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Original Research Article

Genotyping and Phytochemical Analysis of Three Species of Spider Lily (*Hymenocallis* spp.)

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ABSTRACT

Spider lily is a plant that contains phytochemicals for various pharmacological activities. Morphological similarities in several spider lily species make it difficult to identify morphologically. Molecular approaches are recommended for identifying plant species that are difficult to identify morphologically. This study aimed to identify three species of spider lily (*Hymenocallis* spp.) in Denpasar, Bali (Flat Lily, Bangkok Lily, and Small Leaf Lily) using DNA barcoding. The phytochemical of the extract was identified using Gas Chromatography-Mass Spectrometry (GC-MS). The molecular data showed that the three species were genetically close to *Hymenocallis littoralis*, although significant genetic distance was still found. Based on phylogenetic tree construction using the Maximum Likelihood method, the three species in Denpasar are in the same clade as *Hymenocallis littoralis*, *Hymenocallis maximiliani*, and *Hymenocallis caribaea* but still show some relevant genetic variation. Various compounds were identified in the leaf extracts. 2,4,6-Cycloheptatrien, Trehalose and Hydroxymethylfurfural were bioactive compounds with high AUC known with known antioxidant, anti-inflammatory, and neuroprotective potential.

Keywords: Deoxyribose nucleic acid Barcoding, Gas Chromatography-Mass Spectrometry, *Hymenocallis littoralis*, maturase K, Phytochemicals.

Introduction

The spider lily (*Hymenocallis* spp.) has been recognised for its medicinal properties in various traditional practices. These plants are valued for their ornamental appeal and ethnobotanical applications, which include treating a range of ailments. Spider lily has been utilised in traditional medicine to treat skin conditions like freckles and blemishes.[1] Research has highlighted the presence of bioactive compounds in spider lilies that contribute to their medicinal efficacy. Notably, *Hymenocallis littoralis* has been documented to possess antitumor properties, with the active compound lycorine being isolated from its bulbs. Lycorine has demonstrated both antineoplastic and antiviral activities, making it a subject of interest in pharmacological studies.[2] Moreover, lily bulbs are also rich in dietary fibres and starch, which have been shown to modulate gut health and inflammation [3]. Identifying spider lily species can present several challenges, primarily due to their morphological similarities and various pathogens that can obscure their characteristics. One significant issue is the overlap in physical traits among different species, which can lead to misidentification. For instance, *Hymenocallis* and *Crinum* species are often confused due to their similar bulbous structures and flower shapes, which can complicate identification efforts.[1]

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In addition to morphological challenges, the identification of spider lilies can also be hindered by the lack of comprehensive taxonomic keys that account for the variability within species. Many identification methods rely heavily on specific morphological traits, which can vary significantly due to environmental factors or disease.[4] This variability can lead to confusion, particularly in regions where multiple species coexist and hybridisation may occur.[1] In Denpasar, Bali, three species of spider lily have been found, namely Small Leaf Lily (local lily/*Lily Daun Kecil*), Bangkok Lily (*Lily Bangkok*), and Flat Lily (*Lily Gepeng*). Although all three grow in the same climatic conditions, morphological differences such as leaf and flower shape indicate the possibility of genetic variation. Researchers have suggested incorporating molecular techniques, such as DNA barcoding, to address these challenges and complement traditional morphological identification methods. DNA barcoding can provide a more reliable means of distinguishing between closely related species, especially in cases where morphological features are not definitive. This approach has been shown to enhance the accuracy of species identification, particularly in taxa that exhibit high levels of cryptic diversity or sexual dimorphism.[5]This study was conducted to identify the three species of spider lily in Denpasar (Bali) through morphological and molecular approaches, using DNA Barcoding with Maturase K gene markers. Phytochemical compounds were also identified in spider lily extracts using Gas Chromatography-Mass Spectrometry (GC-MS).

Materials and Methods

This research is a descriptive observational study in morphological identification, molecular identification, and GC-MS analysis to determine the function of each bioactive component contained in each species.

