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GENETIC STABILITY AND PHYTOCHEMICAL PROPERTIES OF *DENDROBIUM NOBILE* LINDL. AS AN ANTIVIRAL USING RAPD

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SUMMARY

Summary: The use of silicon is an option for reducing the adverse effects of water deficit conditions. *Dendrobium nobile* Lindl. is a native orchid from Lampung, Indonesia, and one of the most widespread ornamental members of the family Orchidaceae. It contains alkaloids, flavonoids, and terpenoids, which are beneficial as antiviral compounds. Characterization succeeded in determining the potential variations in its genetic stability and phytochemical properties through DNA nucleotide sequence polymorphism analysis using the random amplified polymorphic DNA (RAPD). The presented research involved eight accessions of *D. nobile* collected from the Liwa Botanical Garden, West Lampung, Indonesia. The screening used five primers, viz., OPA-01, OPA-07, OPN-07, OPC-16, and OPD-08. The study revealed a PIC value (≥ 0.3), indicating that these molecular markers were more informative. The amplification process produced 107–134 DNA bands, including 17 polymorphic bands ranging from 200–1000 bp. The polymorphism rate for each primer ranged from 0.31 to 0.40. Phylogenetic analysis grouped the accessions into two clusters with coefficient values at >0.85 and >0.9, while similarity indices were 0.33 and 0.55, respectively. The accessions' further dividing into two subclusters comprised subcluster I (D2, D5, D1, D7, D4, and D8) and subcluster II (D3), based on habitat differences and environmental factors linked to their domestication. The PCR-RAPD proved more effective in characterizing the genetic stability in relation to antiviral phytochemicals.

Keywords: *Dendrobium nobile* Lindl., Orchidaceae, genetic stability, phytochemical and antiviral properties, RAPD, polymorphism

Key findings: The RAPD technique was evidently more effective in characterizing the genetic stability related to antiviral properties of the *Dendrobium nobile* accessions in Lampung, Indonesia.

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INTRODUCTION

Dendrobium nobile Lindl. is a native orchid species from Lampung and a distinctive flora of Southern Sumatera, Indonesia (Mahfut *et al*., 2021a), requiring effective conservation for future use (Putra *et al*., 2024). This orchid serves as a valuable collection and an attraction for visitors at the Liwa Botanical Garden, West Lampung, Indonesia (Mahfut *et al.*, 2020a; 2020b; 2025d). The high genetic variability within populations suggests that in situ conservation strategies could effectively maintain genetic resources. However, targeted ex situ efforts are essential for populations with lower diversity. Beyond its ornamental value, *D. nobile* has long been a recognized plant for its use in traditional medicine, particularly in China (Meitei *et al*., 2023). The plant, known to contain alkaloids, flavonoids, and terpenoids, has reports of exhibiting antiviral compounds (Songjaeng *et al*., 2022).

One of its unique alkaloid derivatives, dendrobine, found exclusively in *D. nobile* Lindl., has demonstrated antiviral activity against various viruses, including H9N2 (Ming *et al.*, 2021), H1N1, H3N2, and SARS-CoV-2 (Rafli *et al*., 2022), and Dengue-2 (Meitei *et al*., 2023). Past studies have shown dendrobine disrupts gene expression by inhibiting RNA polymerase II activity at the gene promoter and suppressing the viral replication (Kinasih *et al*., 2022). Its low IC50 value highlights its potential as an antiviral agent. Furthermore, Fielding *et al*. (2020) reported that alkaloids can also modulate host factors to produce antiviral immunomodulators and reduce viral replication. Alkaloids interfere with the DNA and RNA polymerase activities, thereby preventing the synthesis of new genetic material (Wu *et al*., 2023). In addition to their antiviral activity, terpenoids and flavonoids exhibit antitumor and anti-inflammatory (Ming *et al*., 2021), antioxidant, antibacterial, antiangiogenic, and antimutagenic effects (Wu *et al*., 2023).

