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IMPACT OF NICKEL AND LEAD STRESS ON THE ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *JUSTICIA ADHATODA*

Farha Rehman* and Dr. Anita Chowbey
Govt. MLB Girls PG (Auto) College Bhopal, Madhya Pradesh, India
Corresponding author mail id: farharehma@gmail.com

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Abstract
This study explores the in vitro antioxidant and antimicrobial activities of *Justicia adhatoda* extracts under Nickel and Lead stress. Hydroalcoholic extracts from *Justicia adhatoda* were subjected to various assays to evaluate their antioxidant potential, including the DPPH method and Ferric Reducing Ability of Plasma (FRAP) assay, along with IC50 value determination. Additionally, the antimicrobial efficacy of the extracts was assessed against *Bacillus subtilis* and *Salmonella bongori*. The results revealed that control group extracts exhibited robust antioxidant and antimicrobial activities, with lower IC50 values and significant inhibition zones. In contrast, Nickel-treated extracts showed reduced antioxidant and variable antimicrobial activity, while Lead-treated extracts demonstrated either maintained or enhanced antimicrobial properties at higher concentrations. These findings highlight the complex impact of heavy metal stress on the bioactivity of *Justicia adhatoda*, suggesting potential alterations in its therapeutic efficacy under metal exposure. The study underscores the need for further research to elucidate the specific mechanisms of plant-metal interactions and their implications for medicinal applications.

Keywords: *Justicia adhatoda*, antioxidant activity, antimicrobial activity, Nickel stress, Lead stress, DPPH assay, FRAP assay, IC50 value, *Bacillus subtilis*, *Salmonella bongori.*

Introduction

Justicia adhatoda, commonly known as Malabar nut or Vasaka, is a medicinal plant that holds significant importance in traditional medicine systems such as Ayurveda and Unani. Indigenous to South Asia, particularly India and Sri Lanka, it has been revered for centuries for its therapeutic properties. The plant is characterized by its distinctive lance-shaped leaves and clusters of white or purple flowers, and it has garnered attention for its pharmacological benefits, including antioxidant and antimicrobial activities (Singh et al., 2010; Bafna and Mishra, 2010).

Antioxidants play a crucial role in human health by neutralizing harmful free radicals and reactive oxygen species (ROS) that contribute to oxidative stress. Oxidative stress is implicated in the pathogenesis of various chronic diseases, including cardiovascular disorders, cancer, diabetes, and neurodegenerative conditions like Alzheimer's and Parkinson's diseases. Natural

antioxidants derived from plants offer a promising avenue for therapeutic interventions due to their ability to scavenge free radicals and reduce oxidative damage to cells and tissues.

Justicia adhatoda contains a diverse array of bioactive compounds known for their antioxidant properties. These include phenolic compounds (such as flavonoids, phenolic acids, and tannins), alkaloids (including vasicine and vasicinone), and other phytochemicals. These constituents have been extensively studied for their ability to inhibit lipid peroxidation, chelate metal ions, and modulate enzymatic antioxidant defenses within cells (Gupta et al., 2012; Tiwari and Singh, 2011).

Various in vitro assays are employed to evaluate the antioxidant potential of plant extracts. The DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay is commonly used to assess the ability of antioxidants to scavenge the DPPH radical by donating hydrogen atoms or electrons. The FRAP (Ferric Reducing Antioxidant Power) assay evaluates the reducing power of antioxidants by their ability to reduce ferric ions to ferrous ions. Studies have indicated that extracts from *Justicia adhatoda* possess significant antioxidant activity across these assays, attributed primarily to its high phenolic content. The plant's extracts have demonstrated effective scavenging of free radicals and ROS, thereby protecting cells from oxidative damage. This antioxidant potential underscores its therapeutic potential in mitigating oxidative stress-related diseases (Singh et al., 2010; Bafna and Mishra, 2010).

This research aims to consolidate and summarize the in vitro antioxidant and antimicrobial activity of *Justicia adhatoda* extracts using various methods. It will highlight recent research findings and discuss the implications for its potential therapeutic applications in managing oxidative stress-related disorders.

Material and methods

Collection of plant material

The plants of *Justicia adhatoda* were collected from Vindhya herbal, Bhopal. This study was performed using *Justicia adhatoda* (Malabar nut) during 2021 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. The entire collected sample from each groups evaluated for plant height, weight and No. of leaves. The same interval also selected for wet lab analysis like chlorophyll estimation and study of bioactive constituents using different techniques like spectroscopy and chromatographic techniques.

