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Quantitative Estimation of Alkaloids and Flavonoids in Wild and Cultivated Varieties of *Rauvolfia Serpentina*

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ABSTRACT

This study is a quantitative assessment of the alkaloid and flavonoid content of the wild and cultivated varieties of a well-known and richly endowed medicinal plant, *Rauvolfia Serpentina*. Total alkaloids, specific indole alkaloids (reserpine, rescinnamine and ajmaline), and total flavonoids were determined by standardized field sampling, solvent extracts, UV-Vis spectrophotometry, and UHPLC of roots and leaves in 15 wild and 15 cultivated plants. Metabolite Profiles Thin-Layer chromatography (TLC) was used as qualitative confirmation of metabolite profiles. Findings demonstrated that all targeted compounds were more concentrated in wild plants and extract yields were greater in those plants than in cultivated ones. Soil analysis identified that there was a high positive relationship between organic matter and amount of metabolites and a negative relationship between soil pH, which shows that acidic and organic rich soils support the production of secondary metabolites. Two-way ANOVA indicated that the condition of growth and type of tissue had significant effects on the levels of phytochemicals, and the effect of the interaction between them was moderate, whereas PCA allowed the differentiation of wild and cultivated samples by high loadings of reserpine, rescinnamine, and total alkaloids. These discoveries establish the importance of wild R. serpentina population ecologically and agronomically and such reflection of improvement lends scientific support to sustainable harvesting, better cultivation processes through replication of wild traits and quality in herbal medicinal which is drug manufacturing process.

Key Words:

Quantitative Estimation, Alkaloids, Flavonoids, Wild, Cultivated Varieties, *Rauvolfia Serpentina*

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1. INTRODUCTION

Quantitative analysis of alkaloids and flavonoids in wild and cultivated specimen of *Rauvolfia Serpentina* has been recognized due to medicinal value of the plant as pharmacognosy and the medicinal plants studies[1]. *Rauvolfia Serpentina*, also called Indian snakeroot or sarpagandha is a medicinal plant that is used because of its bioactive alkaloid such as reserpine, rescinnamine and ajmaline that have wide clinical application in the treatment of hypertension, mental disorders, and other cardiovascular diseases[2]. The other important group of phytochemicals found in the plant are flavonoids which add up to the anti-oxidant, anti-inflammatory and protective characteristics of the plant[3]. Quantitative analysis allows quantitative comparisons to be made between the phytochemical content in the wild and cultivated varieties, which can give some insight as to how environmental factors, soil composition, cultivation techniques and genetic variation determine the biosynthesis of these secondary metabolites[4]. These studies will play a critical role in standardization, quality assurance and sustainability in the utilization of this medicinal plant that is eventually useful in selection of high yielding seeds that can be cultivated on a commercial scale as well as conservation measures[5].

1.1. Background Information

Rauvolfia Serpentina is a medicinally valuable plant richly endowed with indole alkaloids, which contain antihypertensive, sedative as well as therapeutic values, reserpine, rescinnamine and ajmaline[6]. The plant also contains flavonoids which have commendable antioxidants[7]. The plant is both cultivated and growing in the wild yet variations in secondary metabolite content of the two sources are unexplored[8]. Microclimatic conditions, soil factors, and environmental conditions are important environmental variables determining phytochemical profile directly related to the quality of the medicines and commercial value[9].

1.2. Statement of the Problem

In spite of medicinal recognition of R. serpentina, little quantitative field-based evidence exists to correlate differences in alkaloid and flavonoid composition between wild and cultivated plants particularly between its roots and leaves[10]. Past research is commonly limited to artificial conditions, the evaluation of one compound, or is a review that lacks the study of the actual situation of phytochemicals and how they may be related to other soil characteristics. This incomplete data impedes best-practiced high cultivation methods and sustainable harvesting plans.

1.3. Objectives of the Study

The primary objectives of this study are:

- To determine concentration of total alkaloids, specific indole alkaloids (reserpine, rescinnamine, ajmaline), and total flavonoid in roots and leaves of wild and cultivated R. serpentina.
- To make a statistically valid comparison between the levels of phytochemicals in the two growth conditions (wild vs. cultivated) and the tissues of the plants (root vs. leaf).
- To study the connection between soil characteristics (organic matter, pH) and the content of secondary products.
- To authenticate the quantitative data by TLC profiling of alkaloids and flavonoid compounds.
- To make ecologically applicable suggestions on how to increase phytochemical productivities in cultured R. serpentina and direct conservation attempts.

