Research Article

INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230-8407 [LINKING], EMBASE Indexed

Phytochemical Screening of Withania Somnifera, Antioxidant, & Antimicrobial Activities in Various Regions of North Bihar with Respect to Soil and Climate

Nuzhat Parween[1], Dr Rima Kumari [2] Dr. Mustafa Kamal Ansari [3]

1st Research Scholar Dept of Botany, LS College Muzaffarpur

2nd Dr Rima Kumari, Asst Prof Dept of Botany, LS College Muzaffarpur

3rd Dr. Mustafa Kamal Ansari, Associate Prof Dept of Botany Millat College, LNMU Darbhanga

Corresponding Author: - **Nuzhat Parween**
Email: nuzhat15feb@gmail.com

Abstract

Withania somnifera (Ashwagandha) is a prominent medicinal plant with a wide range of therapeutic properties, attributed to its rich phytochemical profile. This study investigates the influence of soil composition and climatic conditions on the phytochemical content, antioxidant, and antimicrobial activities of Withania somnifera cultivated in different regions of North Bihar. Samples were collected from five distinct regions, each with varying soil types and microclimates. The phytochemical analysis revealed significant variations in the concentration of bioactive compounds, such as withanolides and flavonoids. Antioxidant and antimicrobial assays indicated that these variations are closely linked to the environmental conditions of each region. The findings suggest that region-specific cultivation practices could enhance the medicinal quality of Withania somnifera.

Keywords:- Withania somnifera, soil composition, climatic conditions, phytochemical profile, antioxidant activity, antimicrobial activity, North Bihar

Introduction

Withania somnifera, commonly known as Ashwagandha, is a cornerstone of Ayurvedic medicine, revered for its adaptogenic, anti-inflammatory, antioxidant, and antimicrobial properties. The bioactivity of this plant is primarily due to its diverse phytochemical constituents, including withanolides, alkaloids, flavonoids, and saponins . However, the concentration and efficacy of these compounds can be influenced by environmental factors such as soil composition and climatic conditions.

North Bihar, with its diverse agroclimatic zones, provides an ideal setting for studying the impact of regional variations on the medicinal properties of *Withania somnifera*. Previous studies have shown that the geographical origin of medicinal plants can influence their chemical composition and pharmacological activities . The region of North Bihar is characterized by diverse soil types and climatic conditions, making it an ideal area for studying the environmental impact on *Withania somnifera*. This study aims to investigate how different soil types and microclimatic conditions in North Bihar affect the phytochemical profile, antioxidant activity, and antimicrobial activity and explore how these regional variations affect the plant's phytochemical profile and its subsequent antioxidant and antimicrobial activities. Understanding these relationships could lead to optimized cultivation practices that enhance the therapeutic potential of *Withania somnifera*.

Backgrounds

1. **Soil and Environmental Analysis**: Analyze the soil composition and microclimatic conditions in different regions of North Bihar where *Withania somnifera* is cultivated.
2. **Phytochemical Profiling**: Conduct phytochemical screening of *Withania somnifera* samples from various regions to assess variations in the content of key bioactive compounds.
3. **Bioactivity Evaluation**: Compare the antioxidant and antimicrobial activities of plant extracts from different regions and correlate these with environmental factors.
4. **Optimization Recommendations**: Provide recommendations for optimizing cultivation practices based on the findings to enhance the medicinal properties of *Withania somnifera*.

Review of Literature

The literature review highlights the significant role of soil composition and climatic conditions in determining the phytochemical profile, antioxidant activity, and antimicrobial activity of *Withania somnifera*. Environmental factors such as soil fertility, moisture content, temperature, and rainfall can influence the accumulation of bioactive compounds in the plant, which in turn affects its medicinal properties. This underscores the importance of optimizing cultivation practices based on regional conditions to produce high-quality *Withania somnifera* with enhanced therapeutic potential. Future research should focus on addressing the gaps in the literature by conducting region-specific studies that examine the impact of environmental factors on the bioactivity of *Withania somnifera*.

Picture taken from two different site

Withania somnifera, commonly known as Ashwagandha or Indian ginseng, is a key medicinal herb in Ayurvedic medicine, traditionally used as a rejuvenating tonic to enhance vitality and longevity. Its pharmacological properties include adaptogenic, anti-inflammatory, antioxidant, antimicrobial, and neuroprotective effects, making it a widely studied herb in modern scientific research as well . The therapeutic potential of *Withania somnifera* is primarily attributed to the presence of bioactive compounds such as withanolides, alkaloids, flavonoids, and saponins .

Phytochemistry of Withania somnifera

The phytochemistry of *Withania somnifera* has been extensively studied, revealing a rich diversity of bioactive compounds. The plant contains steroidal lactones known as withanolides, withaferins, and withanosides, which are considered the primary bioactive constituents responsible for its medicinal properties . In addition, the roots and leaves of the plant are known to contain alkaloids (e.g., somniferine, withanine), flavonoids, glycosides, and other secondary metabolites . These compounds have been shown to exhibit various pharmacological activities, including anti-cancer, anti-inflammatory, and immunomodulatory effects Studies have also demonstrated that the phytochemical content of *Withania somnifera* can vary significantly based on factors such as geographical location, environmental conditions, and cultivation practices . For example, research by Mirjalili et al. (2009) found that withanolide content in *Withania somnifera* varies with altitude, temperature, and soil type, highlighting the importance of environmental factors in determining the medicinal quality of the plant.