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).

***In vitro* antioxidant activity of *Justicia adhatoda* extracts using different method**

Antioxidants are the key constituents that defend our bodies from the damages caused by free radical-induced oxidative stress. Plants provide many important compounds which offer resistance against oxidative stress by scavenging free radicals, preventing lipid peroxidation, and other mechanisms. The micronutrients such as vitamin C and E, *β*-carotene, and other important ingredients such as phenolic and flavonoids from plants are helpful to reduce oxidative stress. The present study was undertaken to compare the antioxidant activity using lead and nickel exposure on the growth and physiological attributes of *Justicia adhatoda* extract.

DPPH method

The spectrophotometer was used to measure the DPPH scavenging activity with a little modification (Jain and Parkhe, 2018). 1.5 ml of the stock solution (6 mg in 100 ml methanol) was made so that it produced an initial absorbance when combined with 1.5 ml of methanol. After 15 minutes, a decrease in absorbance was seen when sample extract at various concentrations (10-100 µg/ml) was present. After diluting 1.5 ml of the DPPH solution with methanol to make 3 ml, the absorbance was measured right away at 517 nm for the control reading. In a series of volumetric flasks, 1.5 ml of DPPH and 1.5 ml of the test sample at various concentrations were added. Each concentration was measured using absorbance at zero time. After 15 minutes, at 517 nm, DPPH absorbance finally decreased with the sample at a varied concentration. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%.

Reducing power (Ferric reducing ability of plasma assay)

The ferric ion reducing power of extracts was determined by measuring the absorbance of chromophore. Different concentrations of extracts or standard, vitamin C (1 mL, 10-100 µg/mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6), potassium ferric cyanide (2.5 mL, 1% w/v) and incubated at 50 ± 2°C for 20 min. Trichloroacetic acid (2.5 mL, 10% w/v) was added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5mL) was separated and mixed with 2.5 mL of distilled water and ferric chloride (0.5 mL, 0.1% w/v). Then, the absorbance was measured at λ_{max} 700 nm using UV-Vis spectrophotometer (Labindia 3000 plus, India) (Benzie and Strain, 1996).

In vitro* antimicrobial activity of hydroalcoholic extract of *Justicia adhatoda

Pathogenic microbes used

The reference microbial species; Microbial Type Culture Collection (MTCC) of *Bacillus subtilis* (MTCC- 441), *Salmonella bongori* (MTCC- 3858)*, Aspergillus niger* (MTCC-281), were collected from Bacteriology Unit of the Microbiology Laboratory.

Media preparation

At first, all instruments which were used in laboratory were made sterile, all glassware's like Erlenmeyer flask, graduated cylinders, stirring rods, beakers, test tubes, petri dishes, inoculating loops, that were used in the assay were placed in an autoclave at 121°C under 15 psi pressure for 25 min by using Autoclave and followed aseptic technique method. Nutrient agar media (NAM) was prepared for growing of bacteria and potato dextrose agar (PDA) for growing of fungus inside the laboratory. The standard size (100mm× 15mm) petri dishes as required for whole experiment. For preparation of NAM, 13 gram powder and for PDA, 24 gram powder was mixed with 1000 ml of distilled water and stirred to obtain homogenized mixture. After which, NAM

and PDA mixture were placed in Autoclave under 15 psi pressure, at 121°C for 25 min for sterilization of media. After that poured the culture media into petri dishes at ratio of 20 ml/dish and was left half covered on the table to let the agar cool down and solidify at room temperature.

Well diffusion assay

The antimicrobial activity is employed on to the all the microbes used under present study with control group and metal group of hydroalcoholic extract of *Justicia adhatoda*. 100µl fresh microbial culture was spread on an agar plate with non-toxic swab. Four wells of 6-mm diameter were punched off into the agar medium with sterile cork-borer (6 mm) and filled with 100 µl (25, 50 and 100mg/ml) of each group extract by using a micropipette in well under aseptic conditions. The plates were allowed to stand for 1 h to allow for pre-diffusion of the extract into the medium. The plates were incubated aerobically in an upright position at 37±2°C for 24-48 h. After incubation, plates were observed to see the antimicrobial activity of extracts towards test microorganism at particular concentration in the form zone of inhibition (mm).