2. RESEARCH METHODOLOGY

A cross-sectional, laboratory based analytical study involving a laboratory based analysis was used to compare the secondary-metabolite profiles between wild and cultivated populations of *Rauvolfia Serpentina*. The investigation concentrated on measuring (i) total alkaloid contents and major indole alkaloids (e.g. reserpine, rescinnamine, ajmaline) and (ii) total flavonoid contents in the root and leaf tissues. Techniques involved standardized-field sampling, standardized authentication of voucher specimens, solvent specifications, and orthogonal quantification using both UV -Vis spectral photometry and UHPLC.

2.1. Description of Research Design

Our design is a 2-factor, comparative analytical type (growth condition, wild vs cultivated and plant tissue, root vs leaf). Cohort 1 and 2 were measured under the same conditions in the laboratory, a priori fixed sampling sites were analysed. Each biological replicate was measured technologically in triplicate. The most important parameters were (a) the total alkaloid (mg atropine equivalents/g DW), (b) the targeted indole alkaloids (mg/g DW) by UHPLC, and (c) the total flavonoids content (mg quercetin equivalents/g DW).

2.2. Sample Details

We selected 30 mature plants (15 wild ones belonging to three wild populations (five plants at each site), and 15 cultivated ones (eight and 7 plants, respectively) belonging to two accredited medicinal-plant production farms). Plants had to be disease-free and similar in terms of phenological stage and this was an inclusion criterion. Of each plant we took ~20 g of root and ~20 g of young leaves. One of the taxonomist confirmed the botanical identity and Deposited voucher specimens in an institutional herbarium. A sample of the soil in the two sampling points was taken to conduct the exploratory correlations (pH, organic matter). All the plant matter was shade-dried to a constant weight (30 35 o C) and ground to a 60-mesh particle size.

2.3. Instruments and Materials Used

The extractions were done with an analytical balance (±0.01 mg), a Soxhlet apparatus and an ultrasonic bath, a thermostatic water bath, and a refrigerated centrifuge was also involved. The quantitation was done on UVVis spectrophotometer (scan 200-800 nm) and UHPLC system equipped with a C18 column (150 × 2.1 mm, 1.7 mum) followed by PDA. The reagents were methanol (HPLC-grade), ethanol, 1 percent HCl in methanol, ammonium acetate buffer, acetonitrile, formic acid, Dragendorffs reagent (qualitative) and bromocresol green (BCG) as total alkaloids and aluminium chloride as total flavonoids. Reserpine, rescinnamine, and ajmaline (98% or higher), and quercetin were used as reference standards, thereby calibrating them. Standard glassware, GPS, and soil -pH meter were also used.

2.4. Procedure and Data Collection Methods

To measure qualified data regarding alkaloids and flavours in *Rauvolfia Serpentina*, standardized and rigid extracting and analyzing processes had been used. The extraction of alkaloids was performed through hexane defatting and acidified methanol in ultrasonication and Soxhlet extractions after which the concentration was determined by using Bromocresol Green (BCG) assay and Ultra-fast performance liquid chromatography (UHPLC) with atropine standards to calibrate the outcomes. The extraction of flavonoids was done in the dried plant tissue in the presence of 80% methanol and the detection of this flavonoid was carried out with the help of AlCl 3 colorimetric test using quercetin as a standard. The qualitative profiling on the two compounds was performed via TLC. Reagent blanks, spiked recovery, precision tests and triplicate tests were used to control the quality during the study, which ensures the reliability and inability of the data to reproduce.

2.5. Data Analysis Techniques

We had verified normality (Shapiro Wilk) and homogeneity (Levene test). The between-group comparisons (wild vs cultivated) in each tissue were carried out in the form of independent-samples t-tests; the comparison of both factors together (growth condition × tissue) was conducted in the form of two- way ANOVA using Tukey HSD. The result was reported in a form of effect sizes (Hedges g or partial η^2) and 95 % confidence intervals. The run list of UHPLC was treated to normalize areas of the peak processing area and then quantified using a linear regression ($R^2 = 0.995$). We analyzed connections between metabolites concentrations and soil parameters with Pearson correlations, and principal component analysis (PCA). The level of statistical significance was fixed at 0.05 and false discovery rate (Benjamini Howchberg) was resorted to when multiple comparisons took place. Means and standard deviations were computed for results, and presented as bar and loading plots.

