

CHARACTERIZATION OF AROMATIC, MORPHOLOGICAL, AND GENETIC DIVERSITY IN GARDENIA (*Gardenia jasminoides*) FOR SUSTAINABLE FRAGRANCE APPLICATIONS

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Introduction

Aromatic plants are widely distributed in nature, with many species cultivated specifically to produce essential oils. Gardenia (*Gardenia jasminoides*) is a flowering perennial with white, sweet-scented flowers, which belongs to the family of Rubiaceae [1]. The plants grow to between two and five feet tall and have glossy light green leaves. It is cultivated largely for decor, perfume, and even the pharmaceutical industry. As an indigenous plant, its utilization aligns with the growing emphasis on sustainable and eco-friendly agricultural practices. Gardenia essential oil is prioritized for its sweet, floral fragrance and is commonly used in perfumery and aromatherapy due to its calming and mood-uplifting properties [2]. This study aims to evaluate the morphological and chemical characterization of these species, utilizing DNA markers and Gas Chromatography-Mass Spectrometry (GC-MS) to achieve precise species identification and detailed profiling of volatile organic compounds (VOCs) present in Gardenia to support its sustainable use and value-added applications. Understanding the genetic diversity and chemical profile is essential for ensuring the optimal use of Gardenia in both perfumery and medicinal fields. The findings could support future breeding programs and the genetic enhancement of fragrance, contributing to both the ornamental and fragrance industries.

Materials and Methods

Selection of Gardenia

Thirty-eight aromatic plant parts were collected from home gardens and public markets in Jaffna and Mullaitivu. Ten men and ten women (22 - 27 years of age) were selected randomly for the sensory evaluation. They evaluated aromatic preference and aroma intensity of aromatic plant parts. A sensory evaluation was conducted using a 0-10 points hedonic intensity scale, in which a score of 0 represented an extremely unpleasant aroma with no detectable odor, while a score of 10 indicated a sublimely pleasant aroma with overwhelmingly strong intensity. This scale enabled precise assessment of both aroma preference and intensity across a broad sensory spectrum [3].

Identify and categorize the different morphotypes of G. jasminoides

Distinct types of Gardenia were identified based on flower fragrance and morphological features, particularly flowers and leaves, in Jaffna, Mullaitivu, and Kilinochchi districts.

Molecular characterization of G. jasminoides

DNA extraction was done by using the modified CTAB method [4]. PCR was performed by using barcoding primers ITS5 F: 5¹-GGA AGT AAA AGT CGT AAC AAG G-3¹, and ITS4 R: 5¹ TCC TCC GCT TAT TGA GC-3¹ with a pre-denaturation 95 °C for 5 min, denaturation 95 °C for 30 sec, annealing at 50 °C for 1 min, and extension 72 °C for 1 min. The final extension was carried out at the same temperature for 10 min to amplify the ITS regions to confirm the species identification [6]. Positive amplicons were sent to Macrogen Inc. (Geumcheon-gu, Seoul, South Korea) for Sanger sequencing.

Phylogenetic analysis

Phylogenetic analysis explored genetic relationships and evolutionary divergence among different accessions of *G. jasminoides*. The sequences of *G. jasminoides*. used in this study were collected from three geographic regions: China, France, and Sri Lanka. The study included six sequences in total, with one originating from our study in Sri Lanka. The sequences were retrieved from the NCBI GenBank database using accession numbers for each respective region. The details of them are given in Table 1. The Neighbor-Joining algorithm was chosen for phylogenetic tree construction.

Table 1. *G. jasminoides* sequences used for phylogenetic analysis

Accession number	Country
KP092802.1	China
KP092801.1	China
MK123595.1	France
MK165332.1	France
KP092786.1	China
PQ386341	Sri Lanka

GC-MS Analysis of G. jasminoides

20 g of cleaned flowers were air-dried under shade for 3 days and placed in a bottle with a lid. 100 mL of hexane (b.p. 40-60 °C) was added to cover the sample fully, and it was kept on a shaker for 72 hours. The extract was filtered and transferred to a tube with a lid. Volatile compounds were analyzed using GC–MS equipped with a capillary column (SCION 8900 TQ GC Mass Spec), and identification was based on retention indices.

Essential oil production of *G. jasminoides*

Fresh flowers of *G. jasminoides* were collected. A thin layer of refined coconut oil was spread on a plate, and a sieve was placed above the plate. The flowers were then placed on the sieve, allowing the volatile compounds to infuse into the oil. After 10 - 12 hours, the infused oil was collected and stored in a glass bottle. The fragrance of the infused Gardenia oil was evaluated by 15 persons from the first day of production up to six months of storage.



Figure 1. Essential oil production of Gardenia (A- collection of Gardenia flowers, B - Placing of flowers on the sieve, C- Allowing the volatile compounds to infuse into the oil)

Results and Discussion

Selection of *G. jasminoides*

G. jasminoides showed a moderate preference, with 8 out of 20 evaluators favoring its fragrance. Its relatively high intensity indicates that it is a widely acceptable scent, making it suitable for applications requiring broad consumer appeal. *G. jasminoides* has a pleasant aroma with calming effects, often used in perfumes but underutilized compared to popular options like Rose or Jasmine. This indigenous nature could mean it is more sustainable and locally accessible.