Results and Discussion

The growth parameters of *Justicia adhatoda* seedlings, including plant height, weight, and number of leaves, were notably affected by metal treatments. Increasing concentrations of heavy metals (nickel and lead) led to a general decline in these parameters compared to the control. This decrease can be attributed to the toxic effects of heavy metals, which impair plant physiological processes and reduce overall plant vigor. Heavy metals can interfere with nutrient uptake, enzyme activity, and other critical physiological functions, leading to stunted growth.

The antioxidant activity of *Justicia adhatoda* extracts was evaluated using the DPPH assay and the Ferric Reducing Ability of Plasma (FRAP) assay. The results showed that the hydroalcoholic extract of *Justicia adhatoda* exhibited significant free radical scavenging activity. This activity was concentration-dependent, with higher concentrations showing greater inhibition of DPPH radical formation. The IC50 values for the control group indicate a relatively potent antioxidant activity.

When exposed to nickel and lead, the antioxidant activity of the extracts was affected, though the degree varied by metal and concentration. For nickel, the IC50 values increased with higher concentrations, indicating reduced antioxidant efficiency. In contrast, the lead-treated groups showed improved antioxidant activity with increasing metal concentrations, suggesting a potential adaptive response or a differential impact of lead on antioxidant pathways compared to nickel.

The FRAP assay further corroborated the antioxidant activity observed in the DPPH assay. The control group's extracts displayed a concentration-dependent increase in ferric reducing power, confirming their ability to reduce ferric ions to ferrous ions. The metal treatments, however, influenced this activity differently. Nickel exposure resulted in a general decline in reducing power, while lead exposure showed an increase at certain concentrations. These results align with the DPPH findings, indicating that heavy metals can alter the antioxidant capacity of plant extracts.

The antimicrobial activity of the hydroalcoholic extracts was assessed using the well diffusion method against selected microbes. The extracts from the control group showed moderate antimicrobial activity, with zones of inhibition varying by concentration. Nickel and lead treatments affected the antimicrobial properties differently. Nickel-treated extracts exhibited reduced antimicrobial activity, particularly at higher concentrations. Lead-treated extracts showed varying degrees of activity, with some concentrations demonstrating enhanced inhibition against certain microorganisms.

Table 1: % Inhibition of ascorbic acid and hydroalcoholic extract of Control group

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Control group
1	10	34.96	22.79
2	20	46.15	32.16
3	40	67.97	45.73
4	60	83.77	64.47
5	80	85.73	76.92
6	100	88.11	79.72
IC_{50} value		**22.23**	**46.28**

Table 2: % Inhibition of hydroalcoholic extract of Nickel group

S. No.	Concentration (µg/ml)	% Inhibition		
		Nickel 50mg	Nickel 100mg	Nickel 150mg
1	10	14.965	14.406	14.965
2	20	18.182	17.203	18.182
3	40	30.350	27.972	31.748
4	60	48.951	37.343	45.734
5	80	68.112	48.951	54.546
6	100	83.357	69.091	67.972
IC_{50} value		**59.28**	**75.82**	**70.36**

Table 3: % Inhibition of hydroalcoholic extract of Lead group

S. No.	Concentration (µg/ml)	% Inhibition		
		Lead 50mg	Lead 100mg	Lead 150mg
1	10	21.958	8.811	12.587
2	20	34.965	18.182	19.720
3	40	52.727	32.168	30.350
4	60	67.972	52.587	46.154
5	80	75.664	68.112	67.972
6	100	84.895	83.636	83.916
IC_{50} value		**42.43**	**58.94**	**59.89**

Table 4: IC 50 values of control and treated groups

Groups	Ascorbic acid	Control group	Nickel 50mg	Nickel 100mg	Nickel 150mg	Lead 50mg	Lead 100mg	Lead 150mg
IC_{50}	22.23	46.28	59.28	75.82	70.36	42.43	58.94	59.89

Table 5: Ferric reducing ability of plasma (FRAP) activity of hydroalcoholic extract of Control group with reference to ascorbic acid

S. No.	Concentration (µg/ml)	Ascorbic acid	Control group
		Absorbance	
1	10	0.265	0.165
2	20	0.312	0.185
3	40	0.445	0.236
4	60	0.585	0.385
5	80	0.589	0.468
6	100	0.592	0.559
7	120	0.593	0.632
8	150	0.593	0.633
9	200	0.594	0.637

Table 6: Ferric reducing ability of plasma (FRAP) activity of hydroalcoholic extract of Nickel group with reference to ascorbic acid