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Collection of Plant Samples

Samples of three species of spider lily were obtained from three different growing locations in South Denpasar, Bali, in June – July 2024. Flat lily was collected from Pamogan village (8°43'3.21 "S, 115°12'13.74 'T), Bangkok lily was collected from Sesetan village (8°42'46.40'S, 115°13'33.01 'T) and small leaf lily was collected from Pedungan village (8°40'51.28'S, 115°12'25.65 "T). Morphological identification of the three samples was carried out through observation to compare the differences in morphological characters in stems, bulbs, leaves, and flowers. Samples were stored at the Genetic Resources and Molecular Biology Laboratory, Udayana University (voucher no. SDGBM/78/24/037 (flat lily), SDGBM/78/24/043 (Bangkok lily), and SDGBM/78/24/044 (small leaf lily)).

Isolation and Amplification of matK Gene by PCR (Polymerase Chain Reaction)

Spider lily DNA was isolated, and total DNA was extracted using Quick DNA Plant/Seed (Zymo Research, D6020). The isolated DNA was amplified using the KOD FX NEO PCR method (Toyobo, KFX-201) with the primer pair *matK* forward 3F-R (5'-CGT ACA GTA CTT TTG TGT TTA CGA G – 3') and reverse *matK*-IR-F (5'ACC CAG TCC ATC TGG AAA TCT TGG TTC -3'), producing a fragment of 850 bp. The total volume of the sample analysed was 50 µL, with PCR conditions as follows: pre-denaturation at 95°C for 2 minutes for one cycle, denaturation at 94°C for 1 minute, annealing at 58°C for 45 seconds, extension at 72°C for 45 seconds, and post-extension at 72°C for 5 minutes for 35 cycles. The resulting amplicon was visualised by electrophoresis on 1% TBE agarose gel with a 100 bp DNA ladder. Electrophoresis was carried out at 90 V for 30 minutes. The DNA bands formed were observed using a UV-transilluminator. The purified PCR products were then sequenced using the bi-directional sequencing technique.

Phylogenetic Construction

The nucleotide sequences from the sequencing results were compared with the database available at NCBI (www.ncbi.nlm.nih.gov) to find sequences homologous to the basil sequences from Bali by performing nucleotide BLAST analysis. Accessions with the highest similarity or identity percentage were selected, and sequence alignment was performed using BioEdit and ClustalW programs. A phylogenetic tree was constructed in MEGA 11 using the Maximum Likelihood method and the Tamura 3-parameter substitution model. The accuracy of the tree was evaluated using a 1000x bootstrap analysis. Phylogenetic construction aimed to determine the kinship level of the spider lily types and observe their evolutionary rates.[6]

GC-MS (Gas Chromatography-Mass Spectrometry) Analysis

Dried leaf samples were extracted using the maceration method with 96% ethanol for three days. The filtrate obtained was evaporated using a vacuum rotary evaporator (Buchi Rotavapor R-200, Büchi Labortechnik AG, Swiss) to produce crude extract for GC-MS Analysis. GC-MS analysis was performed using an Agilent 7890B MSD5977B (Agilent Technologies, USA) with a Wakosil ODS/5C18-200 silica column, sized 4.6 x 200 mm. Samples were injected with a volume of 1 µL into the GC-MS column at an injection temperature of 290°C for 27 minutes. Phytochemical compounds were identified using Willey database version 7.0 by comparing the mass spectrum and fragmentation patterns of reference compounds stored in Willey's library.

Results and Discussion

Morphological characterisation of the three spider lily species showed similarities and apparent differences. The stems of spider lilies are typically erect and can reach heights of about 60 cm. They are generally slender and may be somewhat fleshy, supporting the flower clusters that emerge from their tops. The stem structure is crucial for supporting the large, often fragrant flowers that characterise these species.[1,7] The stem's morphology can vary slightly depending on environmental conditions and specific species, but it generally maintains a robust and upright posture. The three spider lily species' stem morphology (caulis) (Figure 1) shows the same characteristics. The stems of spider lilies appear round and white. However, it is a layered bulb with modified tunica or squama (scales), a metamorphosis of leaves and a place to store food reserves.