Further to its phytochemical contents, characterizing genetic stability is essential for understanding variations in phytochemical composition (Tikendra *et al*., 2021). The RAPD has proven to be an efficient technique for characterizing the genetic stability by examining the DNA nucleotide sequence polymorphism using single primers (Guo *et al*., 2023). This technique has also proceeded in studying genetic diversity phylogenetic relationships (Asadudin *et al*., 2024; Putera *et al*., 2024), genetic mapping, DNA fingerprinting, and genetic stability (Guo *et al*., 2023). Characterizing genetic stability is particularly important for understanding the phytochemical variations due to environmental factors and habitat differences (Sukmawati *et al*., 2021; Aritonang *et al*., 2024; Nuraini *et al*., 2024; Simamora *et al*., 2024).

RAPD markers have been a widely used tool for their simplicity, cost-effectiveness, and ability to detect polymorphisms without prior genomic information, making them ideal for studying non-model species like orchids. The genetic variations detected using RAPD markers align with observed differences in phytochemical profiles reported in previous studies (Liu *et al*., 2020; Tikendra *et al*., 2021). Accessions with higher polymorphism levels may exhibit greater phytochemical diversity, enhancing their potential as antiviral agents. This correlation underscores the importance of integrating genetic and phytochemical analyses to comprehensively characterize *D. nobile*. Further metabolomic profiling studies could validate the link between genetic stability and phytochemical composition, enabling targeted conservation and breeding strategies.

Research on the genetic stability and phytochemical analysis of *D. nobile* remains limited. Tikendra *et al.* (2021) reported a genetic stability analysis of *D. nobile* regenerated in vitro using RAPD markers that showed 97% DNA profile similarity among the regenerants, with a PIC value of 0.92. The study also revealed linear data in phytochemical analysis, indicating homogeneity among micropropagated plants and their parental genotypes. Transcriptomic analysis using 454 pyrosequencing revealed 69.97% sequence alignment with the database. These findings demonstrated the reliability of the RAPD technique for

characterizing genetic stability and its implications for understanding phytochemical variations through genetic diversity analysis.

Ethnomedicinal studies exploring the potential antiviral properties of native *D. nobile* remain scarce despite anecdotal reports from Lampung, Indonesia. Based on our previous studies, conservation efforts at the Liwa Botanical Garden have focused on identifying the native orchids through morphological (Mahfut, 2020, 2021; Mahfut *et al*., 2024a), anatomical (Mahfut *et al*., 2023a, 2023b), and molecular (Mahfut *et al*., 2023c) analyses. Additional protective measures have progressed through the identification of disease infections (Mahfut *et al*., 2023d; Anbiya *et al*., 2024; Septiana *et al*., 2024). Despite the ecological and medicinal significance of *D. nobile*, limited research existed in integrating its genetic diversity with phytochemical profiling to evaluate antiviral potential. This study aims to bridge this gap by investigating the potential of native orchids in Lampung while supporting their conservation. Likewise, it builds on previous research by identifying phytochemical compounds with antiviral potential and characterizing genetic stability to understand variations in phytochemical content using RAPD analysis.

MATERIALS AND METHODS

Plant material

The survey and sample collection of *D. nobile* Lindl. commenced in the greenhouse at the Liwa Botanical Garden, West Lampung, Indonesia. Leaf samples' collection followed the methodology described by Mahfut *et al.* (2021b).

Genomic DNA isolation

Genomic DNA isolation began as the initial stage, followed by the DNA quality and quantity assessment and subsequent amplification using RAPD (Windiyani *et al*., 2022). The isolation process employed the cetyltrimethylammonium bromide (CTAB) method (Mahfut *et al*., 2024b). The DNA quality assessment ran through 1.5% agarose gel electrophoresis at 100 V for 30 min, while the DNA quantity measurement used a nanodrop spectrophotometer at A260/280 (Sari *et al*., 2025).

DNA amplification

DNA amplification ensued using a Thermal Cycler T100 (BioRAD) with five RAPD primers (OPN-07, OPA-04, OPB-19, OPC-16, and OPA-07) (Mahfut *et al*., 2024b). The PCR reaction mixture (20 µl total volume) comprised 2 µl primer, 10 µl PCR mix, 6 µl ddH_2O, and 2 µl DNA sample. The thermal cycling conditions included an initial denaturation at 94 °C for three minutes, followed by 30 cycles of denaturation at 94 °C for one minute, annealing at the primer's TM temperature, extension at 72 °C for two minutes, and a final extension at 72 °C for five minutes.