S. No.	Concentration (µg/ml)	Nickel 50mg	Nickel 100mg	Nickel 150mg
		Absorbance		
1	10	0.225	0.248	0.285
2	20	0.315	0.365	0.388
3	40	0.456	0.485	0.496
4	60	0.558	0.595	0.612
5	80	0.612	0.645	0.669
6	100	0.695	0.689	0.685
7	120	0.698	0.699	0.685
8	150	0.699	0.702	0.688
9	200	0.702	0.705	0.689

Table 7: Ferric reducing ability of plasma (FRAP) activity of hydroalcoholic extract of Lead group with reference to ascorbic acid

S. No.	Concentration (µg/ml)	Lead 50mg	Lead 100mg	Lead 150mg
		Absorbance		
1	10	0.285	0.235	0.228
2	20	0.315	0.315	0.345
3	40	0.468	0.415	0.436
4	60	0.552	0.545	0.525
5	80	0.615	0.636	0.663
6	100	0.645	0.662	0.678
7	120	0.658	0.692	0.688
8	150	0.659	0.699	0.689
9	200	0.659	0.699	0.692

Table 8: Antimicrobial activity of standard drug against selected microbes

S. No.	Name of drug	Microbes	Zone of Inhibition (mm)		
			10 µg/ml	20 µg/ml	30 µg/ml
1.	Ciprofloxacin	*Bacillus subtilis*	14±0.5	20±0.74	26±0.47
		Salmonella bongori	17±0.57	23±0.86	25±0.5

***Average of three determination, Mean ± SD**

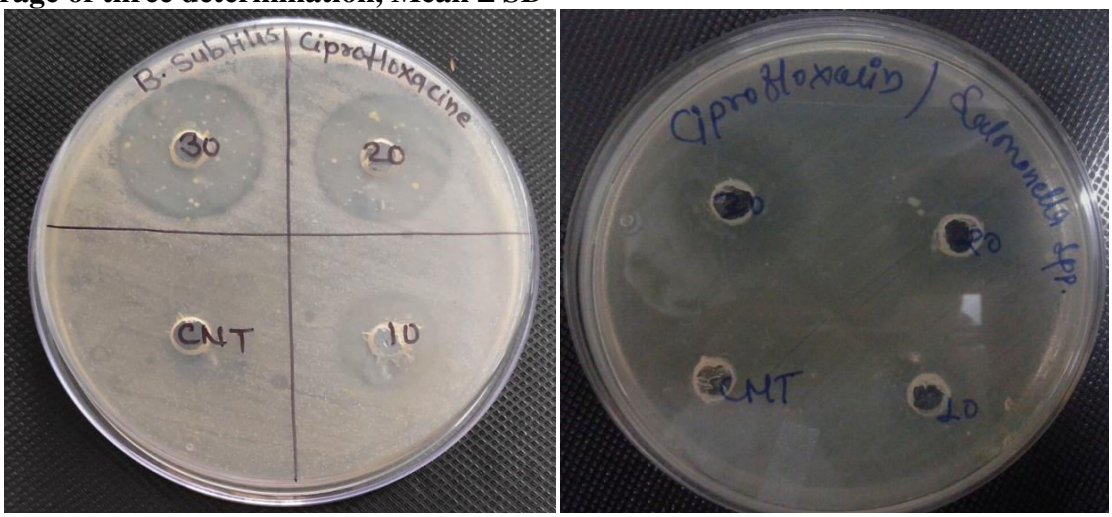


Figure 6.8: Antimicrobial activity of *standard drug* against selected microbes

Table 9: Antimicrobial activity of hydroalcoholic extract of control group against selected microbes

Sr. No.	Name of Organism	Zone of Inhibition (mm)		
		25 mg/ml	50 mg/ml	100 mg/ml
1.	*Bacillus subtilis*	9±0.5	11±0.74	15±0.57
2.	*Salmonella bongori*	8±0.94	9±0.86	12±0.47

