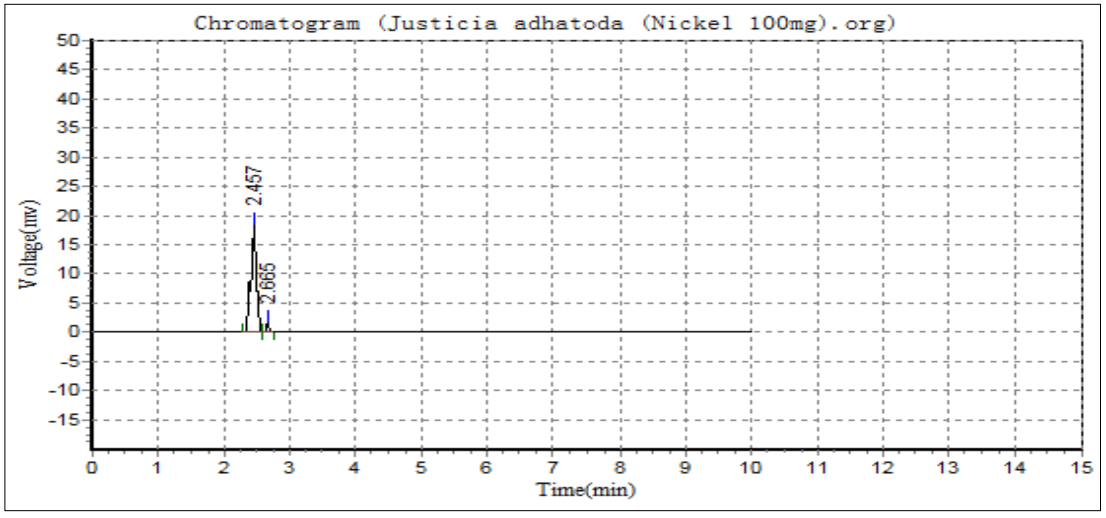

Figure 4: Chromatogram of hydroalcoholic extract (Nickel 100mg)

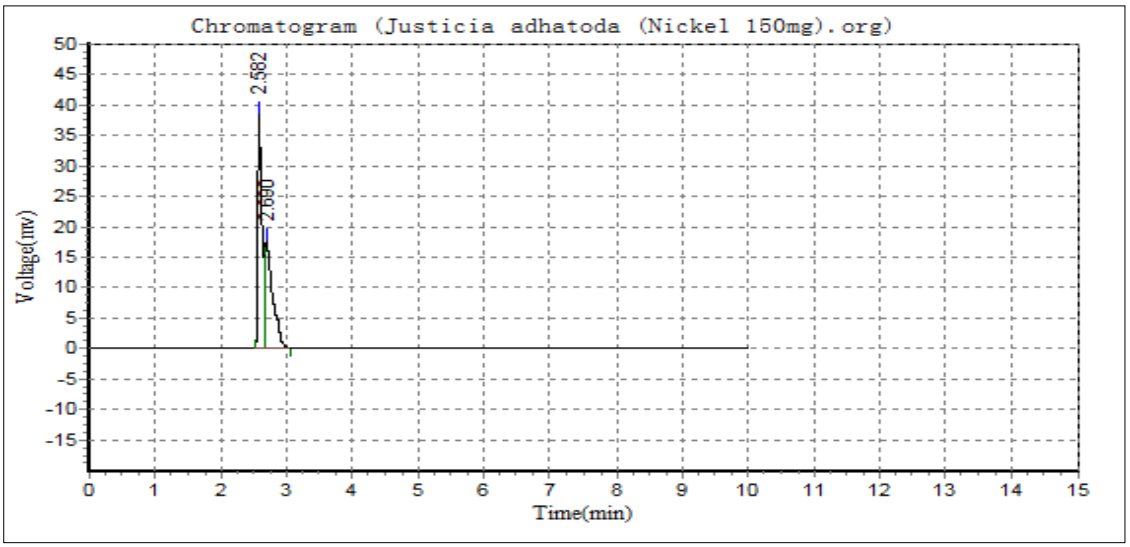

Figure 5: Chromatogram of hydroalcoholic extract (Nickel 150mg)