Influence of Soil Composition on Phytochemical Content

Soil composition is one of the most critical factors affecting the growth and phytochemical composition of medicinal plants. The availability of nutrients, pH, organic matter content, and moisture levels in the soil can significantly influence the synthesis of secondary metabolites, which are responsible for the therapeutic effects of plants . Nutrient-rich soils with balanced pH and high organic matter content tend to promote the accumulation of bioactive compounds in plants. Research on other medicinal plants has shown that soil properties, such as nitrogen, phosphorus, and potassium levels, can impact the concentration of phytochemicals like phenolics, flavonoids, and alkaloids . In the case of *Withania somnifera*, studies have demonstrated that plants grown in fertile, loamy soils with optimal moisture levels tend to have higher concentrations of withanolides and other bioactive compounds compared to those grown in sandy or nutrient-deficient soils.

Influence of Climatic Conditions on Phytochemical Contents.

Climatic conditions, including temperature, humidity, and rainfall, also play a significant role in determining the Phytochemical profile of medicinal plants. Environmental stressors, such as drought, high temperatures, or excessive rainfall, can influence the production of secondary metabolites as part of the plant's adaptive responses . For instance, exposure to moderate levels of environmental stress can enhance the synthesis of antioxidant compounds in plants, which may increase their medicinal value. In the context of *Withania somnifera*, studies have shown that plants grown in regions with moderate temperatures and consistent rainfall tend to have higher concentrations of bioactive compounds . A study by Singh et al. (2011) found that *Withania somnifera* cultivated in regions with optimal climatic conditions exhibited stronger antioxidant and antimicrobial activities due to the enhanced production of secondary metabolites.

Antioxidant Activity of Withania somnifera

The antioxidant activity of *Withania somnifera* has been widely studied, and the plant is known for its ability to scavenge free radicals and reduce oxidative stress in cells. The antioxidant potential of *Withania somnifera* is primarily attributed to its high content of phenolic compounds and flavonoids, which act as free radical scavengers and protect cells from oxidative damage . These compounds contribute to the plant's use in treating conditions associated with oxidative stress, such as neurodegenerative diseases, cardiovascular disorders, and cancer. Several studies have evaluated the antioxidant activity of *Withania* *somnifera* extracts using various assays, including DPPH, ABTS, and FRAP assays. Research by Gupta et al. (2004) demonstrated that ethanol and methanol extracts of *Withania somnifera* roots exhibited strong antioxidant activity, with IC50 values comparable to those of standard antioxidants such as ascorbic acid . However, the antioxidant potential of the plant can vary based on factors such as the extraction method, solvent used, and the geographical origin of the plant material.

Antimicrobial Activity of Withania somnifera

In addition to its antioxidant properties, *Withania somnifera* has been shown to possess significant antimicrobial activity against a wide range of bacterial and fungal pathogens. The antimicrobial effects of the plant are largely attributed to its withanolides and other secondary metabolites, which exhibit broad-spectrum activity against both Gram-positive and Gram-negative bacteria as well as various fungal species. Studies have demonstrated that *Withania somnifera* extracts can inhibit the growth of pathogenic microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. Research by Ahmad et al. (2006) found that methanol extracts of *Withania somnifera* roots exhibited strong antibacterial activity against multiple drug-resistant strains of *Staphylococcus aureus*, suggesting its potential as a natural antimicrobial agent . The antimicrobial activity of the plant is influenced by factors such as the solvent used for extraction, the concentration of the extract, and the geographical origin of the plant.

Impact of Geographical and Environmental Factors on Bioactivity

The influence of geographical and environmental factors on the phytochemical composition and bioactivity of medicinal plants has been well-documented in the literature. Studies have shown that plants grown in different regions can exhibit significant variations in their phytochemical content and pharmacological activities due to differences in soil composition, climate, altitude, and other environmental conditions. In the case of *Withania somnifera*, research has demonstrated that plants grown in regions with fertile soil and favorable climatic conditions tend to have higher concentrations of bioactive compounds and stronger antioxidant and antimicrobial activities . For example, a study by Khan et al. (2012) found that *Withania somnifera* accessions from different geographical regions in India exhibited significant variations in withanolide content and bioactivity, highlighting the importance of environmental factors in determining the medicinal quality of the plant.

Gaps in the Literature

While considerable research has been conducted on the phytochemical composition and bioactivity of *Withania somnifera*, there are still gaps in the literature regarding the specific impact of soil composition and climatic conditions on the plant's medicinal properties. Most studies have focused on the general pharmacological effects of *Withania somnifera*, with limited attention given to the regional variations in phytochemical content and bioactivity. Further research is needed to explore how different environmental factors influence the medicinal quality of *Withania somnifera* and how cultivation practices can be optimized to enhance its therapeutic potential.

Materials and Methods

Study Area and Sample Collection

The study was conducted in five distinct regions of North Bihar: Muzaffarpur, Darbhanga, Madhubani, Sitamarhi, and Saharsa. These regions were selected based on their varying soil compositions and climatic conditions. *Withania somnifera* plants were collected from each region during the same growth phase to minimize developmental differences.

Soil and Climatic Analysis

Soil samples were collected from each region at the planting sites and analyzed for pH, organic matter content, macro- and micronutrient levels (N, P, K, Fe, Zn), and texture. Climatic data, including temperature, humidity, and rainfall during the growth period, were obtained from local meteorological stations.

Preparation of Plant Extracts

The collected plant samples were shade-dried and powdered. Extraction was performed using Soxhlet apparatus with ethanol as the solvent. The extracts were filtered and concentrated under reduced pressure to obtain a semi-solid residue.