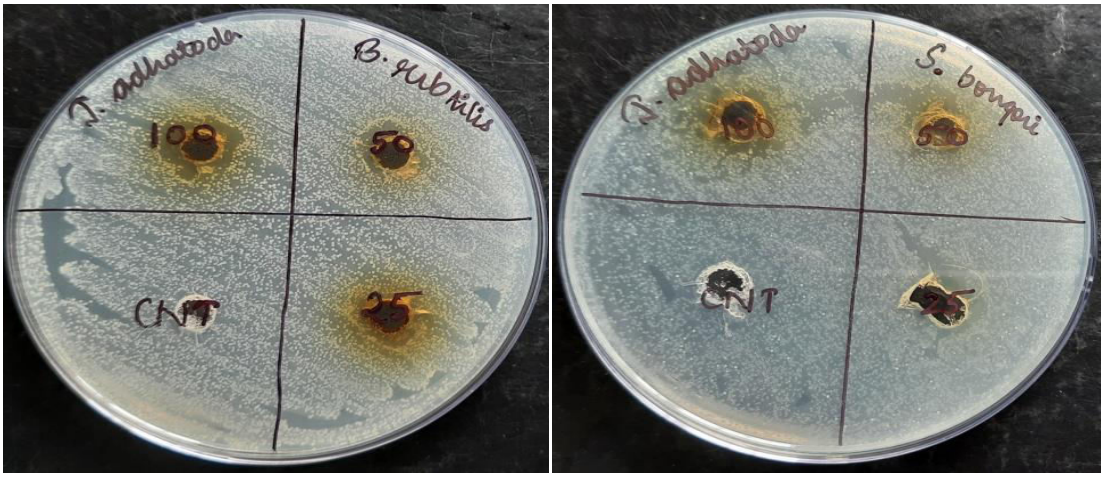
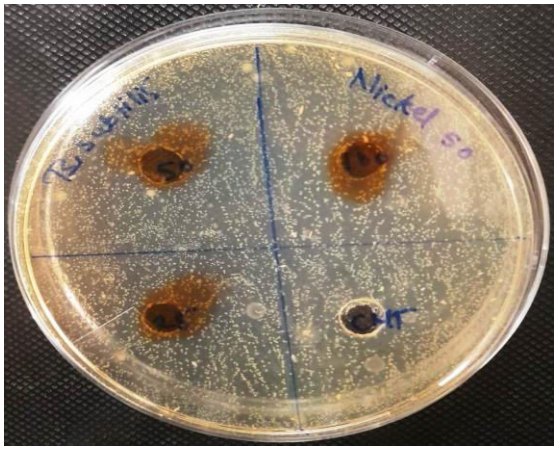


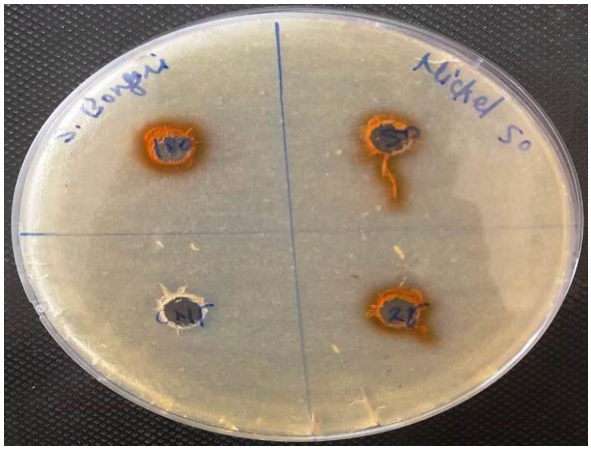
Figure 1: Photoplates of antimicrobial activity of hydroalcoholic extract of control group against selected microbes

Table 10: Antimicrobial activity of hydroalcoholic extract of Nickel group against selected microbes

S. No.	Name of microbes	Zone of inhibition (mm)		
		Hydroalcoholic extract of Nickel 50mg		
		25mg/ml	50 mg/ml	100mg/ml
1.	*Bacillus subtilis*	8±0.47	9±0.47	12±0.86
2.	*Salmonella bongori*	9±0.5	10±0.74	11±0.94
		Hydroalcoholic extract of Nickel 100mg		
1.	*Bacillus subtilis*	6±0.74	8±0.47	11±0.57
2.	*Salmonella bongori*	8±0.5	9±0	10±0
		Hydroalcoholic extract of Nickel 150mg		
1.	*Bacillus subtilis*	7±0.47	8±0.74	9±0.86
2.	*Salmonella bongori*	6±0	9±0.5	9±0.47