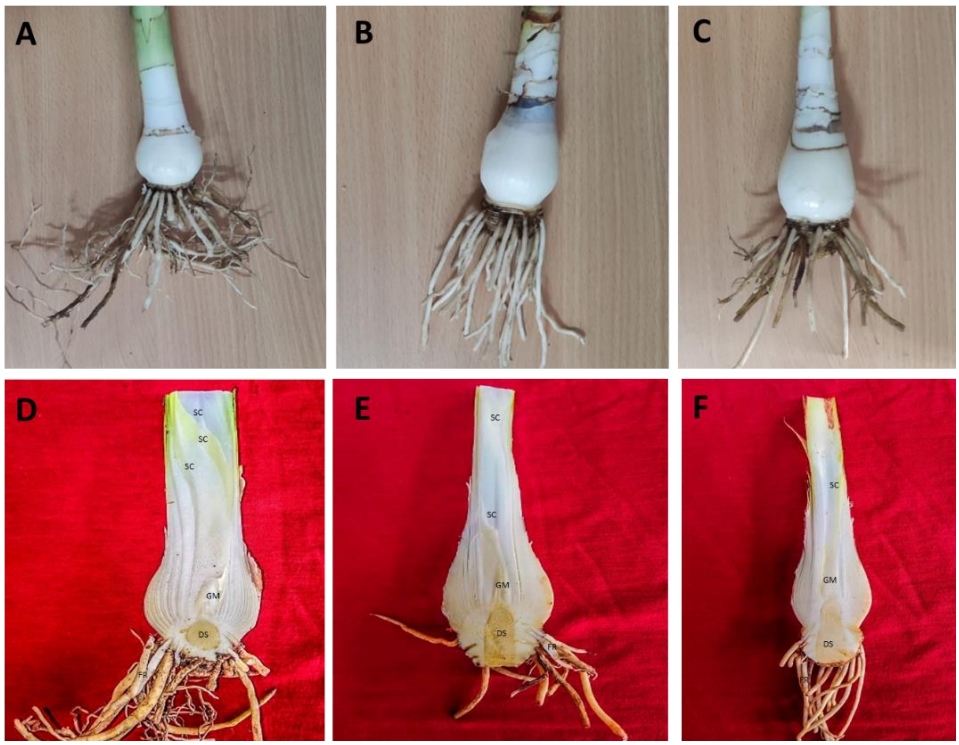

Figure 1: Morphological appearance of stems and bulbs of flat lily (A, D), Bangkok lily (B, E), and Small Leaf Lily (C, F). The bulbs of the three spider lily species (D, E, F) show the presence of scales (SC), gemmae/buds (GM), discus (DS), and fibrous roots (FR).

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The bulbs of spider lilies are bulbous and fleshy, serving as storage organs for nutrients. These bulbs are usually located underground and are responsible for the plant's vegetative propagation. The bulb's morphology can vary among species, with some exhibiting a more rounded shape while others may be elongated. The bulb's size and health are critical for the plant's flowering capacity, as larger bulbs tend to support more vigorous growth and earlier flowering. The bulbs also play a role in the plant's drought resistance, as they store carbohydrates essential for sustaining growth during dry periods.[8] All three types of spider lily have the same form of branching, similar to onions, with the loss in apical dominance and lateral initiation occurring after the development of two or three leaves. The apical meristem is divided into two parts, with new leaves and lateral buds sprouting at the branching. The bulbs are similar to onions with a disc-like structure (discus), branched and show white buds between the segments (Figure 1). Identification shows several forms of stem modification, such as discus and scales (tunica/squama). The leaves of spider lilies are narrow and strap-like, typically arranged in a rosette pattern at the base of the plant. These evergreen leaves are usually green and can vary in length depending on the species. The leaf morphology is adapted to maximise photosynthesis while minimising water loss, which is particularly important for plants in warmer climates.[1] The leaves also play a role in the plant's overall health, as they are involved in nutrient uptake and photosynthesis, contributing to the bulb's energy reserve. In this research, spider lily samples show dense crowned leaves with an elongated flat leaf shape, smooth leaf surface, and pointed leaf tips; leaf length ranges from 30 to 90 centimetres. The leaf morphology (Folium) shows similar characteristics with different parts (Figure 2).