Data analysis

The DNA amplification bands' analysis followed the methods described by Windiyani *et al*. (2022). Scoring of DNA bands continued using a binary system (1 for the presence of a band and 0 for its absence). Assessing DNA band patterns utilized the Multivariate Statistical Package, Version 3.2 (MVSP). Cluster analysis employed the Unweighted Pair-Group with Arithmetic Average (UPGMA). The Polymorphic Information Content (PIC) value's calculation helps the assessment of polymorphic bands (Mahfut *et al*., 2024b).

RESULTS AND DISCUSSION

DNA amplification

The collection process resulted in seven accessions of *D. nobile* from the Liwa Botanical Garden. DNA amplification using five primers across all samples yielded a total of 17 DNA bands (Table 1). The DNA amplification demonstrated that all RAPD markers successfully recognized and amplified DNA

Table 1. The PIC values and primers with polymorphic bands.

No.	Primers	Band size (bp)	Total number of bands	Number of polymorphic bands	PIC values
1	OPA-1	200–500	125	4	0.38
2	OPA-7	200–600	116	2	0.31
3	OPN-7	200–1000	134	4	0.40
4	OPC-16	200–400	111	4	0.36
5	OPD-8	200–500	109	3	0.32

sequences within the orchid genome, generating 107–134 DNA bands, including 17 polymorphic bands, ranging from 200–1000 bp.

The polymorphism level of each marker, as determined by the Polymorphism Information Content (PIC) values, ranged from 0.31 to 0.40. This result aligns with the principle of the RAPD technique, which uses short and arbitrary primers to amplify random segments of genomic DNA. The distinct banding patterns obtained reflect the genetic diversity among *D. nobile* accessions. This genetic diversity is crucial because it directly impacts the variability in phytochemical composition, including compounds like alkaloids, flavonoids, and terpenoids, which exhibit antiviral properties. The RAPD technique's ability to detect these polymorphisms allows for the identification of genetic markers associated with the production of these compounds. Previous studies detailed PIC values for *D. nobile,* ranging from 0.24 to 0.85, with an average of 0.61 (Tikendra *et al.*, 2021; Mahfut *et al*., 2025c). These findings indicate high genetic diversity in *D. nobile* populations and emerged associated with antiviral compounds, important for conservation and breeding programs. This connection underscores the genetic variation revealed in this study that supports the conservation and breeding of *D. nobile*, aimed at optimizing its potential as a source of antiviral agents. The distinct banding patterns observed provide insights into genetic regions potentially linked to the biosynthesis of these antiviral compounds, offering a pathway for further research into enhancing their production.

The number of DNA bands generated by RAPD depends on the selection of primers, as each primer binds to specific complementary DNA sequences. Variations in band patterns indicate polymorphism and reflect the genetic diversity. The implications of high/low genetic diversity will affect the conservation status or resistance of *D. nobile* to environmental change. The observed clustering pattern results suggest that the possible reasons are geographic isolation and selective pressure. Primers with unique nucleotide sequences produce distinct amplification patterns, enabling the identification of polymorphic and monomorphic bands. Higher genome coverage leads to increased polymorphism levels. Singh (2020) mentioned the amplification variability results from variations in genomic sequences where primers anneal, leading to the magnification of distinct regions and varying levels of polymorphism. Mismatches between the primer and the template can result in absent or reduced PCR product, highlighting RAPD's sensitivity to sequence variations. The primers' selection and genome coverage significantly influence the detection of polymorphic markers in the RAPD analysis (Babu *et al*., 2021).

The PIC values for individual primers were as follows: OPA-01 (0.38), OPA-7 (0.31), OPN-07 (0.40), OPC-16 (0.36), and OPD-08 (0.33). The highest PIC value was evident for OPN-07 (0.40). Based on classifications, markers with PIC values >0.60 are highly informative, 0.3–0.59 are moderately informative, and <0.30 are less informative. All primers in this study were moderately informative molecular markers. The PCR-RAPD results for *D. nobile* are illustrative in Figure 1. Variations in PIC values had influences from the crop species, cultivars analyzed, and marker types. Liu *et al.* (2020) reported PIC values obtained from PCR-RAPD molecular markers used to assess genetic stability in *D.*