***Average of three determination, Mean ± SD**





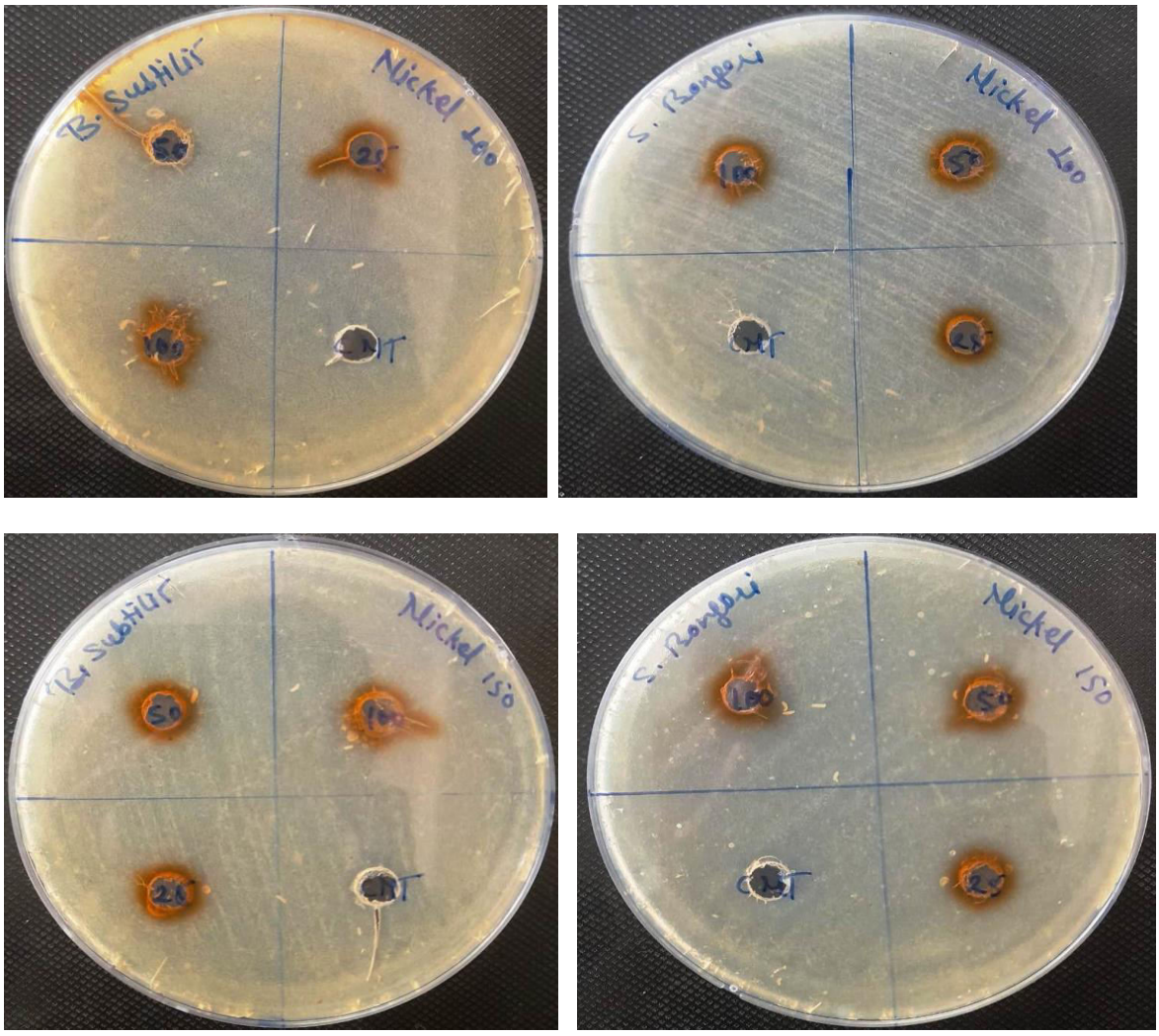


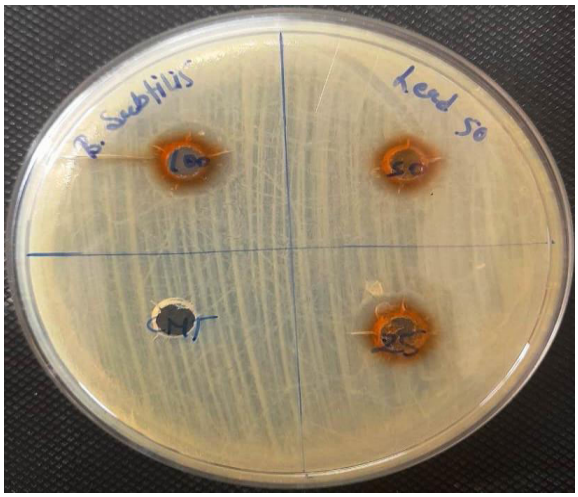
Figure 2: Photoplates of antimicrobial activity of hydroalcoholic extract of Nickel group against selected microbes