Identify and categorize the different morphotypes of *G. jasminoides*

Two morphologically distinct Gardenia species, *Gardenia jasminoides* and *Gardenia taitensis*, were identified in the Jaffna and Mullaitivu districts based on floral and foliar characteristics. Both of them produce white flowers; however, there are notable differences in their fragrance profiles. *G. jasminoides* is characterized by a strong, sweet fragrance, which is more intense compared to that of *G. taitensis*.



Figure 2. Morphotypes of Gardenia (A- *G. jasminoides*, B- *G. taitensis*)

Molecular characterization of *G. jasminoides*

PCR amplification using ITS barcoding primers yielded amplicons of approximately 650 bp, which was successfully visualized on a 1.5% agarose gel. As the fragrance of

G. jasminoides was preferred by evaluators, molecular analyses were focused on *G. jasminoides*, whereas *G. taitensis* was excluded from further molecular characterization.

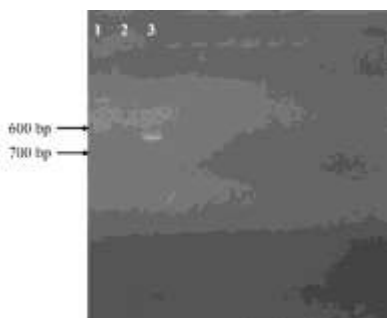


Figure 3. Evaluation of PCR-labeled probes by agarose gel electrophoresis of *G. Jasminoids*

Agarose gel (1.5%) analysis of PCR amplicons from DNA extracted from *G. jasminoids* using ITS 4 and ITS 5 primer pair. Lane 1- DNA Ladder(100bp), Lane 2- negative control, Lane 3 - *G. jasminoids*.

Gardenia GenBank accession number of the sequence is PQ386341.

Table 2. Comparison of morphological identification and BLAST results

Morphological Identification	Gen Bank Accession of the matched sequenced	Corresponding Taxon	Similarity percentage
Gardenia	KP092802.1	<i>Gardenia jasminoides</i>	98.46%

Phylogenetic analysis

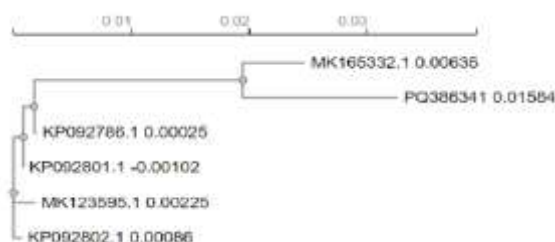


Figure 4. Phylogenetic analysis of *G. jasminoides* sequences from China, France, and Sri Lanka produced a tree with two major clades

The presence of two clades indicates significant genetic divergence between *G. jasminoides* plants in these regions. The Sri Lankan sample clusters with the Chinese group but displays genetic differentiation, pointing to some degree of local adaptation or isolation.

GC-MS Analysis G. jasminoides

GC-MS analysis of *G. jasminoides* revealed the presence of 48 distinct volatile organic compounds (VOCs) with a rich composition of fatty acids and esters. The results present a notably diverse and complex chemical profile compared to previous research. While prior studies emphasized floral and green aroma compounds such as α -Farnesene and Linalool, these results show a dominance of n-Hexadecanoic acid (31.37%) and 9,12-Octadecadienoic acid (Z,Z)- (10.27%), compounds that are either absent or present in lower quantities in earlier reports. The presence of these long-chain fatty acids and methyl esters adds depth to the scent profile, suggesting a fragrance that may be more long-lasting and richer compared to other *G. jasminoides* samples. α -farnesene (0.23%), farnesol (0.20%), benzyl benzoate (0.10%), α -cadinol (0.06%), hexadecanal (0.45%), 9,17- octadecadienal (Z) (0.73%), and neophytadiene (2.52%) are the key aroma VOCs. The presence of minor compounds like Neophytadiene (2.52%), isoeugenol (0.12%), and Alpha-Cadinol (0.06%) suggests potential applications in anti-inflammatory and antimicrobial treatments. The floral, sweet, and slightly spicy aroma profile, contributed by compounds such as Phenylethyl alcohol (0.08%), Benzaldehyde (0.09%), and Benzyl alcohol (0.26%) makes it suitable for fragrance applications in perfumes and aromatherapy.

Essential oil production of G. jasminoides

Essential oil production from *G. jasminoides* was successfully achieved. The gardenia fragrance was effectively infused with refined coconut oil. The infused oil was then carefully collected from the plate and stored in small glass bottles for further use. Sensory panel evaluators confirmed that the fragrance of the infused Gardenia oil was retained for up to six months.

Conclusions and Recommendations

This study provided an integrative characterization of *G. jasminoides* through morphological, molecular, and chemical analyses. Two morphotypes were identified. DNA barcoding confirms *G. jasminoides* species, and phylogenetic analysis reveals genetic divergence among global accessions. GC-MS profiling identified 48 VOCs, dominated by fatty acids and esters of potential value in fragrance, skincare, and medicinal industries. Moreover, the novel infusion method using refined coconut oil demonstrated a cost-effective approach for essential oil production, with fragrance stability maintained for up to six months. These findings underscore the potential of *G. jasminoides* as a sustainable resource for perfumery and related applications.

References

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