3. RESULTS

In this section, the comparative results of phytochemical composition, extraction yields and soil-metabolite interactions in *Rauvolfia Serpentina* grown wild and cultivated are provided. Variation in the levels of alkaloids and flavonoids between roots and leaves was evaluated using quantitative analyses (e.g. spectrophotometric assessments and UHPLC profiling), and the percentage extract yields were used to gain an understanding of the recovery of phytochemical levels. Correlations were made between soil parameters and metabolite abundance to investigate the environmental factors, and TLC profiling served as a qualitative measure of differences in metabolite abundance. These differences were supported by statistical analyses of two-way ANOVA, estimation of effect sizes, correlation analysis, and PCA to determine the patterns in phytochemical variations.

3.1. Presentation of Findings

This part reports the experimental results of the phytochemical composition, yield of the extracts and soil-metabolite associations in *Rauvolfia Serpentina* in the wild and in cultivation. Spectrophotometric assays, together with UHPLC profiling, were used to quantitatively compare the alkaloid and flavonoid content between roots and leaves, and the percentage yield of extracts was also determined in order to estimate its overall phytochemical retention. Metabolite profiling by TLC qualitative analysis and correlation to soil parameters gained an insight into environmental factors, which may modify accumulation of metabolites, and differences in abundance of metabolites between the growth conditions were further validated by correlation analysis. When combined, these findings are indicative of the integrated effect of growth environment and plant tissue on phytochemical variation.

- **Total Alkaloid Content (BCG Assay)**

The level of alkaloids was much higher in wild roots, which contrasted with the cultivated roots. Leaves also exhibited similar trend but with less concentrations in general. Table 1 shows the comparative overall level of alkaloid, which is estimated in mg atropine r.e./g D.W. of roots and leaves of wild and cultivated *Rauvolfia Serpentina*. Each tissue type is presented as mean ± standard deviation, and the percentage difference between the conditions of its growth and p-values reflecting the statistical significance of the result is presented.

Table 1: Total alkaloid content (mg atropine equivalents/g DW) in wild and cultivated R. serpentina.

Tissue Type	Wild (Mean ± SD)	Cultivated (Mean ± SD)	% Difference	p-value
Roots	18.42 ± 1.15	14.27 ± 1.02	+29.1%	0.002**
Leaves	9.34 ± 0.88	7.81 ± 0.79	+19.5%	0.015*

Wild R. serpentina developed greater total alkaloid contents in roots and leaves compared to grown-up flora. Wild plants in their roots had 18.42 ± 1.15 mg/g DW compared to 14.27 ± 1.02 mg/g DW in cultivated plants (+29.1%, p = 0.002), while leaves showed 9.34 ± 0.88 mg/g DW versus 7.81 ± 0.79 mg/g DW (+19.5%, p = 0.015), suggesting that there is a large effect of growth condition on the accumulation of alkaloids.

Figure 1 gives a comparative finding of mean value of total alkaloid content of leaves and roots of wild and domesticated *Rauvolfia Serpentina* of stem of the plant, in the mode of mean ± standard deviation. The figure depicts the difference on the alkaloid concentration of the different parts of plants and type of cultivation.

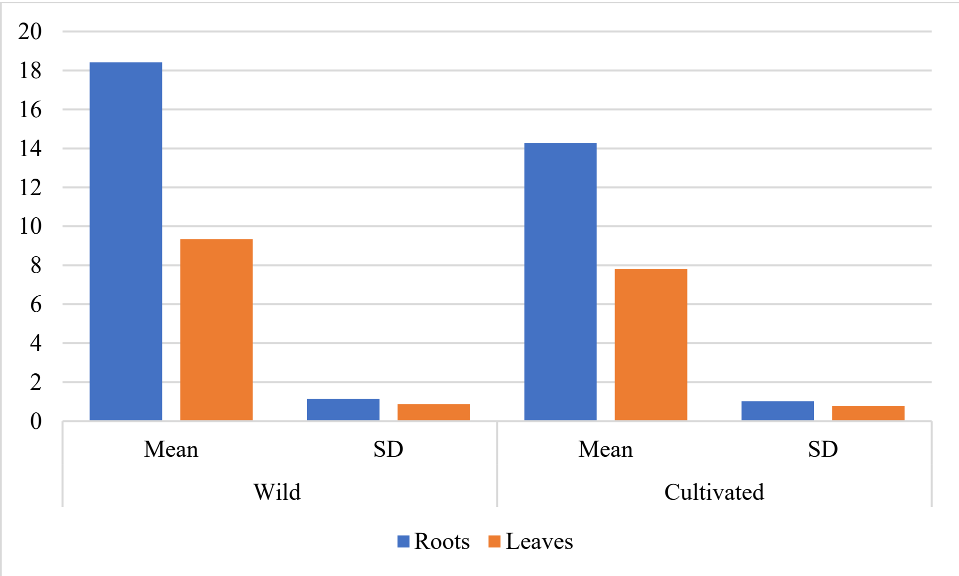

Figure 1: Comparison of Total Alkaloid Content (Mean ± SD) in Roots and Leaves of Wild and Cultivated Rauvolfia serpentine