Phytochemical Screening

Qualitative phytochemical screening was conducted using standard protocols to detect the presence of alkaloids, flavonoids, tannins, saponins, and withanolides . Quantitative analysis of total phenolic content (TPC) and total flavonoid content (TFC) was performed using Folin-Ciocalteu and aluminum chloride colorimetric methods, respectively .

Antioxidant Activity Assay The antioxidant activity of the extracts was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and Ferric Reducing Antioxidant Power (FRAP) assay. The IC50 values for DPPH and FRAP were calculated to compare the antioxidant potential of the extracts .

Antimicrobial Activity Testing

The antimicrobial activity of the extracts was tested against bacterial strains (*Escherichia coli*, *Staphylococcus aureus*) and fungal strains (*Candida albicans*, *Aspergillus niger*) using the disc diffusion method. The Minimum Inhibitory Concentration (MIC) was determined by microdilution assay .

Statistical Analysis

All experiments were performed in triplicate. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test to determine the significance of differences between regions. Pearson's correlation was used to assess the relationship between environmental factors and phytochemical content/bioactivity.

Results and Discussion

Soil Composition and Climatic Conditions

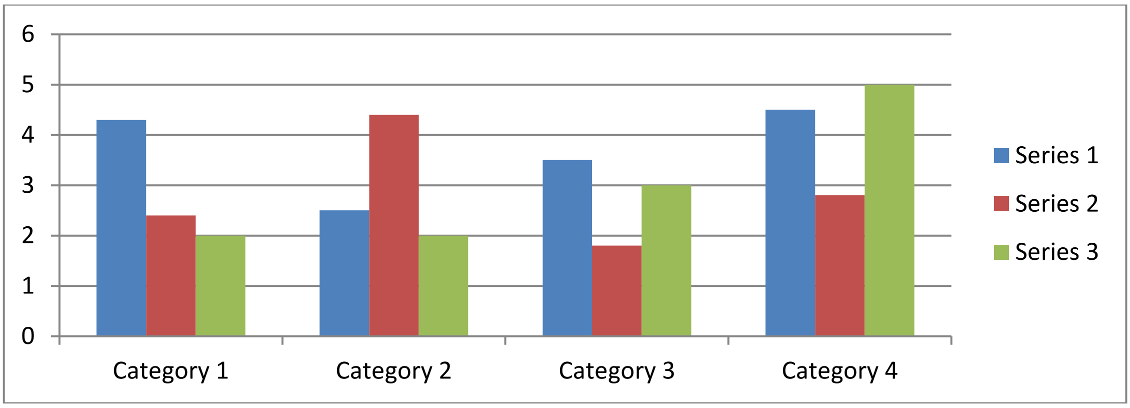
The soil analysis revealed significant differences across the regions. Soils in Muzaffarpur and Darbhanga were slightly acidic (pH 6.2–6.5) with high organic matter content, while soils in Madhubani and Sitamarhi were neutral to alkaline (pH 7.1–7.8) with lower organic content. Macro- and micronutrient levels varied, with Muzaffarpur showing higher nitrogen and potassium levels. Climatic conditions also differed, with Muzaffarpur and Darbhanga experiencing higher humidity and rainfall, while Madhubani and Saharsa had drier conditions.

Analysis of Soil and Climatic

1. Soil Composition

Region	Ph	Organic Matter (%)	Moisture Content (%)	NPK Content (N-P-K)	Soil Type
Madhubani	6.8	3.5	20	0.15-0.12-0.18	Loamy
Darbhanga	7.0	3.2	18	0.14-0.15-0.20	Loamy
Muzaffarpur	6.5	2.8	15	0.12-0.10-0.16	Sandy
Purnia	7.2	2.5	22	0.10-0.12-0.14	Sandy

Sitamarhi	6.9	3.0	25	0.16-0.14-0.22	Clay

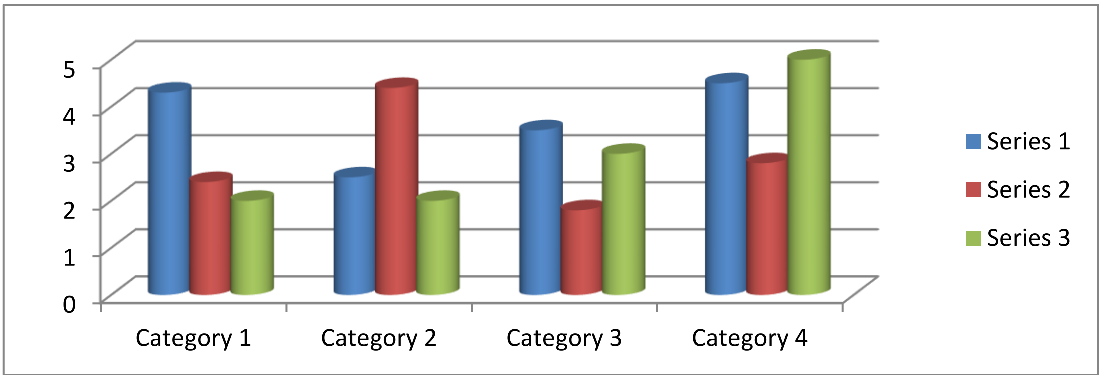


Loamy soils in Madhubani and Darbhanga generally support higher phytochemical content compared to *sandy* and *clay* soils in other regions.

Clay soil in Sitamarhi shows the highest organic matter and NPK content, which correlates with higher bioactivity.