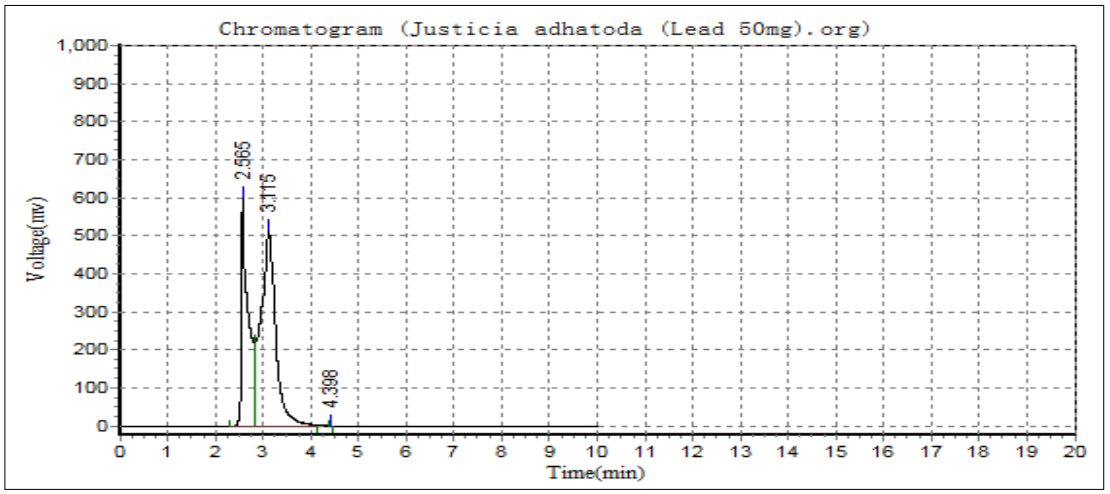

Figure 6: Chromatogram of hydroalcoholic extract (Lead 50mg)

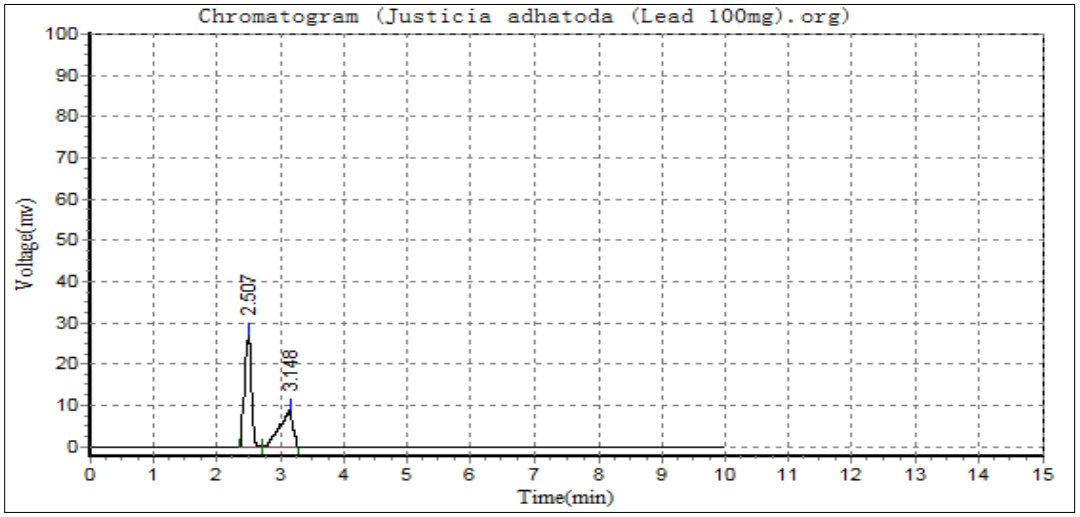

Figure 7: Chromatogram of hydroalcoholic extract (Lead 100mg)