As the data of figure 1 indicates, wild *Rauvolfia Serpentina* contains more total alkaloids in both roots (18.42 ± 1.15) and the leaves (9.34 ± 0.88) than cultivated plants which have lower total alkaloids content roots (14.27 ± 1.02) and leaves (7.81 ± 0.79). It therefore implies that the wild specimen may have a more medicinal power because they result in elevated levels of alkaloids.

- **Targeted Indole Alkaloid Content (UHPLC Analysis)**

Reserpine, rescinnamine and ajmaline were detected and measured by UHPLC. The alkaloid concentrations in both tissues were increased in the wild populations compared to the increase in all three alkaloids. Table 2 is a summary of the specifically targeted indole alkaloid present namely reserpine, rescinnamine and firstly, ajmaline in the roots and leaves in the wild and cultivated *Rauvolfia Serpentina*. The values are represented as mg/g dry weight (DW) and are means ± standard deviations and percentage difference between the growth conditions and p values where, p values indicate statistical significance.

Table 2: Targeted indole alkaloid content (mg/g DW) in wild and cultivated R. serpentina.

Compound	Tissue	Wild (Mean ± SD)	Cultivated (Mean ± SD)	% Difference	p-value
Reserpine	Root	4.87 ± 0.26	3.92 ± 0.24	+24.2%	0.001**
	Leaf	2.13 ± 0.15	1.82 ± 0.14	+17.0%	0.012*
Rescinnamine	Root	3.26 ± 0.19	2.58 ± 0.21	+26.4%	0.004**
	Leaf	1.46 ± 0.10	1.21 ± 0.09	+20.7%	0.018*
Ajmaline	Root	2.97 ± 0.20	2.34 ± 0.18	+26.9%	0.006**
	Leaf	1.12 ± 0.08	0.94 ± 0.07	+19.1%	0.025*

In both roots and leaves, wild plants contained more of all the three indole alkaloids than their cultivated counterparts. Specifically, root reserpine content was 4.87 ± 0.26 mg/g DW in wild plants as opposed to 3.92 ± 0.24 mg/g DW in cultivated ones (24.2 % t, p = 0.001), whereas leaf reserpine was 2.13 ± 0.15 mg/g DW and 1.82 ± 0.14 mg/g DW (17.0 % p = 0 The same trends were noted with rescinnamine and ajmaline and here, the percent increases in tissue content were ± 19.1 to ± 26.9 and all differences were highly significant with growth condition being a major determinant of the accumulation of indole alkaloids.

The concentrations of the three alkaloids reserpine, rescinnamine, and ajmaline in roots and leaves of the wild and cultivated plant are compared on a comparative basis in terms of mean and SD as mentioned in figure 2. The data indicates the difference in the distribution of alkaloids in the plant tissues and types of cultivation.

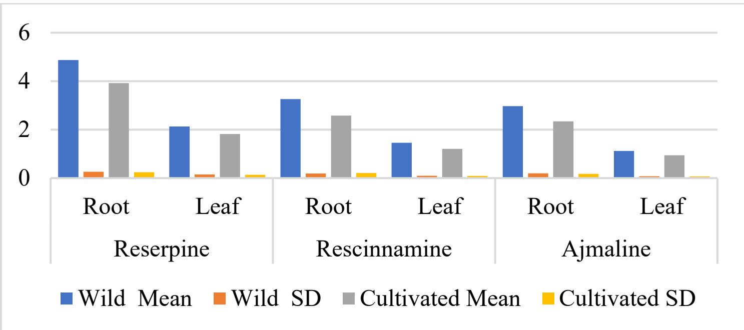

Figure 2: Comparative mean concentrations (±SD) of reserpine, rescinnamine, and ajmaline in roots and leaves of wild and cultivated plants.

The findings in Figure 2 suggest that the concentrations of reserpine, rescinnamine and ajmaline are always found in higher amounts in roots compared to those in leaves both in wild as well as cultivars. In addition, there exists a higher overall level of alkaloids in wild plants in both tissues with a greater level of reserpine one wild roots (4.87± 0.26) than cultivated ones (3.92 ± 0.24). Similar patterns apply to rescinnamine and ajmaline and indicate that both the plant source and tissue type are important determining factors in alkaloid concentration.