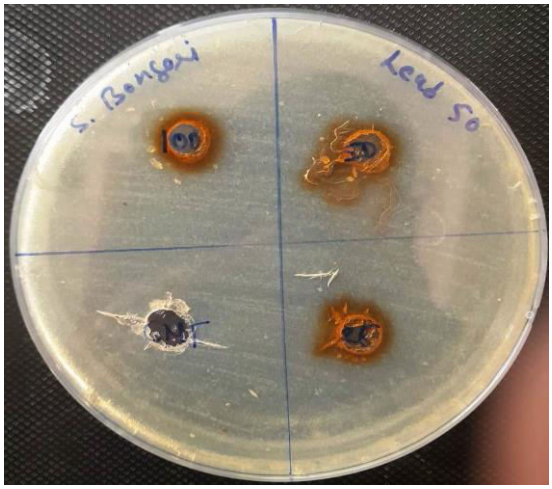
Table 11: Antimicrobial activity of hydroalcoholic extract of Lead group against selected microbes

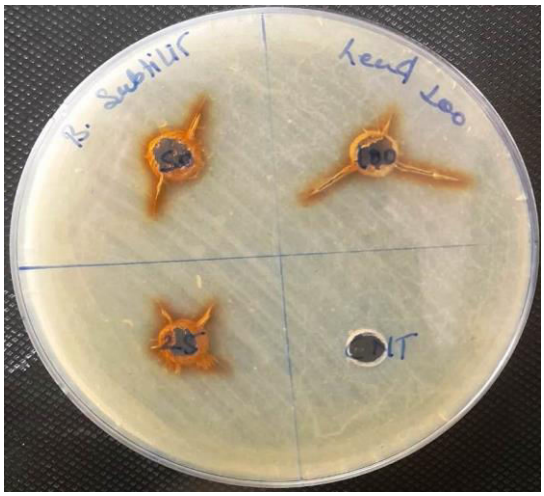
S. No.	Name of microbes	Zone of inhibition (mm)		
		Hydroalcoholic extract of Lead 50mg		
		25mg/ml	50 mg/ml	100mg/ml
1.	*Bacillus subtilis*	7±0.57	10±0.94	13±0.5
2.	*Salmonella bongori*	7±0	8±0.47	11±0.57
		Hydroalcoholic extract of Lead 100mg		
1.	*Bacillus subtilis*	8±0.5	9.6±0.74	12±0.47
2.	*Salmonella bongori*	7±0.94	8.6±0.5	10.6±0.57
		Hydroalcoholic extract of Lead 150mg		

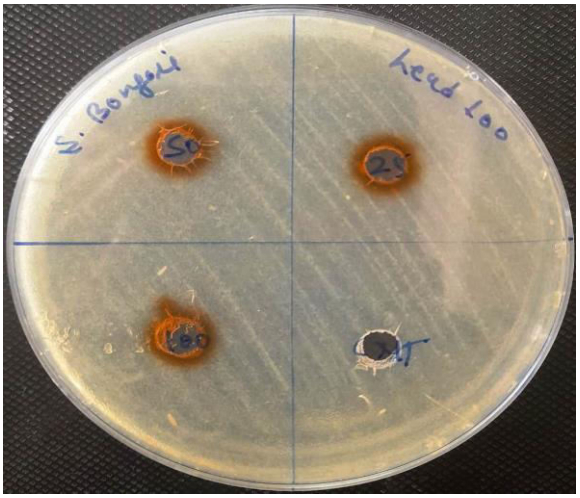
1.	*Bacillus subtilis*	9±0.86	11±0.74	12.6±0.47
2.	*Salmonella bongori*	8±0.5	10±0.47	11.6±0.57