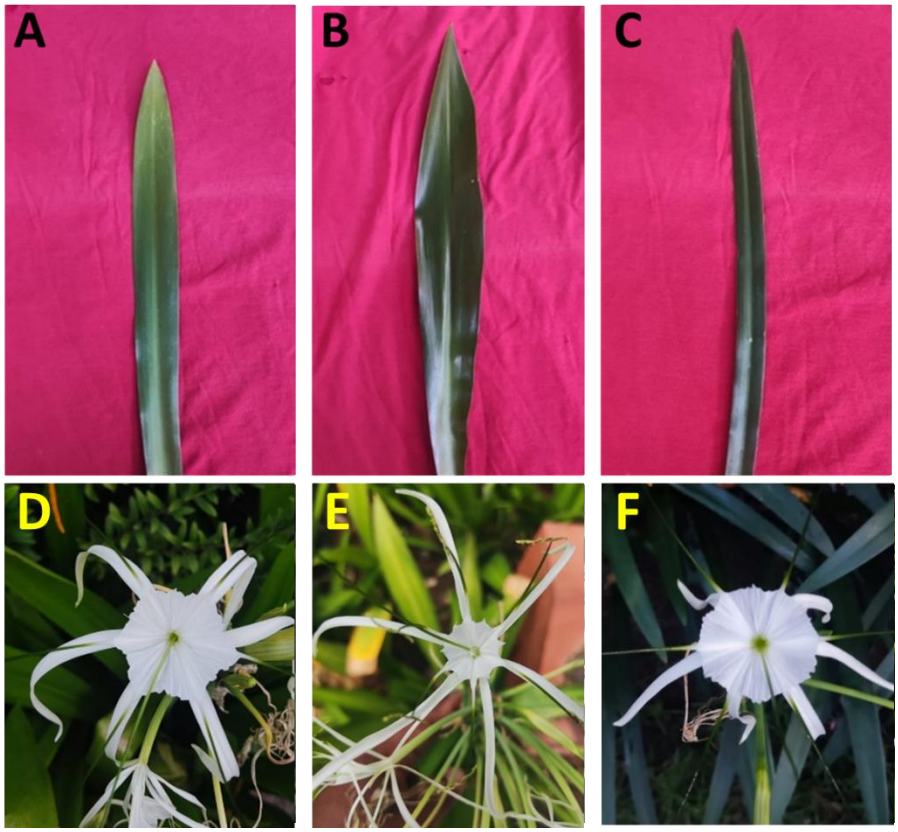

Figure 2: Morphological appearance of the leaves and flowers of Flat Lily (A, D), Bangkok lily (B, E), and Small Leaf Lily (C, F).

The leaves of the Bangkok lily are larger, with a width of 3-7 centimetres, while the leaves of the flat lily and the small leaf lily are straight and pointed. Flat lily leaves are larger (2.5-4 cm) than small leaf lily (1.5-2.5 cm). These three leaves have the same green colour and similar thickness, about 0.2-0.4 cm. The flowers of spider lilies are one of their most striking features. They typically appear in clusters and are characterised by their unique shape, which resembles the legs of a spider, hence the common name. The flowers have slender, recurved petals that are often white, although some species may exhibit variations in colour. Each flower is borne on a long stalk that rises from the centre of the leaves, creating a visually appealing display.[1,7] The flowers are known for their fragrance, which can attract pollinators, and they often bloom in the warmer months, adding to their ornamental value.[1] The characteristics of the flowers (flos) on the three types of spider lily show similarities with non-significant differences (Figure 3). The difference shown on the sprawling lily flower is similar to the small leaf lily flower but slightly different from the Bangkok lily. The Bangkok lily has the smallest flowers compared to the flat lily and small leaf lily, and the Bangkok lily has a slightly different flower shape. All three have the same white flower colour. Amplification was performed using primers matK forward 3F-R and reverse gene matK-IR-F. PCR results were visualised through gel electrophoresis and UV transilluminator. In the electrophoresis results of the DNA of three types of spider lily growing in Denpasar, some bands show the amplified DNA fragments, indicating the number of base pairs that have been successfully cut (Figure 4). Table 1 shows that the three spider lily species have the smallest genetic distance from *Hymenocallis littoralis*: (JX903566.1) 0.8993 for sprawling lily, 0.8842 for Bangkok lily, and 0.9049 for small leaf lily. The largest percentage identity was also with *Hymenocallis littoralis*: 51.11% for flat lily, 51.34% for Bangkok lily, and 50.99% for small leaf lily. The phylogenetic tree of sprawling lily, Bangkok lily, small leaf lily and others constructed by the Maximum Likelihood method and the 3-parameter Tamura model (MEGA 11) showed that the closest species to the three types of spider lily were *Hymenocallis littoralis* (JX903566.1) with *Hymenocallis maximiliani* (JX464596.1) and *Hymenocallis caribaea* (AB017288.1) in one group (Figure 5).

