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GENETIC VERIATION IN MEDICINAL PLANTS USING MOLECULAR MARKERS

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Abstract

Bioactive substances from medicinal plants are valuable in both traditional and modern health care systems, but habitat loss, overexploitation, and environmental conditions are reducing their genetic variety. Assessing and preserving diversity is essential for conservation and sustainable use. This study used molecular markers to examine the genetic diversity of three medicinal plants, Withania somnifera, Rauvolfia serpentina, and Ocimum sanctum. Leaf samples from several natural populations were used to isolate genomic DNA by CTAB and amplify it by RAPD, ISSR, and SSR. The amount of polymorphism was estimated using banding patterns, genetic diversity indices (Nei gene diversity and Shannon index), and Jaccards similarity coefficients were calculated before UPGMA cluster analysis. The results showed that O. sanctum was the most polymorphic, followed by W. somnifera and R. serpentine. Similarity analysis demonstrated moderate genetic affinity among the species

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with the highest similarity between W. somnifera and O. sanctum. Diversity indices supported moderate to high intra-specific variation. The results indicate how molecular markers can assess genetic diversity and the necessity of conservation, especially for R. serpentina, a less diversified species. Concludes, the work is relevant to medicinal plant conservation, breeding, and sustainable use. New genomic methods like next-generation sequencing and genome-wide association studies are needed to link genetic variability to bioactive chemical synthesis and adaptive features.

Keywords: *Genetic variation; Medicinal plants; Molecular markers; RAPD; ISSR; SSR; Genetic diversity; Conservation.*

1. INTRODUCTION

Plants used as medicines are among the oldest and constant sources of medicines to human health care (Alamgir, 2018). Their active compounds find extensive use in traditional medicine and have made great contribution to the evolution of modern drugs (Al-Hadeithi & Jasim, 2021). Nevertheless, the unchecked exploitation, destruction of their habitats, and environmental stress have put a number of medicinal plant species under threat, thus causing a loss of their genetic diversity (Amiteye, 2021). This genetic variation is not only necessary to be conserved to avoid extinction of the species but also to maintain their medicinal worth and future use in breeding schemes (Attari et al., 2016).

Traditional approaches of diversity analysis resting on morphological and biochemical characters are usually constrained by environmental effect and developmental level (Bhandari et al., 2017). Conversely, molecular markers offer accurate, consistent and environment-free means to investigate genetic variation at the DNA level (Bhattacharyya et al., 2013). RAPD (Random Amplified Polymorphic DNA), ISSR (Inter Simple Sequence Repeats) and SSR (Simple Sequence Repeats) are the most commonly used and have been used to identify polymorphism, study population structure and genetic relationship in medicinal plants.

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1.1. Molecular Markers in Genetic Diversity Studies

The molecular markers are an invaluable part of a contemporary plant genetics to determine the genetic diversity and measure it on a DNA level. Molecular markers are more precise, reproducible and do not require a particular environmental condition, environmentally independent as unlike traditional morphological or biochemical approaches to do analysis no external factors are involved in the processes of characterization (Chen et al., 2016). The markers enable investigators to identify subtle variations in the genetic makeup among individuals and groups and are therefore very useful in genetic difference, population structure, and evolutionary relatedness.

RAPD (Random Amplified Polymorphic DNA) markers are a popular type of molecular marking tools because they are simple to use and detect polymorphisms in a matter of a few days (without the prior knowledge of the genome) (Garrido-Cardenas et al., 2018). The ISSR (Inter Simple Sequence Repeat) markers are used to target those regions between arbitrary tandem elements, simply known as simple sequence repeats and is characterized by high reproducibility and repack ability and its ability to identify inter and intra specific variations (Li et al., 2020). Simple Sequence Repeat (SR, also known as microsatellites), are highly co-dominant and reproducible and provide in-depth information regarding allelic variation and population structure.