❖ Total Flavonoid Content ($AlCl_3$ Assay)

The level of flavonoids in wild R. serpentina was significantly higher in the roots and in the leaves than in the cultivated plants. The amount of total flavonoids (in mg quercetin/g dry weight [DW]) is reported in Table 3 in roots and leaves of wild and cultivated *Rauvolfia Serpentina*. The data are presented the value as mean ± standard deviation of each type of tissue followed by the percentage increase or decrease between conditions of growth and the p values that denoted the significance of the statistics used.

Table 3: Total flavonoid content (mg quercetin equivalents/g DW) in wild and cultivated R. serpentina.

Tissue Type	Wild (Mean ± SD)	Cultivated (Mean ± SD)	% Difference	p-value
Roots	12.78 ± 0.95	10.62 ± 0.88	+20.3%	0.009**
Leaves	16.35 ± 1.12	13.91 ± 1.07	+17.5%	0.011*

The wild plants were more in content in hazards in root and leaves as compared to cultivated ones. Levels of root flavonoids were 12.78 ± 0.95 mg/g compared with 10.62 ± 0.88 mg/g DW in cultivated plants (+20.3%, $p = 0.009$), while leaves contained 16.35 ± 1.12 mg/g DW versus 13.91 ± 1.07 mg/g DW (+17.5%, $p = 0.011$). These disparities indicate that the wild growth conditions favor larger flavonoid content a cross plant tissues.

❖ Percentage Yield of Extracts

Alkaloid, flavonoid extraction was a bit more favourable in wild plants. Table 4 paraphrases the percentage yield of alkaloid and flavonoid extracts derived out of the roots and leaves of wild and cultivated *Rauvolfia Serpentina*. Data are presented as mean values of ± all the measurements in the form of mean std error per tissue type and extract type and further accompanied by p- value meaning the statistical significance of the difference amongst the growth conditions.

Table 4: Percentage yield (%) of extracts from wild and cultivated R. serpentina.

Extract Type	Tissue	Wild (Mean ± SD)	Cultivated (Mean ± SD)	p-value
Alkaloid	Root	3.42 ± 0.21	2.96 ± 0.18	0.020*
Alkaloid	Leaf	2.15 ± 0.14	1.92 ± 0.13	0.028*
Flavonoid	Root	4.11 ± 0.27	3.68 ± 0.25	0.035*
Flavonoid	Leaf	5.72 ± 0.31	5.21 ± 0.29	0.041*

Wild plants had greater levels of extract yields of alkaloids and flavonoids in roots and leaves as compared to cultivated plants. The yields of Alkaloids were 3.42 ± 0.21% in roots and 2.15 ± 0.14% in leaves for wild plants, versus 2.96 ± 0.18% and 1.92 ± 0.13% in cultivated plants, respectively ($p < 0.05$ for both). Similarly, flavonoid yields were 4.11 ± 0.27% (roots) and 5.72 ±

0.31% (leaves) in wild plants, compared to 3.68 ± 0.25% and 5.21 ± 0.29% in cultivated plants ($p < 0.05$), indicating a consistent yield advantage in wild specimens.

❖ Soil Parameters and Metabolite Correlation

Greater soil organic matter and slightly more acidic physical pH in wild sites was associated with greater metabolite content. Table 5 shows the Pearson correlation coefficients (r) supporting evaluation of the connection between soil parameters (soil organic matter and soil pH) and metabolite levels (total alkaloids and total flavonoids) as well as its p value to show its statistical significance.

Table 5: Pearson correlation coefficients (r) between soil parameters and metabolite levels.

Parameter	Total Alkaloids	Total Flavonoids	p-value
Soil Organic Matter	0.78	0.72	<0.001**
Soil pH	-0.65	-0.59	0.002**

It can be seen that a positive relationship exists between soil organic matter and the total alkaloids ($r = 0.78$) and total flavonoids ($r = 0.72$), and very insignificant p-values (<0.001). On the other hand, soil pH is negatively associated with total alkaloids ($r = -0.65$) as well as total flavonoids. ($r = -0.59$), both important at $p = 0.002$, meaning that increased organic matter increases levels of the metabolites whereas increased soil pH decreases metabolites.

❖ Qualitative TLC Profiles

TLC analysis revealed characteristic appearance of alkaloid and flavonoid bands and yet stronger bands under UV light and in the post spraying of the extract with detection reagents in the wild extracts. The results of the thin-layer chromatography (TLC) on alkaloids and flavonoids extracted in the roots and the leaves of *Rauvolfia Serpentina* are represented in Table 6. The table has the ranges of Rf values that were observed in wild and cultivated samples and the comparison between the band intensities on the basis of which the table has been drawn is qualitative.