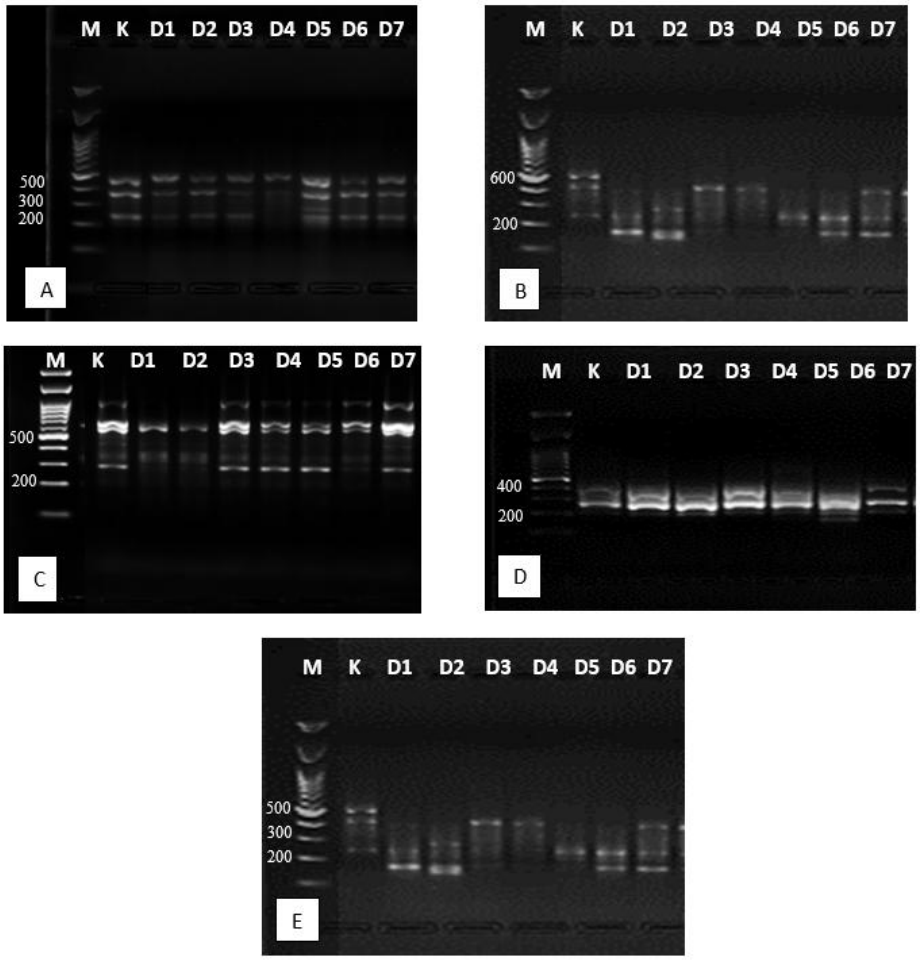

Figure 1. The DNA band pattern resulting from PCR-RAPD amplification of the *Dendrobium nobile* Lindl. accessions in Lampung, Indonesia. A (OPA-01), B (OPA-07), C (OPN-07), D (OPC-16), E (OPD-08), K (Control), and M (Marker 1000 bp).

nobile, showing highly informative PIC values (PIC > 0.5) and moderate values (PIC = 0.3–0.5).

The amplified DNA bands predominantly displayed clear and bright patterns, indicating high DNA concentration, whereas the faint bands suggested lower DNA concentration. Smear bands were likely a result of contamination with proteins, RNA, polysaccharides, and polyphenols. This is consistent with the findings of Mahfut *et al.* (2025a) that the clear DNA bands in gel electrophoresis reflect the highest DNA concentration, whereas the faint bands indicate lower DNA concentration. The smeared DNA bands are often due to contamination by proteins, RNA, and other substances. Polysaccharides and polyphenols can interfere with DNA purity, leading to tarnished bands during electrophoresis. These findings highlight the importance of DNA purity and for obtaining high-quality results.