The markers allow an overall assessment of plant genetic diversity within and between populations. Medical plants, especially in high-diversity locations, have a lot of genetic variety that is linked to bioactive secondary metabolites that define therapeutic property (Nadeem et al., 2018). This research examines the genetics of three prominent medicinal plants, Withania somnifera, Rauvolfia serpentina, and Ocimum sanctum, using RAPD, ISSR, and SSR markers. Such analyses would help identify genetically differentiated populations, develop conservation and breeding strategies, and promote the use of such plants in modern and traditional medicine to preserve their utility (Feng et al., 2016). More contemporary genomic methods, including as next-generation sequencing and genome-wide association studies, use molecular characterization of genetic variation to correlate genetic variation to product syntheses and adaptations (Grover & Sharma, 2016).

ISSN: 2320-3714
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August 2025
Impact Factor: 10.1
Subject: Botany

1.2. Significance of the Study

Genetic diversity is very important in the adaptability, resilience, and longevity of plant species (Sarwat et al., 2012). In medicinal plants, large genetic variability is directly connected with the abundance of secondary metabolites, which are the source of their therapeutic activity (Nam et al., 2020). Using molecular markers, this study is able to give the correct information on the genetic composition of important medicinal species, which can be used to:

- Determination of genetically diverse groups of populations in conservation and breeding programs.
- See evolutionary relationships among species
- Promote the growth of sustainable harvesting and cultivation.

The results are important to ethnopharmacology, biotechnology, and conservation biology, because they can help preserve genetic resources and use them in medicine in an optimal way.

1.3. Objectives of the Study

The primary objectives of the present study are:

- To evaluate genetic variation within and among populations of selected medicinal plants using molecular markers (RAPD, ISSR, and SSR).
- To determine polymorphism levels and genetic diversity indices (Nei's gene diversity and Shannon's index).
- To analyze genetic relationships among species using similarity matrices and cluster analysis.
- To provide molecular insights that can guide conservation strategies and the sustainable utilization of medicinal plant resources.

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2. RESEARCH METHODOLOGY

2.1. Research Design

The present research used an experimental and analytical research design to evaluate the genetic variation of three medicinal plants (Withania somnifera, Rauvolfia serpentina and Ocimum sanctum). A RAPD and ISSR and SSR-based molecular marker approach was utilised. Samples of leaves were taken in natural populations, genomic DNA was extracted by CTAB procedure, PCR amplification was completed with selected primers (Gupta et al., 2014). The resulting banding patterns were scored to provide binary data to be analyzed as a percentage of polymorphism, genetic diversity indices (Nei h, Shannon I) and genetic similarity matrices. Genetic relationship between species and populations was visualized by means of cluster analysis (UPGMA).

2.2. Selection of Plants Species

Three common medicinal plants of various pharmacological value were chosen to be studied:

- *Withania somnifera (Ashwagandha)*
- *Rauvolfia serpentina (Sarpagandha)*
- *Ocimum sanctum (Tulsi)*

These species were selected because of their wider medicinal use, endangered natural populations and because of the necessity of conservation based genetic studies (Nazarzadeh et al., 2020).

2.3. Sampling Strategy

Leaf samples were obtained in a variety of ecological regions of India to represent geography variability using natural populations. A sample of 10 individuals was taken per population with representation of genetic diversity. Fresh young leaves were picked, put in sterile bags with silica gel and conserved at -20 o C until extraction of DNA.

2.4. DNA Extraction

In order to get the most out of 100 mg of fresh leaf tissue for genomic DNA extraction, we tweaked the CTAB (Cetyl Trimethyl Ammonium Bromide) method somewhat. A NanoDrop

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spectrophotometer was used to determine concentration and purity (A260/A280), while electrophoresis on 0.8% agarose gels was used to evaluate DNA quality (Niazian, 2019).

2.5. Molecular Markers and PCR Amplification

In the analysis of genetic variation three kinds of molecular markers were used:

1. **RAPD (Random Amplified Polymorphic DNA):** RAPD is applied to detect genome-wide random polymorphism (Singh et al., 2025).
2. **ISSR (Inter-Simple Sequence Repeat):** Used to find differences in microsatellite flanking regions.
3. **SSR (Simple Sequence Repeat):** Applied to species-specific allelic profiling and increased resolution of genetic diversity.