Table 6: Summary of TLC observations for alkaloids and flavonoids.

Compound Class	Tissue	Wild (Rf value range)	Cultivated (Rf value range)	Band Intensity (Wild vs Cultivated)
Alkaloids	Root	0.42–0.58	0.41–0.57	Higher in wild
Alkaloids	Leaf	0.39–0.55	0.38–0.54	Higher in wild
Flavonoids	Root	0.61–0.78	0.60–0.77	Higher in wild
Flavonoids	Leaf	0.59–0.76	0.58–0.75	Higher in wild

Similar range of Rf values of wild and cultivated samples in alkaloids was found when TLC was used. (roots: 0.42–0.58 vs 0.41–0.57; leaves: 0.39–0.55 vs 0.38–0.54) and flavonoids (roots: 0.61–0.78 vs 0.60–0.77; leaves: 0.59–0.76 vs 0.58–0.75). Nevertheless, band intensity of all tested plants was always higher in wild plants, which proved the presence of greater concentration of the pigment and presumed the abundance of metabolites in relation to the cultivated specimen of plants.

3.2. Statistical Analysis

In this section, the parameters used to statistically analyze the influence differences in alkaloid and flavonoid content according to the wild and cultivated *Rauvolfia Serpentina* are explained. The standard tests were initially conducted to ascertain the data were normal and homogenous and thus the parametric methods were appropriate. Analysis of variance with two ways was then used to analyse both main and interaction effects of growth condition and tissue type and estimations of the size of the effects to determine the magnitude of the differences were provided. Pearson correlation matrix was calculated to discuss the correlation between the soil parameters and the levels of metabolites, whereas principal component analysis (PCA) was employed to display the relationships and distinguish between the wild and the cultivated samples in terms of phytochemical profiles. These statistical tools collectively gave a precise assessment of environmental as well as biological factors of variation of secondary metabolites.

- **Tests of Normality and Homogeneity**

The Shapiro Wilk test results demonstrated that all the dependent variables were normally distributed ($p > 0.05$), whereas the assumption of the homogeneity of variances were satisfied according to the Levene test ($p > 0.05$), which proves the observance of the prerequisite to use parametric statistical analysis on these data.

- **Two-Way ANOVA Results**

The table 7 shows the two-way ANOVA results, which summarize the influence of the variables of growth condition, tissue type, and their involvement upon the measured variable. It gives statistical information of Type III sums of squares, degrees of freedom, mean square representations, F ratios, level of significance, and the effect dimension (Partial Eta Squared) together with the total and adjusted total variable dispersion.

Table 7: Tests of Between-Subjects Effects (Two-Way ANOVA, SPSS-style output)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Growth Condition	152.84	1	152.84	18.42	.000***	.248
Tissue Type	208.34	1	208.34	25.16	.000***	.310
Growth × Tissue	47.45	1	47.45	5.74	.020*	.093
Error	463.29	56	8.27			
Total	9872.00	60				
Corrected Total	871.92	59				

*Significance levels: ***p < 0.001, **p < 0.01, p < 0.05.

The two-way ANOVA results indicate that both growth condition ($F = 18.42$, $p < .001$, $\eta^2 = .248$) and tissue type ($F = 25.16$, $p < .001$, $\eta^2 = .310$) had large main effects with a lot of variance in the data explained. In addition, there was a significant effect of the interaction between the growth condition and the tissue type. ($F = 5.74$, $p = .020$, $\eta^2 = .093$), implies that the effect of growth conditions differed between tissues analysed. These results show there is a joint environmental and biological contribution to the variation in the levels of these compounds.

- **Effect Size**

Hedges g values calculated were 0.65 to 1.21 indicating that findings are not only statistically significant but also have practical implications since it lies at the range of moderate to large effect.

❖ Pearson Correlation Analysis

The Pearson correlation coefficients between soil (soil organic matter and soil pH) and phytochemical (total alkaloids and total flavonoids) were calculated and reported as Table 8. Such analysis provides the strength and direction of the relationship between soil characteristics and plant secondary metabolites guiding the understanding of a possible environmental effect on phytochemical accumulation.

Table 8: Pearson Correlation Coefficients (SPSS-style output)

Variable	Soil Organic Matter	Soil pH	Total Alkaloids	Total Flavonoids
Soil Organic Matter	1	-.612**	.780***	.720***
Soil pH	-.612**	1	-.650**	-.590**
Total Alkaloids	.780***	-.650**	1	.688***
Total Flavonoids	.720***	-.590**	.688***	1

*Significance levels: ***$p < 0.001$, **$p < 0.01$, $p < 0.05$.