***Average of three determination, Mean ± SD**









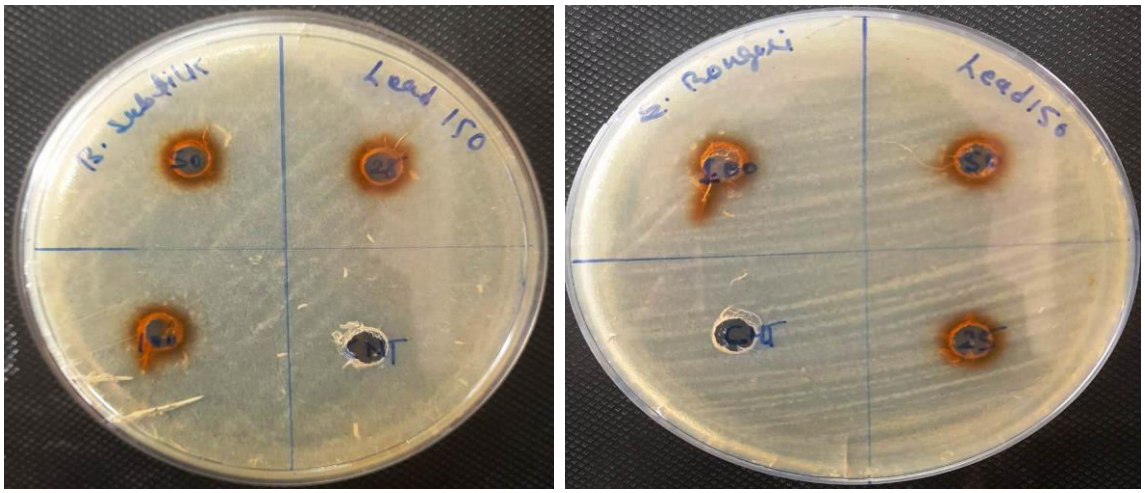


Figure 3: Photoplates of Antimicrobial activity of hydroalcoholic extract of Lead group against selected microbes

Conclusion

The research investigated the in vitro antioxidant and antimicrobial activities of *Justicia adhatoda* extracts, focusing on how Nickel and Lead stress impacts these properties. The study employed various assays to assess antioxidant potential, including the DPPH method and Ferric Reducing Ability of Plasma (FRAP) assay, and measured IC50 values to gauge antioxidant efficacy. The DPPH method demonstrated that the control group extracts had substantial free radical scavenging activity, with lower IC50 values indicating higher antioxidant potency. In contrast, extracts from Nickel and Lead-treated groups showed varying levels of inhibition, suggesting that metal stress influences the plant's antioxidant capacity, generally reducing its effectiveness. In terms of FRAP activity, the control group exhibited robust reducing power, comparable to ascorbic acid, highlighting its strong ability to neutralize ferric ions. The Nickel and Lead-treated extracts showed varied responses, reflecting how different metals impact the plant's redox balance and antioxidant mechanisms differently. This nuanced response underscores the complex interaction between metal stress and plant antioxidant defense systems. Antimicrobial activity was assessed using the well diffusion method against *Bacillus subtilis* and *Salmonella bongori*. Ciprofloxacin, the standard antimicrobial drug, showed significant inhibition of both microorganisms. The hydroalcoholic extracts from the Control group exhibited consistent and dose-dependent antimicrobial activity, reinforcing the potential of *Justicia adhatoda* for microbial control. Nickel-treated extracts showed variable antimicrobial responses with fluctuating zones of inhibition, while Lead-treated extracts generally maintained or even enhanced their antimicrobial activity at higher concentrations.

In conclusion, the study provides a comprehensive view of how Nickel and Lead stress affects the antioxidant and antimicrobial activities of *Justicia adhatoda*. The observed changes in bioactivity under metal stress highlight the intricate nature of plant-metal interactions and their impact on medicinal properties. These findings underscore the need for further research to

understand the molecular mechanisms underlying these effects, which could have implications for the use of *Justicia adhatoda* in medicine and microbial control.

References

- Singh S, Singh A, Singh B, Gupta RS. *Justicia adhatoda* Linn.: phytochemical and pharmacological review. J Pharm Res. 2010;3(8):1885-1887.
- Gupta S, Sharma SB, Singh UR. Antioxidant and anti diabetic activities of aqueous extract of *Justicia adhatoda* leaves in Alloxan-induced diabetic rats. Int J Pharm Sci Res. 2012;3(11):4326-4330.
- Bafna AR, Mishra SH. In vitro antioxidant activity of *Justicia adhatoda* leaf extracts. Int J Pharm Sci Res. 2010;1(2):80-86.
- Tiwari S, Singh S. In vitro antioxidant activities of *Justicia adhatoda* leaf extracts. J Appl Pharm Sci. 2011;1(8):157-160.
- Abubakar AR, Haque M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. J Pharm Bioallied Sci. 2020; 12(1): 1–10.
- Parkhe G, Jain P. Study of antioxidant potential of hydroalcoholic extract of *Anethum graveolens*. Career. Int J Sci Technol. 2018; 1(2):39-45.
- Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of ‘‘power’’: The FRAP assay. Anal Biochem 1996; 239:70-76.