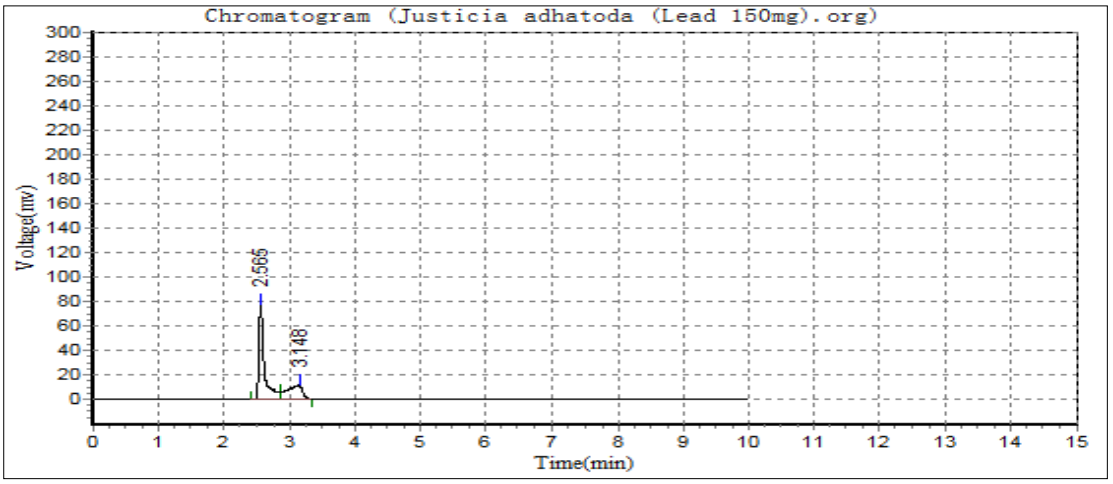

Figure 8: Chromatogram of hydroalcoholic extract (Lead 150mg)

Table 6: Quantitative estimation of Quercetin in extracts (AUC)

S. No.	Standard / Hydroalcoholic extract	RT	Area Under Curve
1.	Quercetin	2.440	2598.850
2.	Control group	2.762	2445.560
3.	Nickel 50mg	2.540	2145.650
4.	Nickel 100mg	2.457	2050.658
5.	Nickel 150mg	2.582	2010.365
6.	Lead 50mg	2.565	2289.887
7.	Lead 100mg	2.507	2174.658
8.	Lead 150mg	2.565	1985.452

Table 7: Quantitative estimation of Quercetin in extracts (% Assay)

S. No.	Standard / Hydroalcoholic extract	RT	% Assay
1.	Quercetin	2.440	-
2.	Control group	2.762	0.0941
3.	Nickel 50mg	2.540	0.0826
4.	Nickel 100mg	2.457	0.0789
5.	Nickel 150mg	2.582	0.0774
6.	Lead 50mg	2.565	0.0881
7.	Lead 100mg	2.507	0.0837
8.	Lead 150mg	2.565	0.0764

4. CONCLUSION

The study on *Justicia adhatoda* reveals that exposure to heavy metals such as nickel and lead significantly affects the plant's yield, phytochemical composition, and bioactive compound content, particularly flavonoids and phenols. In particular, lead exposure demonstrated a more detrimental effect on the production of key bioactive compounds, leading to a reduction in the total flavonoid content (TFC) and total phenolic content (TPC), as well as a decrease in quercetin levels. Nickel exposure, while still impacting the plant's metabolism, had a milder effect on flavonoid and phenolic biosynthesis compared to lead. The changes in the extraction yield and bioactive compounds point to the negative impact of environmental pollutants on the therapeutic potential of medicinal plants. Despite the reductions in key bioactive compounds under metal stress, *Justicia adhatoda* appears to retain a portion of its antioxidant properties, especially under nickel stress. This suggests that while the plant may still have medicinal value, its efficacy could be compromised in environments with high levels of contamination, particularly lead.

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