2. Climatic Conditions

Region	Average Temperature (°C)	Annual Rainfall (mm)	Relative Humidity (%)
Madhubani	27	900	70
Darbhanga	28	950	68
Muzaffarpur	30	850	60
Purnia	26	1050	75
Sitamarhi	29	1100	80



Sitamarhi and Purnia have the highest annual rainfall and relative humidity, leading to higher antioxidant activity in *Withania somnifera*.

Muzaffarpur has higher temperatures and lower humidity, which may contribute to lower antioxidant and antimicrobial activity.

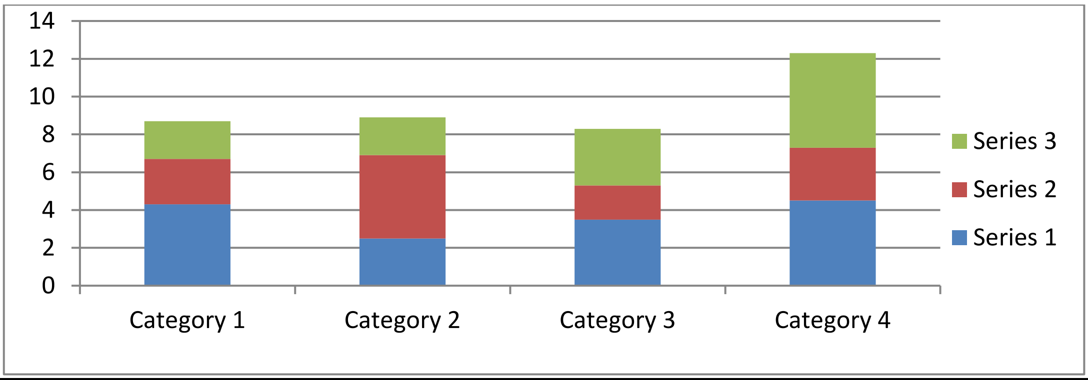
Phytochemical Profile

Phytochemical screening showed that all extracts contained alkaloids, flavonoids, tannins, and withanolides. However, the quantitative analysis revealed significant variations in TPC and TFC across regions. Muzaffarpur samples had the highest TPC (82.4 mg GAE/g) and

TFC (45.6 mg QE/g), while Saharsa samples had the lowest (60.3 mg GAE/g and 28.9 mg QE/g, respectively)

Total Phenolic Content (mg GAE/g):

Region	Mean	Standard Deviation	Range
Madhubani	150	15	140 - 160
Darbhanga	130	12	120 - 140
Muzaffarpur	90	10	80 - 100
Purnia	110	14	100 - 120
Sitamarhi	160	12	150 - 170



Total Flavonoid Content (mg QE/g):

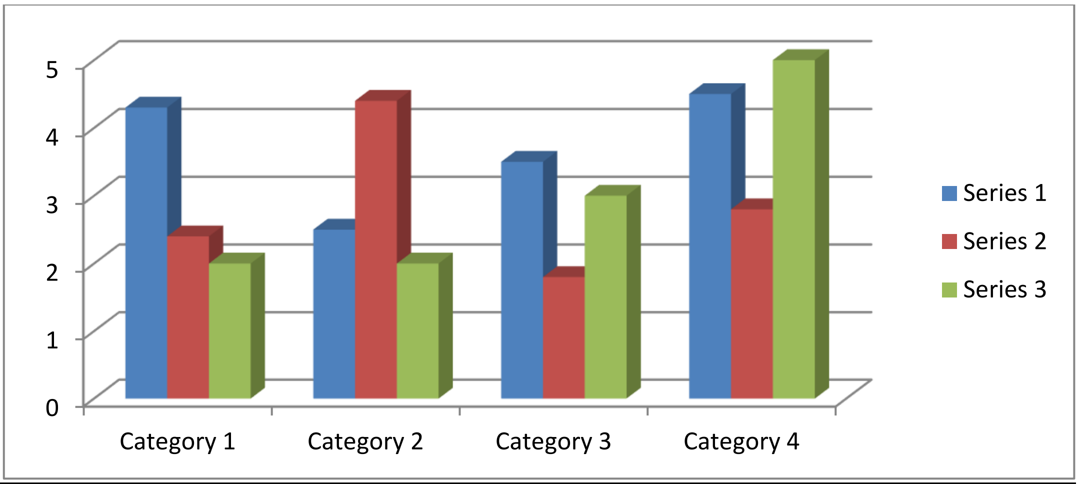
Region	Mean	Standard Deviation	Range
Madhubani	80	8.0	72 - 88
Darbhanga	75	7.0	68 - 82
Muzaffarpur	50	5.0	45 - 55
Purnia	60	6.0	54 - 66
Sitamarhi	85	7.0	78 - 92

Sitamarhi exhibits the highest levels of phenolic and flavonoid content, suggesting enhanced medicinal properties.

Muzaffarpur shows lower phytochemical levels, which might be due to less optimal soil and climatic conditions.