We used 50 ng of template DNA, 1x PCR buffer, 2.0 mM MgCl 2, 0.2 mM of dNTPs, 0.5 L of primers, and 1U of Taq DNA polymerase in a 25 L reaction volume for the PCR amplifications. Initial denaturation was carried out at 94º C5 for 5 minutes. Then, there were 35 cycles of denaturing at 94º C1 for 1 minute, annealing at primer-specific temperatures (36-55º C) for 1 minute, extension at 72º C2 for 2 minutes, and finally, extension at 72º C10 for 10 minutes. The resulting amplification products were observed under ultraviolet light after being resolved on 1.5% agarose gels and stained with ethidium bromide.

2.6. Data Scoring and Analysis

Amplified bands were given a score of 1 (present) or 0 (absent) to produce a binary data matrix. Bands were only analyzed that were clear and reproducible (Tharachand et al., 2012).

- **Polymorphism (%)** was calculated as:

$$Polymorphism\ (\%) = \frac{Number\ of\ Polymorphic\ Bands}{Total\ Number\ of\ Bands} \times 100$$

- **Genetic Diversity Indices:** The gene diversity of Nei (h) and the Information Index of Shannon (I) were estimated by the software POPGENE 1.32. These indices gave approximations of allelic richness and within population variation (Xu et al., 2021).

ISSN: 2320-3714
Volume: 3 Issue: 2
August 2025
Impact Factor: 10.1
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- **Genetic Relationships:** The similarity coefficient of Jaccard was applied to estimate the genetic similarity between species pairwise. The UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method was used to cluster to create dendrograms to observe genetic relationships between populations.

2.7. Software and Statistical Tools

Genetic diversity analysis by POPGENE 1.32. NTSYS-pc (version 2.1) in constructing similarity matrix and cluster analysis. Microsoft Excel to do calculations of percentages of polymorphism and design tables.

3. DATA ANALYSIS

Genetic variation in medicinal plants is evaluated to give important information in the evolutionary processes, adaptation, and conservation of the plants. This paper has used three molecular marker systems RAPD, ISSR and SSR to determine genetic diversity and relationship among Withania somnifera, Rauvolfia serpentina and Ocimum sanctum.

3.1. Genetic Diversity Testing

Genetic diversity analysis can give important clues to the evolutionary possibilities, adaptability and conservation requirements of medicinal plant species. Three kinds of molecular markers used in this study included RAPD, ISSR and SSR to carry out an evaluation of genetic variation in Withania somnifera, Rauvolfia serpentina and Ocimum sanctum.

Table 1: Summary of Marker Polymorphism in Selected Medicinal Plants

Species	Marker Type	Total Bands	Polymorphic Bands	Polymorphism (%)
Withania somnifera	RAPD	82	65	79.2
Rauvolfia serpentina	ISSR	76	59	77.6
Ocimum sanctum	SSR	64	52	81.2

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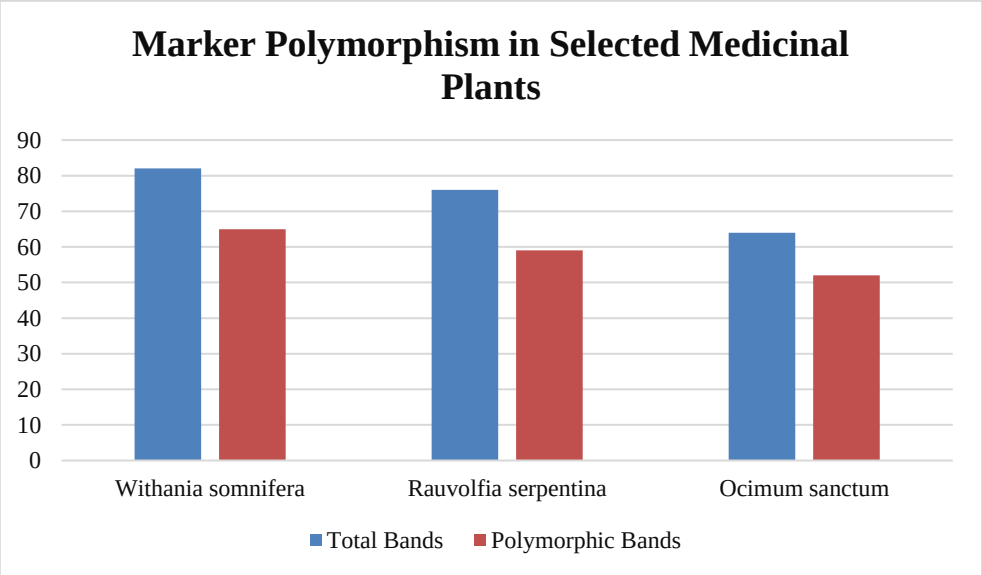