Primer-specific amplification patterns were as follows: OPA-01: 125 DNA bands, four polymorphic bands (200–500 bp); OPA-07: 116 DNA bands, two polymorphic bands (200–600 bp); OPN-07: 134 DNA bands, four polymorphic bands (200–1000 bp); OPC-16: 111 DNA bands, four polymorphic bands (200–400 bp); and OPD-08: 109 DNA bands, three polymorphic bands (200–500 bp). These variations in DNA band sizes, including distinct

polymorphic bands, reflect genetic variation and phylogenetic relationships among *D. nobile* accessions. Previous research (Bhattacharyya *et al.*, 2014) has shown the usefulness of RAPD markers, including the primers used in this study, to analyze genetic variability in *D. nobile.*

The band profiles generated by RAPD markers revealed patterns, such as shared bands, band loss, and the emergence of new bands. The loss and gain of DNA bands refer to the variations in the genetic sequence, such as deletions, duplications, base substitutions, insertions, and translocations, which often occur as plants adapt to environmental factors. Liu *et al*. (2020) reported that plants frequently endure both biotic and abiotic stressors and have evolved sophisticated adaptation and defense mechanisms. Fielding *et al.* (2020) further demonstrated that epigenetic modifications, including DNA methylation, histone modifications, and RNA modifications, enable crop plants to dynamically and heritably adjust gene expression in response to environmental conditions. Stress-induced heritable changes in DNA methylation patterns could play a key role in the adaptation to recurring stresses (Mahfut *et al*., 2025b). Collectively, these findings suggested the genetic and epigenetic variations are significant contributors to plant adaptation under changing environmental conditions.

The DNA amplification observed through the PCR-RAPD technique demonstrated that using informative markers (OPA-01, OPA-07, OPN-07, OPC-16, and OPD-08) is an effective method for characterizing the genetic stability, which has direct links to the consistency of antiviral phytochemicals in *D. nobile* accessions from Lampung, Indonesia. Tikendra *et al.* (2021) similarly utilized RAPD markers to assess the genetic fidelity of in vitro regenerated *D. nobile*, confirming the technique's efficacy in assessing the genetic stability and phytochemical consistency. The presence of high concentrations of dendrobine and alkaloids suggests a strong potential for antiviral applications, corroborating findings from Liu *et al*. (2020). Bhattacharyya *et al.* (2014) constructed the genetic linkage maps for *D. nobile* using RAPD markers, highlighting the utility of these markers in genetic characterization. Tikendra *et al*. (2021) investigated the genetic stability of *Dendrobium Bobby Messina* following the cryopreservation, demonstrating RAPD's effectiveness in assessing genetic fidelity. Joshi *et al.* (2023) also assessed the genetic stability of *Dendrobium transparens* using RAPD and ISSR markers, supporting the applicability of these techniques in genetic characterization. The promising study further underscores the reliability of RAPD markers for evaluating genetic stability, which is crucial for understanding phytochemical attributes, including the production of antiviral compounds, in *Dendrobium* species. The observed genetic diversity may contribute to variations in phytochemical profiles across populations, as suggested by the differing levels of flavonoids and alkaloids in samples.

As genetic variations were evident from polymorphic DNA bands, their implications for phytochemical diversity require further exploration. Previous studies have established a connection between genetic stability and phytochemical properties, emphasizing the role of alkaloids, terpenoids, and flavonoids in antiviral activity. For example, dendrobine synthesis can result from genetic differences among accessions. Polymorphic accessions, such as D3 exhibiting distinct genetic profiles, could harbor unique phytochemical compositions. Variations in environmental factors, such as habitat and cultivation practices, likely contribute to this diversity.

Phylogenetic analysis

Phylogenetic analysis based on DNA band scoring (Figure 2) revealed a similarity index ranging from 0.47 to 1.00, dividing the accessions into two clusters. Cluster A, with a similarity index of 0.33, consisted of a single control accession. Cluster B, with a similarity index of 0.55, further parts into two subclusters. Subcluster I comprised D2, D5, D1, D6, D4, and D7, while Subcluster II included accession D3, grouped separately due to habitat and environmental differences.