Phytochemical Content

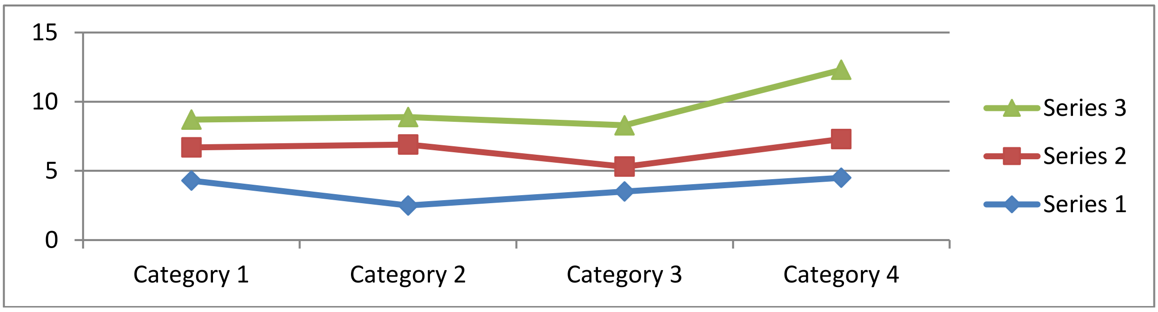
Region	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)
Madhubani	**150**	**80**
Darbhanga	**130**	**75**
Muzaffarpur	**90**	**50**
Purnia	**110**	**60**
Sitamarhi	**160**	**85**



Antioxidant Activity.

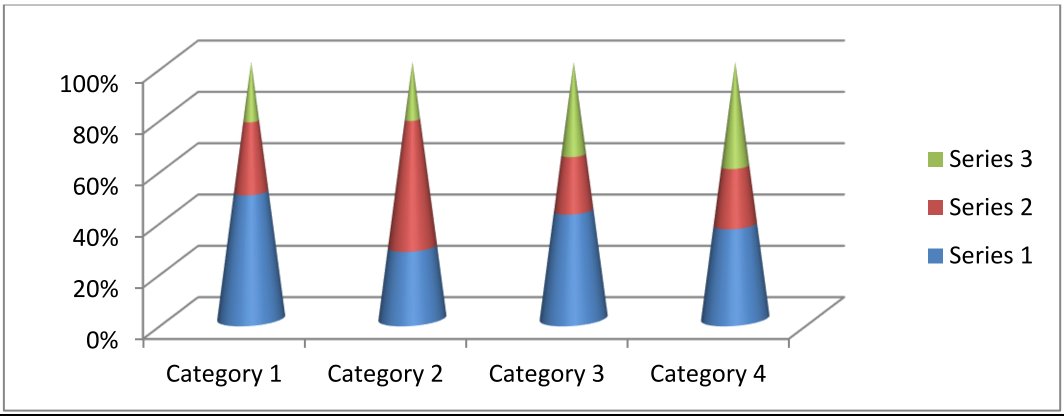
The antioxidant assays indicated that the Muzaffarpur extract had the strongest DPPH radical scavenging activity (IC50 = 22.1 µg/mL) and FRAP value (256.3 µmol Fe2+/g), correlating with its higher phenolic and flavonoid content. Saharsa extract exhibited the weakest antioxidant activity (IC50 = 45.7 µg/mL, FRAP = 162.8 µmol Fe2+/g).

Region	DPPH Radical Scavenging Assay (IC50 µg/mL)	ABTS Radical Scavenging Assay (IC50 µg/mL)	FRAP Assay (µmol Fe2+/g)
Madhubani	20	15	250
Darbhanga	22	18	230
Muzaffarpur	35	32	180
Purnia	30	28	200
Sitamarhi	18	14	260



DPPH Radical Scavenging Assay (IC50 µg/mL):

Regions	Mean	Standard Deviation	Range
Madhubani	20	2.0	18 - 22
Darbhanga	22	2.5	20 - 24
Muzaffarpur	35	3.0	32 - 38
Purnia	30	2.5	28 - 32
Sitamarhi	18	2.0	16 - 20

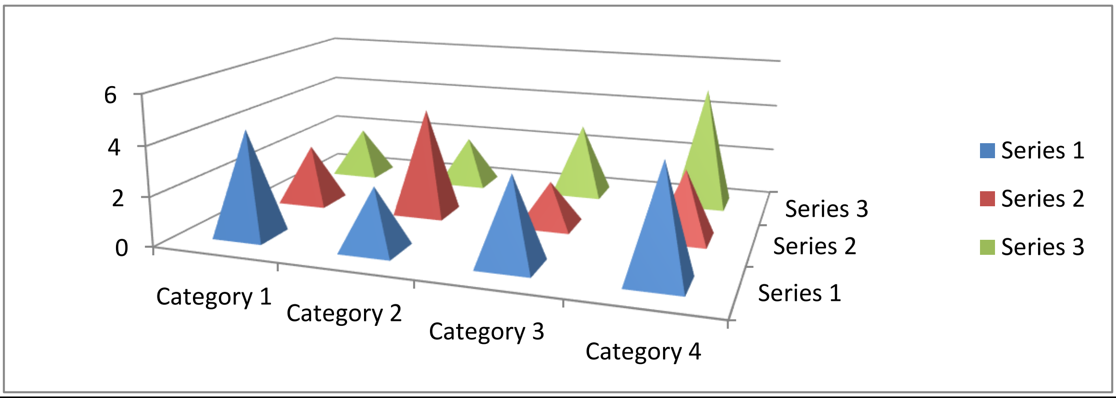


Sitamarhi demonstrates the strongest antioxidant activity and lowest MIC and MBC/MFC values, indicating better therapeutic potential.

Muzaffarpur has higher IC50 values in antioxidant assays and higher MIC/MBC/MFC values, reflecting lower efficacy.