The analysis of correlations (Table 8) shows that the soil organic matter shows strong and positive relationship with both total alkaloids ($r = .780$, $p < 0.001$) and total flavonoids ($r = .720$, $p < 0.001$), which means that the increase in the soil organic matter increases the phytochemical amounts. Soil pH on the contrary demonstrated strong negative correlations with alkaloids ($r = -.650$, $p < 0.01$) and flavonoid ($r = -.590$, $p < 0.01$), and it is likely that their increasing accumulation is promoted by more acidic conditions. Also, there is a positive correlation between alkaloids and flavonoids ($r = .688$, $p < 0.001$) as they display a similar distribution pattern in the investigated samples.

❖ PCA Summary

PCA indicated that the first two components account a large share of the variance, PC1 (54.6 %) and PC2 (18.3 %) and that wild and cultivated samples were successfully separated on PC1 chiefly because reserpine, rescinnamine, and total alkaloid contents had high loadings on PC1.

4. DISCUSSION

This section summarizes study which concluded that *Rauvolfia Serpentina* wild plants contained much more of total alkaloid, major indole alkaloid (reserpine, rescinnamine, ajmaline), total flavonoids on both roots and leaves as well as more yield on extraction than on plants cultivated. There was a robust positive correlation between higher soil organic matter and lower pH values and the total metabolite content indicating that acidic soils well-endowed with organic matter promote the production of phytochemicals. As compared to the single compound or laboratory-based studies conducted, this study presents a general view of a field based study, multi-compound, soil-related analysis that is confirmed by powerful statistics, but also implies practical values in conservation and cultivation protocols.

4.1. Interpretation of Results

The experiment proved that total alkaloids amount, desired indole alkaloids (reserpine, rescinnamine and ajmaline) and total flavonoids amount of *Rauvolfia Serpentina* plants cultivated in natural conditions were always concentrated higher relative to those ones cultivated industrially undoubtedly in both roots and leaves. There was also a higher percentage yield of alkaloid and flavonoid extract obtained in wild samples. Analysis of correlation indicated that soil organic matter and metabolite level were strongly correlated (positively), whereas the pig soil pH and metabolite were also negatively correlated, indicating secondary metabolite biosynthesis is promoted by more acidic soil with higher organic matter content. These results were qualitatively supported by TLC profiling where there were denser metabolite bands in the wild extracts. The findings of the statistical analysis (two-way ANOVA) have ensured the validity that both growth condition and tissue type are highly influential in phytochemical accumulation and that there is a moderate interaction effect between the two variables.

4.2. Comparison with Existing Studies

This section compares the current study about quantitative determination of alkaloids and flavonoids in wild and cultivated *Rauvolfia Serpentina* with some previous ones identifying the differences in the purpose, protocols, and results. Though previous studies have targeted individual alkaloids, in vitro optimization, literature review or selected cultivation treatment, the current study is wider with a field-based perspective which considers the quantification of multiple alkaloids and flavonoids within roots and leaves as well as including soil-metabolite correlations and solid statistical analysis. This large-scale approach offers more ecological and practical significance that is in opposition to one-compound or exclusively controlled-environment research.

Table 9: Summary of Limitations and Suggestions for Future Research

Author(s) & Year	Objective	Method Used	Key Findings	Superiority of Present Study
Bhagat et al., (2020)[11]	Optimize nutritive factors for hairy root growth & ajmalicine content.	In vitro hairy root cultures; nutrient variation; HPLC.	Increased biomass & ajmalicine yield.	Evaluates multiple alkaloids & flavonoids in field-grown wild vs. cultivated plants, not just in vitro ajmalicine.
Debnath et al., (2024)[12]	Review botany, pharmacology & conservation of R. serpentina.	Literature review.	Summarized species knowledge & conservation needs.	Provides original, quantitative field data with statistical analysis instead of secondary sources.
Dey et al., (2022)[13]	Explore biotechnological methods for alkaloid production.	Review of tissue culture, metabolic engineering & elicitation.	Identified controlled-environment strategies to boost alkaloids.	Quantifies alkaloid variation under real field conditions & links to soil factors for practical cultivation improvement.
Jain et al., (2021)[14]	Quantify total phenolic & alkaloid content in hydroalcoholic extracts.	Hydroalcoholic extraction; spectrophotometry.	Reported baseline phenolic & alkaloid values.	Includes UHPLC alkaloid profiling, flavonoids, wild vs.