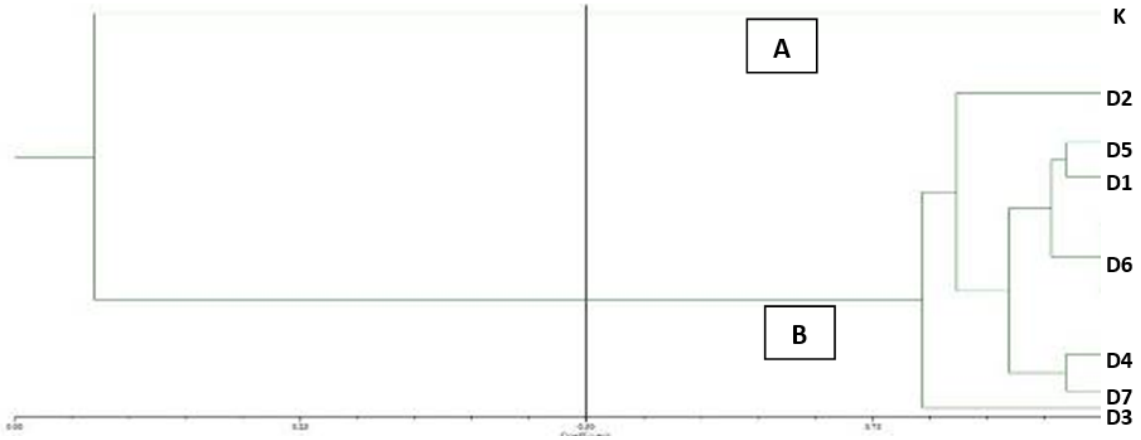

Figure 2. Dendrogram of the phylogenetic relationships of the *D. nobile* Lindl. accessions in Lampung, Indonesia using Jaccard's Coefficient.

Accessions within subclusters I and II also exhibited close genetic relationships, as authenticated by coefficient values exceeding 0.85. Accessions grouped within the same subcluster tended to have a particularly close relationship, with coefficient values above 0.9. In contrast, D3 and the control display distant relationships with the other accessions, as reflected by coefficient values of less than 0.1 and 0.80, respectively.

The cluster analysis revealed all native accessions of *D. nobile* in Lampung form a single group, indicating overall genetic similarity despite some mutation occurring across the accessions. This clustering was likely to refer to the shared habitat and environmental factors, leading to uniformity in the genotypes amplified by the RAPD primer. Six accessions (D2, D5, D1, D6, D4, and D7) separated from accession D3 have experienced habitat variations and environmental adaptation due to domestication and cultivation outside their original habitat.

Tikendra *et al.* (2021) reported that *D. nobile* populations from similar habitats exhibited genetic similarity when assessed using RAPD markers. *D. nobile* accessions from comparable environments tend to cluster together in genetic analysis, underscoring the significant influence of habitat on genetic structure (Mahfut *et al*., 2024c). Bhattacharyya *et al.* (2014) further stated environmental factors and domestication processes can lead to genetic variations in *D. nobile* populations, as evidenced by the distinct grouping of accession D3 in this study. These findings suggested the habitat and environmental conditions play a vital role in shaping the genetic structure of *D. nobile* populations.

The variations among the *D. nobile* accessions, as observed in the dendrogram, reflect diversity from the mutation, which enhances the genetic variability in plants. The control and all other accessions in different clusters indicated the genetic differences caused by point mutations that alter a single base pair without changing the encoded amino acid and generating the genetic diversity. This phenomenon aligns with findings by Bhattacharyya *et al.* (2014), which state plant mutations have various effects on their traits, ranging from positive to negative and even neutral. Chu and Wei (2021) highlight that synonymous mutations, which do not alter the amino acid sequence, however, contribute to the genetic diversity and adaptation in crop plants. Past studies discussed how evolutionary forces, including mutation, can affect the synonymous variations in plant genomes, thereby influencing genetic diversity (Nkurikiyimfura *et al*., 2024). These studies underscore the role of point mutations, including synonymous ones, in generating the genetic diversity within plant populations. Results of this study have some limitations in sample size, RAPD marker resolution, and

limitations in phytochemical screening methods. Further studies using high-throughput sequencing techniques and advanced metabolomics could provide more insights into the genetic and biochemical diversity of *D. nobile*.

CONCLUSIONS

This study successfully revealed the genetic diversity and phytochemical properties of *Dendrobium nobile* Lindl. using the RAPD technique. The analysis indicated a moderate level of polymorphism, with PIC values ranging from 0.31 to 0.40, reflecting genetic diversity that is essential for characterizing genetic stability. Phylogenetic analysis grouped the accessions into two main clusters, with subclusters influenced by environmental and habitat factors. The RAPD technique proved effective in identifying genetic stability and the consistency of antiviral phytochemical compounds, including alkaloids, flavonoids, and terpenoids, which hold potential as antiviral agents. These findings support the conservation and further exploration of the phytochemical potential of *D. nobile* as a source of antiviral medicines.

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