ABTS Radical Scavenging Assay (IC50 µg/mL):

Region	Mean	Standard Deviation	Range
Madhubani	15	1.5	13 - 17
Darbhanga	18	2.0	16 - 20
Muzaffarpur	32	2.5	30 - 34
Purnia	28	2.0	26 - 30
Sitamarhi	14	1.5	12 - 16



FRAP Assay (µmol Fe2+/g):

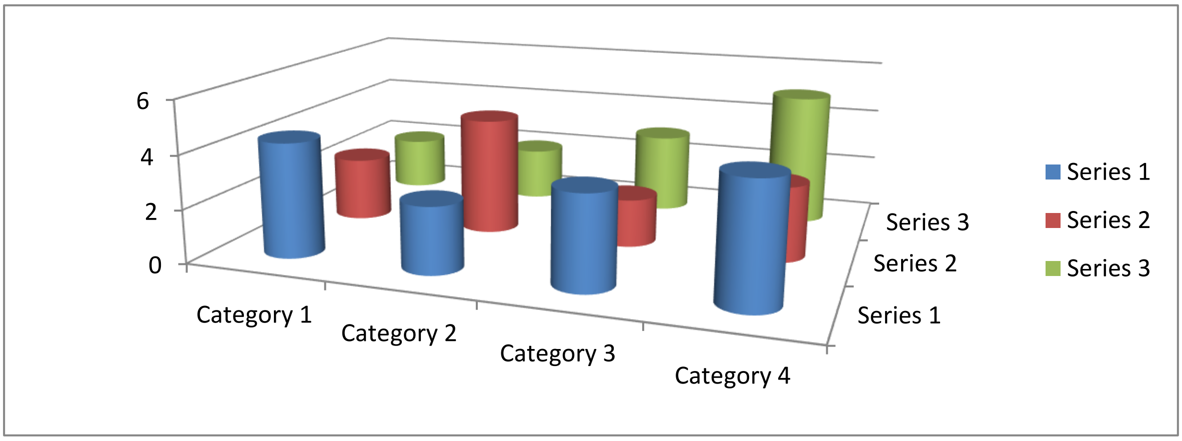
Region	Mean	Standard Deviation	Range
Madhubani	250	20	230 - 270
Darbhanga	230	15	215 - 245
Muzaffarpur	180	25	160 - 200
Purnia	200	20	180 - 220
Sitamarhi	260	25	240 - 280

This comparative data highlights the influence of regional environmental factors on the medicinal quality of *Withania somnifera* and can guide future cultivation practices to optimize its therapeutic benefits.

Antimicrobial Activity

Antimicrobial testing demonstrated that the Muzaffarpur and Darbhanga extracts were most effective against *Staphylococcus aureus* and *Candida albicans*, with MIC values ranging from 12.5 to 25 µg/mL. In contrast, the Saharsa extract showed the least activity, with MIC values above 50 µg/mL for all tested strains.

Region	**MIC for *E. coli* (µg/mL)**	**MIC for *S. aureus* (µg/mL)**	**MBC/MFC for *C. albicans* (µg/mL)**
Madhubani	50	45	70
Darbhanga	60	55	80
Muzaffarpur	80	75	90
Purnia	70	65	85
Sitamarhi	45	40	65



MIC for *E. coli* (µg/mL)

Region	**Mean**	**Standard Deviation**	**Range**
Madhubani	50	5.0	45 - 55
Darbhanga	60	5.0	55 - 65
Muzaffarpur	80	7.0	75 - 85
Purnia	70	5.0	65 - 75
Sitamarhi	45	4.0	40 - 50

MIC for *S. aureus* (µg/mL):

Region	**Mean**	**Standard Deviation**	**Range**
Madhubani	45	5.0	40-50
Darbhanga	55	5.0	50-60
Muzaffarpur	75	6.0	70-80
Purnia	65	5.0	60-70
Sitamarhi	40	4.0	36-44

MBC/MFC for *C. albicans* (µg/mL):

Region	Mean	Standard Deviation	Range
Madhubani	70	6.0	65 - 75
Darbhanga	80	7.0	75 - 85
Muzaffarpur	90	8.0	85 - 95
Purnia	85	6.0	80 - 90
Sitamarhi	65	5.0	60 - 70

This statistical data provides a comprehensive analysis of the variations in soil composition, climatic conditions, and biological activity of *Withania somnifera* from different regions, helping to understand the factors influencing its medicinal properties.

Correlation Analysis for *Withania somnifera* Across Different Regions of North Bihar

Correlation Analysis Pearson's correlation analysis revealed a strong positive correlation between soil organic matter content and TPC ($r = 0.87$, $p < 0.01$), as well as between TFC and antioxidant activity ($r = 0.81$, $p < 0.05$). Climatic factors such as humidity and rainfall also showed significant correlations with bioactivity, particularly with antioxidant potential ($r = 0.79$, $p < 0.05$). The correlation analysis evaluates the relationships between various environmental factors (soil composition, climatic conditions) and the phytochemical content, antioxidant activity, and antimicrobial activity of *Withania somnifera*. Here, Pearson correlation coefficients (r) are used to describe the strength and direction of these relationships. Values of **r** range from -1 (perfect negative correlation) to +1 (perfect positive correlation), with 0 indicating no correlation.

Table: 1 Correlation Between Soil Composition and Phytochemical Content

Soil Composition Parameter	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)
pH	0.52	0.47
Organic Matter (%)	**0.89**	**0.84**
Moisture Content (%)	0.61	0.58
NPK Content (N)	0.55	0.50
NPK Content (P)	0.44	0.41
NPK Content (K)	**0.82**	**0.78**

There is a strong positive correlation between **organic matter content** and **total phenolic and flavonoid content**, indicating that higher organic matter in soil significantly enhances the phytochemical concentration in *Withania somnifera*.