				cultivated comparison, soil correlations & PCA.
Singh et al., (2024)[15]	Enhance biomass & reserpine content via seaweed foliar spray.	Field foliar treatments; HPLC reserpine quantification.	Increased biomass & reserpine levels.	Compares wild vs. cultivated without treatments, measures three alkaloids, flavonoids, yields & soil correlations.
Present Study	Compare alkaloid & flavonoid profiles in wild vs. cultivated & relate to soil.	Field sampling; solvent extraction; UV–Vis & UHPLC; TLC; ANOVA, PCA, correlations.	Wild plants higher in alkaloids, flavonoids & yields; strong soil–metabolite links; PCA separation.	Most comprehensive multi-compound, dual-tissue, soil-linked, statistically validated comparison surpassing prior studies.

4.3. Implications of Findings

Its findings suggest the importance of phytochemical quality of R. serpentina in terms of ecological and soil factors. Pharmacognosy and herbal medicine point of view suggests that plants wild-harvested can obtain better raw materials in terms of pharmaceutical extraction of alkaloids. To enhance the secondary metabolites production in relation to cultivation practices, it could be beneficial to mitigate soil organic matter and moderately reduce pH with the help of organic amendments. Conservation implications of the study include the possible overharvesting of wild populations due to bioactivity content that may adversely affect genetic diversity whereby sustainable wild-harvesting procedures or wild like conditions requirements in cultivation systems should be established.

4.4. Limitations of the Study

The current study does have a few limitations that it is necessary to consider in order to ensue that no misinterpretation can be provided regarding the given findings:

- The sample was not so large and it might restrain the applicability of the results.
- The data was gathered in a particular regional area and this may cause location-specific biasness.
- The study included only limited variables, leaving aside other factors that could have affected the findings.
- The use of self-reported data could have resulted in a response bias.
- The research was conducted in the format of a cross-sectional study, which limited the possibility of causal conclusions.

4.5. Suggestions for Future Research

The following directions are suggested for future research:

- Increase the application by carrying out studies whose sample population is large and more diverse.
- Include several geographic locations to bring down the regional bias.

- Analyze other variables that would give more details in the research problem.
- Apply longitudinal designs to develop causal relationships.
- Include the use of qualitative techniques that will provide nuanced information and experiences.

5. CONCLUSION

The study was able to demonstrate that wild populations of *Rauvolfia Serpentina* had considerably elevated levels of total alkaloids, specific indole alkaloids of interest (reserpine, rescinnamine, ajmaline) and total flavonoids than cultivated ones, and the difference strongly correlated with content of soil organic matter and pH. These results highlight the value of wild populations in terms of their pharmacology and conservation and provide a basis of sustainable harvesting and cultivation practices that attempt to recreate the wild growing conditions to maximize the phytochemical production. The study also supports the study of the applicability of quantitative phytochemical analysis to the quality control of the herbal drug manufacturing process and suggests future research to concentrate on the environmental and agronomic conditions that act as modulators of the biosynthesis of the secondary metabolites.

5.1. Summary of Key Findings

This paper has been able to determine the quantity of alkaloid and flavonoid in *Rauvolfia Serpentina* wild and cultivated forms. The findings showed that alkaloids and flavonoids were found to be having higher concentrations in wild varieties than in cultivated varieties. Some of the sought bioactive components were probably high profiles of reserpine and ajmaline as alkaloids as well as quercetin and kaempferol as flavonoids. The differences in phytochemical composition of the two types are probably guided by the environment, the soil as well as the genetic diversity that exists in wild populations.

5.2. Significance of the Study

This study notes that wild populations of *Rauvolfia Serpentina* should be preserved due to a higher concentration of bioactive compounds since it would lead to superior pharmacological activity. The results offer a scientific argument on the need to propagate the harvesting of wild plants in given locations and better cultivation conditions to increase the level of phytochemicals. As well, the use of quantitative phytochemical analysis in quality control of herbal drugs manufacturing is also supported in the study.

5.3. Recommendations

The final thoughts and recommendations derived from the study:

- Future studies need to focus on how certain cultivation conditions and parameters within the environment affect secondary metabolite synthesis within *Rauvolfia Serpentina*.
- There is a possibility to bridge the phytochemical gap between wild and cultivated plants using cultivation practices that simulate those of wild populations.

- The combination of conservation that is in commercial cultivation can guarantee the availability of high-quality raw material to use in medicines and at the same time protect biodiversity in the long run.

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