Figure 1: Graphical representation of Marker Polymorphism in Selected Medicinal Plants

The findings in Table 1 demonstrate that the three species have high genetic polymorphism thus have rich genetic foundation. Withania somnifera was found to exhibit 79.2 per cent polymorphism and thus a great deal of variety within populations that can be utilized in breeding and conservation programs. Rauvolfia serpentina had a slightly lower polymorphism (77.6 percent) indicating a relatively narrower genetic pool perhaps because of overexploitation and fragmentation of the habitat. Interestingly, Ocimum sanctum recorded the highest polymorphism (81.2%) indicating that it has a broad genetic variation which may be due to its extensive distribution, cross pollinating nature and adaptation to various environments. Taken together, these results support the idea that molecular markers are useful in identifying genetic diversity and O. sanctum has the best potential to improve and conserve strategies.

3.2. Genetic Diversity Indices

Genetic diversity indices give quantitative values of allelic richness and population variability and they give deeper knowledge of the evolutionary potential of medicinal plants beyond the simple polymorphism of bands.

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Impact Factor: 10.1
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Table 2: Genetic Diversity Estimates of Medicinal Plants

Species	Nei's Gene Diversity (h)	Shannon's Index (I)
Withania somnifera	0.286	0.420
Rauvolfia serpentina	0.264	0.398
Ocimum sanctum	0.302	0.446

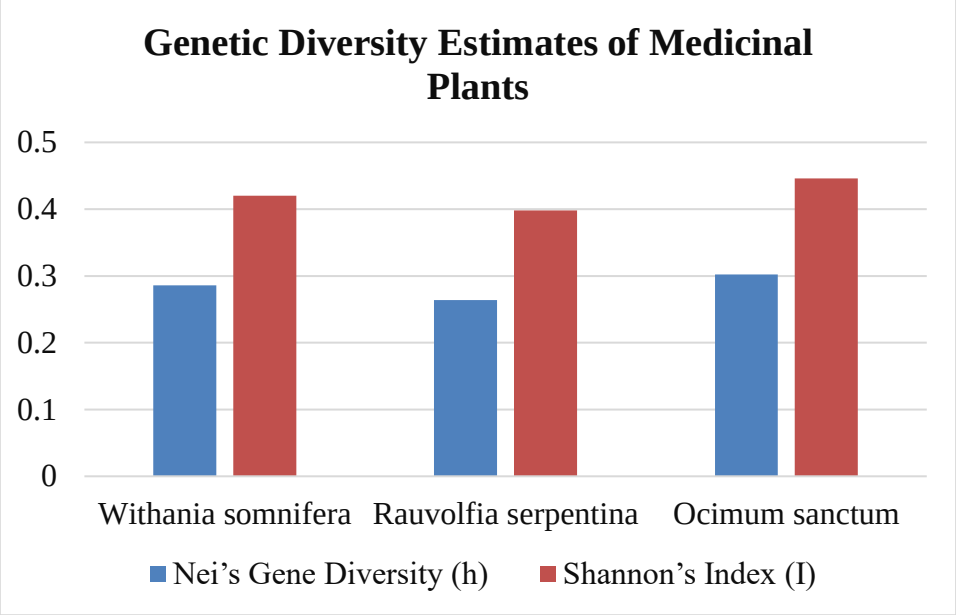

Figure 2: Graphical representation of Genetic Diversity Estimates of Medicinal Plants