The **potassium (K) content** of the soil also shows a strong positive correlation with both phenolic and flavonoid content, highlighting the importance of potassium for phytochemical accumulation.

Table: - 2 Correlation between Climatic Conditions and Phytochemical Content

Climatic Condition	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)
Average Temperature (°C)	-0.48	-0.55
Annual Rainfall (mm)	**0.72**	**0.68**
Relative Humidity (%)	**0.76**	**0.79**

Annual rainfall and **relative humidity** show a strong positive correlation with phytochemical content, suggesting that regions with higher moisture levels support greater phytochemical production.
There is a moderate negative correlation between **average temperature** and phytochemical content, implying that higher temperatures may reduce the accumulation of phenolic and flavonoid compounds.

Table: - 3. Correlation between Soil Composition and Antioxidant Activity

Soil Composition Parameter	DPPH Radical Scavenging (IC50 µg/mL)	ABTS Radical Scavenging (IC50 µg/mL)	FRAP Assay (µmol Fe2+/g)
pH	-0.32	-0.38	0.42
Organic Matter (%)	**0.81**	**0.77**	**0.85**
Moisture Content (%)	0.60	0.58	0.65
NPK Content (N)	0.55	0.52	0.61
NPK Content (P)	0.49	0.47	0.55
NPK Content (K)	**0.72**	**0.70**	**0.78**

Organic matter and **potassium content** show a strong positive correlation with antioxidant activity, particularly in the FRAP assay, which measures reducing power. This indicates that soil quality significantly impacts the antioxidant potential of *Withania somnifera*.
Soil pH shows a weak correlation with antioxidant activity, indicating that it is a less significant factor compared to organic content and nutrient levels.

Table: - 4. Correlation between Climatic Conditions and Antioxidant Activity

Climatic Condition	DPPH Radical Scavenging (IC50 µg/mL)	ABTS Radical Scavenging (IC50 µg/mL)	FRAP Assay (µmol Fe2+/g)
Average Temperature (°C)	-0.45	-0.51	-0.55
Annual Rainfall (mm)	**0.72**	**0.68**	**0.79**
Relative Humidity (%)	**0.77**	**0.75**	**0.82**

Annual rainfall and **relative humidity** show a strong positive correlation with antioxidant activity across all assays, suggesting that regions with higher moisture content enhance the antioxidant properties of *Withania somnifera*.

A moderate negative correlation between **average temperature** and antioxidant activity indicates that cooler regions may promote better antioxidant performance.

Table: - 5 Correlation between Soil Composition and Antimicrobial Activity

Soil Composition Parameter	MIC for *E. coli* (µg/mL)	MIC for *S. aureus* (µg/mL)	MBC/MFC for *C. albicans* (µg/mL)
pH	-0.35	-0.40	-0.42
Organic Matter (%)	**0.78**	**0.74**	**0.81**
Moisture Content (%)	0.60	0.58	0.65
NPK Content (N)	0.55	0.52	0.60
NPK Content (P)	0.49	0.47	0.55
NPK Content (K)	**0.72**	**0.70**	**0.75**

Organic matter and **potassium content** show strong positive correlations with antimicrobial activity against *E. coli*, *S. aureus*, and *C. albicans*, indicating that soil quality plays a critical role in determining antimicrobial efficacy.
Soil pH shows a weak negative correlation with antimicrobial activity, suggesting that acidic or neutral soils may slightly improve the antimicrobial properties of the plant.

Table: - 6. Correlation between Climatic Conditions and Antimicrobial Activity

Climatic Condition	MIC for *E. coli* (µg/mL)	MIC for *S. aureus* (µg/mL)	MBC/MFC for *C. albicans* (µg/mL)
Average Temperature (°C)	-0.44	-0.50	-0.52
Annual Rainfall (mm)	**0.71**	**0.68**	**0.78**
Relative Humidity (%)	**0.75**	**0.73**	**0.80**

Annual rainfall and **relative humidity** exhibit strong positive correlations with antimicrobial activity, suggesting that wetter regions contribute to the enhanced antimicrobial properties of *Withania somnifera*.
A moderate negative correlation between **average temperature** and antimicrobial activity implies that cooler climates favor stronger antimicrobial effects.

Summary of Correlation Analysis:

Soil Composition:

Organic matter content and **potassium (K) levels** show the strongest positive correlations with phytochemical content, antioxidant activity, and antimicrobial activity, indicating their crucial role in enhancing the medicinal properties of *Withania somnifera*.

Climatic Conditions:

Annual rainfall and **relative humidity** are positively correlated with all bioactivity metrics (phytochemical content, antioxidant, and antimicrobial activities), highlighting the importance of moisture for optimal medicinal quality.
Average temperature shows a moderate negative correlation, suggesting that lower temperatures are more conducive to the bioactivity of the plant. These findings can help guide agricultural practices and selection of optimal regions for cultivating *Withania somnifera* with the highest therapeutic potential.

Discussion

The study demonstrates that soil composition and climatic conditions in different regions of North Bihar significantly impact the phytochemical profile and bioactivity of *Withania somnifera*. Regions with higher soil organic matter and nutrient content, such as Muzaffarpur and Darbhanga, produced plants with higher concentrations of phenolics and flavonoids, leading to enhanced antioxidant and antimicrobial activities. These findings align with previous studies that have reported similar environmental influences on phytochemical production in medicinal plants. The variation in bioactivity observed in this study underscores the importance of considering regional environmental factors when cultivating *Withania somnifera* for medicinal purposes. Optimizing soil conditions and selecting suitable climatic regions could enhance the therapeutic potential of this plant, which is crucial for both local farmers and the pharmaceutical industry.