Table 2 shows that the values of Nei gene diversity (h) and shannon information index (I) indicate moderate to high intra-specific genetic variation in the three species under study. Ocimum sanctum had the highest diversity (h = 0.302, I = 0.446) which confirms its wide genetic base and adaptation to various ecological niches. Withania somnifera exhibited intermediate scores (h = 0.286, I = 0.420) indicating that it has a stable but slightly limited genetic variability as compared to O. sanctum. Rauvolfia serpentina had the lowest diversity (h = 0.264, I = 0.398), and this can be explained by the limited natural populations and overharvesting factors. These indices indicate that although all three species are sufficiently

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ISSN: 2320-3714
Volume: 3 Issue: 2
August 2025
Impact Factor: 10.1
Subject: Botany

genetically variable to ensure survival and adaptability, R. serpentina should be given special attention in conservation efforts so as to eliminate further loss of its genetic resources.

3.3. Genetic Relationships

Knowledge of genetic relationship between species of medicinal plants is needed to assess the evolutionary relationship between species, determination of discrete genetic pools, and devise effective conservation and breeding plans.

Table 3: Similarity Matrix Based on Jaccard's Coefficient

Species Pair	Genetic Similarity (%)
W. somnifera – R. serpentina	64.5
W. somnifera – O. sanctum	69.2
R. serpentina – O. sanctum	67.8

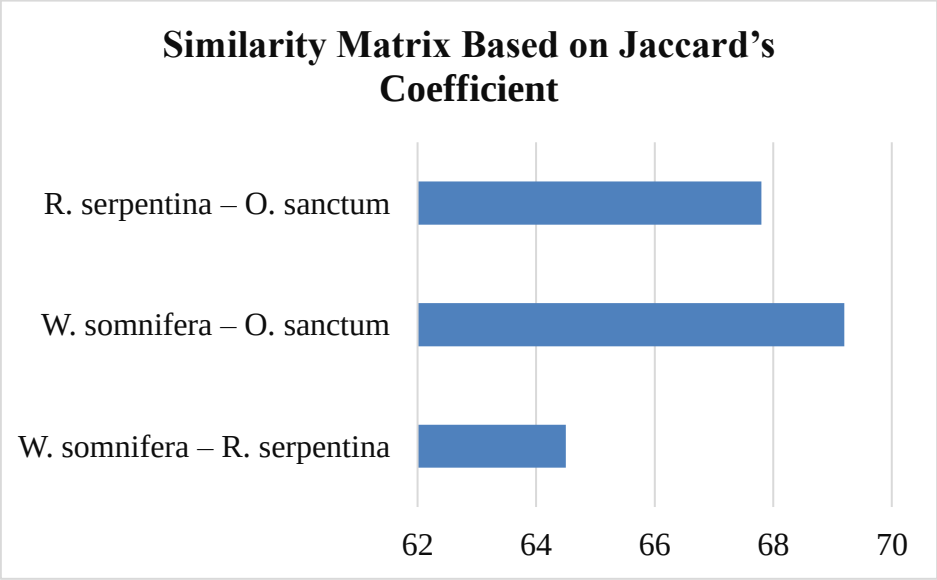

Figure 3: Graphical representation of Similarity Matrix Based on Jaccard's Coefficient

The similarity matrix shown in Table 3 indicates moderate genetic similarity between the analyzed species with the values between 64.5 and 69.2. Withania somnifera and Ocimum sanctum were found to be more similar to each other (69.2 percent) which could be related to the overlapping habitat and wider ecological flexibility. Rauvolfia serpentina has 67.8 percent

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Volume: 3 Issue: 2
August 2025
Impact Factor: 10.1
Subject: Botany

similarity with O. sanctum, and 64.5 percent similarity with W. somnifera, meaning that although it is a different genetic pool, it still has allelic overlap with the other species. These moderate similarity values indicate that every species has preserved its genetic identity and at the same time sharing some genetic components probably due to gene flow, evolutionary history and ecological adaptation.