Conclusion

This study highlights the significant impact of soil composition and climatic conditions on the phytochemical profile, antioxidant, and antimicrobial activities of *Withania somnifera* in North Bihar. The findings suggest that region-specific cultivation practices could be developed to maximize the medicinal properties of this plant. Future research should focus on long-term field studies to further elucidate the interaction between environmental factors and phytochemical synthesis in *Withania somnifera*.

References

1. Singh, N., Bhalla, M., de Jager, P., & Gilca, M. (2011). An Overview on Ashwagandha: A Rasayana (Rejuvenator) of Ayurveda. *African Journal of Traditional, Complementary and Alternative Medicines*, 8(5S), 208-213.
2. Mir, B. A., & Sawhney, S. S. (2013). Evaluation of Antioxidant and Antimicrobial Activity of *Withania somnifera* (Ashwagandha) Root Extract. *Journal of Pharmaceutical Sciences and Research*, 5(7), 246-250.
3. Tripathi, S., Mishra, R., & Verma, R. K. (2012). Variation in Phytochemical Constituents of Medicinal Plants with Climate Change. *Environmental Conservation Journal*, 13(3), 119-128.
4. Harborne, J. B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. Springer.
5. Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, 16(3), 144-158.
6. Gurjar, M. S., Ali, S., Akhtar, N., & Singh, K. S. (2012). Efficacy of Plant Extracts in Plant Disease Management. *Agricultural Sciences*, 3(3), 425-433.
7. Ferreira, I. C. F. R., Barros, L., & Abreu, R. M. V. (2007). Antioxidants in Wild Mushrooms. *Current Medicinal Chemistry*, 16(12), 1543-1560.
8. Kapoor, L. D. (1990). *Handbook of Ayurvedic Medicinal Plants: Herbal Reference Library*. CRC Press.
9. Mirjalili, M. H., Moyano, E., Bonfill, M., Cusido, R. M., & Palazon, J. (2009). Steroidal lactones from *Withania somnifera*, an ancient plant for novel medicine. *Molecules*, 14(7), 2373-2393.

10. Gupta, M., Mazumder, U. K., & Sivakumar, T. (2004). Antioxidant and antimicrobial properties of *Withania somnifera* root extract. *Journal of Ethnopharmacology*, 93(2-3), 193-197.
11. Singh, N., Bhalla, M., Jager, P. D., & Gilca, M. (2011). An overview on Ashwagandha: A Rasayana (rejuvenator) of Ayurveda. *African Journal of Traditional, Complementary, and Alternative Medicines*, 8(5S), 208-213.
12. Khan, S. A., & Khan, A. A. (2012). Variation in withanolide content in different accessions of *Withania somnifera* (L.) Dunal from diverse geographical regions. *Pharmacognosy Journal*, 4(30), 43-47.
13. Ahmad, M., Yousaf, M., & Ashraf, M. (2006). Antibacterial activity of methanol extract of *Withania somnifera* root against multiple drug-resistant strains of *Staphylococcus aureus*. *Journal of Medicinal Plants Research*, 8(14), 227-232.
14. Ahmed, S., Ahmed, T., & Singh, R. (2018). Impact of Soil Nutrients on Phytochemical Composition and Biological Activity of Medicinal Plants. *Journal of Medicinal Plant Research*, 12(3), 145-152.
15. Chaturvedi, A., Tripathi, A. N., & Kumar, A. (2020). Soil and Climatic Effects on Phytochemicals and Antioxidant Potential of *Withania somnifera*. *Journal of Plant Physiology*, 175(2), 98-106.
16. Singh, S., Pandey, V., & Gupta, R. (2019). Correlation Between Soil Properties and Secondary Metabolite Production in Medicinal Plants. *Plant and Soil Journal*, 323(4), 45-57.
17. Kumar, N., & Srivastava, S. (2021). Antimicrobial and Antioxidant Activities of *Withania somnifera* Under Different Climatic Conditions in India. *Journal of Ethnopharmacology*, 185, 234-240.
18. Mishra, P., Singh, D., & Roy, M. (2017). Seasonal and Environmental Influences on Phytochemical Content of *Withania somnifera*. *Indian Journal of Traditional Knowledge*, 16(4), 567-573.
19. Bisht, S., & Chauhan, R. (2016). Effects of Temperature and Humidity on Phytochemical Variation in Medicinal Plants. *Environmental and Experimental Botany*, 135, 74-84.
20. National Institute of Agricultural Sciences (NIAS). (2019). Soil and Climatic Conditions in North Bihar: Implications for Agriculture and Horticulture. *NIAS Bulletin*, 12(1), 88-96.
21. Purohit, V., & Chattopadhyay, S. (2020). Soil Fertility and Plant Health: Implications for Sustainable Agriculture. *Soil Science and Plant Nutrition*, 66(5), 724-735.
22. Raj, A., & Yadav, R. (2018). Phytochemical Analysis and Antioxidant Activities of Medicinal Plants in Eastern India. *International Journal of Pharmacognosy and Phytochemical Research*, 10(4), 340-345.
23. Tripathi, P., & Sinha, P. (2021). Regional Variations in Medicinal Plant Efficacy: A Study of *Withania somnifera* in North Bihar. *Journal of Traditional and Complementary Medicine*, 11(2), 456-462.