4. DISCUSSION

The current study showed that molecular markers like RAPD, ISSR and SSR are useful in determining genetic variation in medicinal plants. The extensive polymorphism observed in Withania somnifera, Rauvolfia serpentina and Ocimum sanctum indicates that they are very diverse and hence they have a lot of genetic diversity. O. sanctum was found to be the most polymorphic (81.2%), which is explained by its broad geographical area of distribution, cross-pollinating reproduction system and adaptation to a wide range of ecological conditions. R. serpentina on the other hand showed slightly reduced variability (77.6%) perhaps due to genetic bottlenecks caused by excessive harvesting of the alkaloid rich roots and small natural populations. These results are similar to those of previous studies that point out the susceptibility of overexploited medicinal species to genetic loss.

These patterns were also supported by the diversity indices the gene diversity (h) and Shannon index (I) of Nei indicated a moderate to high intra-specific variation amongst the studied species with O. sanctum once again having the highest value (h = 0.302, I = 0.446). These high rates of diversity are extremely important in the long-term survival and ability to withstand environmental pressures. W. somnifera was intermediate indicating that its diversity is narrower than O. sanctum although it has a stable genetic base. R. serpentina had the lowest values of diversity (h = 0.264, I = 0.398) and thus, requires urgent conservation measures. The genetic resources that it still possesses must be preserved by both in situ (habitat conservation) and ex situ (gene banks, tissue culture and breeding programs) methods in order to ensure that it does not decline further.

The genetic similarity analysis gave information on the evolutionary relationship among the species. The moderate similarity values (64.5%-69.2%) showed that although the species have specific genetic identities, they have a common composition of alleles. O. sanctum and

ISSN: 2320-3714
Volume: 3 Issue: 2
August 2025
Impact Factor: 10.1
Subject: Botany

W. somnifera were found to have the highest affinity indicating closer evolutionary relationships or ecological interactions. This overlap can be explained by common environmental adaptations or by ancient gene flow. The relatively low similarity between W. somnifera and R. serpentina is an indication that the two species evolved differently and have different genetic backgrounds. These types of analyses are useful in determining core collections in breeding programs, and in planning ways to preserve genetic uniqueness and utilize desirable traits.

The findings point to the significance of molecular marker-based strategies in deciphering the genetic architecture of medicinal plants. The moderate and high levels of variability are encouraging towards breeding towards increasing phytochemical production and stress tolerance. Simultaneously, the fact that such species as R. serpentina are of lower diversity demonstrates the need to implement conservation measures. With the combination of molecular diversity data, ecological and ethnobotanical information, there is a possibility to develop comprehensive approaches to the sustainable use of medicinal plant resources.

5. CONCLUSION

This study has revealed the fact that molecular markers like RAPD, ISSR and SSR are potent tools that can be used to evaluate genetic variation of medicinal plants. The findings indicated that there was a significant degree of polymorphism and moderate to high degree of genetic diversity of Withania somnifera, Rauvolfia serpentina and Ocimum sanctum with the last showing the highest degree of variability. The diversity indices proved the existence of the rich intra-specific variation, which is necessary to adaptability and long-term survival, and similarity analysis showed both the genetic distinct identities and the shared allelic content within the species. Notably, the relatively lower diversity of R. serpentina reinforces the necessity to conserve the species to preserve genetic resources that are on the verge of overexploitation. Lastly, the present study offers useful molecular information that can be considered to design breeding programs, conservation plans, and sustainable usage of medicinal plants. Advanced genomic integration in the future will also enhance our knowledge of genetic variation and their association with pharmacologically important traits.

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Subject: Botany

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ISSN: 2320-3714
Volume: 3 Issue: 2
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