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Table 1: Pairwise distance and percentage identity of spider lily sequences with species in GenBank

Species	Accession No	Flat Lily		Bangkok Lily		Small Leaf Lily	
		PD	ID	PD	ID	PD	ID
Flat Lily	-	-	-	0.0034	99.66	0.0056	99.65
Bangkok Lily	-	0.0034	99.66	-	-	0.0094	99.08
Small Leaf Lily	-	0.0056	99.65	0.0094	99.08	-	-
Hymenocallis littoralis	JX903566.1	0.8993	51.11	0.8842	51.34	0.9049	50.99
Hymenocallis maximiliani	JX464596.1	0.9079	50.99	0.8924	51.22	0.9135	50.88
Hymenocallis tubiflore	AY434482.1	1.4212	41.74	1.447	41.55	1.4316	41.47
Hymenocallis speciosa	AF223512.1	2.2324	32.73	2.1179	33.03	2.0547	32.97
Hymenocallis caribea	AB017288.1	0.9079	50.88	0.8925	51.11	0.9135	50.76

PD (Pairwise distance), ID (percentage of identity)

The pairwise values show that the three species of lilies sampled from Denpasar Bali show closeness to *Hymenocallis littoralis*, however, with pairwise closeness values that are still quite far away (around 0.8 and 0.9). Morphological characteristics of the three spider lily samples observed through this study showed similar characteristics to *Hymenocallis littoralis* as described by.[1,7]

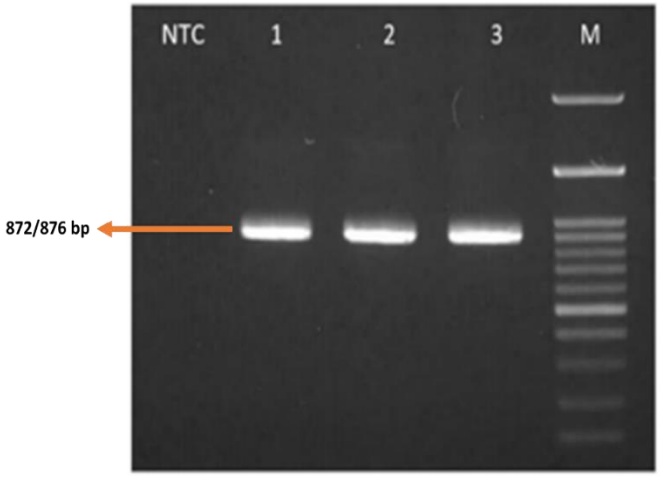

Figure 3: Electrophoresis result of PCR amplification using matK gene

DNA barcoding has been recognised as a highly effective tool for species identification, offering high reliability and accuracy across various taxonomic groups. This molecular method employs short, standardised DNA sequences to distinguish species, enabling rapid and precise identification, particularly in cases where morphological traits are ambiguous, or species exhibit cryptic characteristics.[9,10] DNA barcoding is a widely accepted method for species identification that utilises short, standardised regions of DNA to differentiate between species. The reliability of DNA barcoding can be influenced by several factors, including the choice of genetic markers and the quality of the reference database used for comparison. Certain markers may have higher resolution for distinguishing closely related species than others. Environmental factors, geographical variations, and the physiological state of the samples can also affect the DNA sequences obtained, potentially leading to misleading similarity scores.

Variations in storage conditions and processing methods can introduce errors in the DNA extraction and sequencing processes, further complicating the interpretation of results.[11,12] The matK gene is a chloroplast DNA marker that has emerged as a significant candidate for DNA barcoding in plants due to its potential for species identification and phylogenetic studies. This gene is particularly valuable because of its relatively high rates of nucleotide substitution, which enhance its ability to differentiate closely related species.[13,14] The matK region has been widely recognised for its discrimination power in various plant families.[15,16]

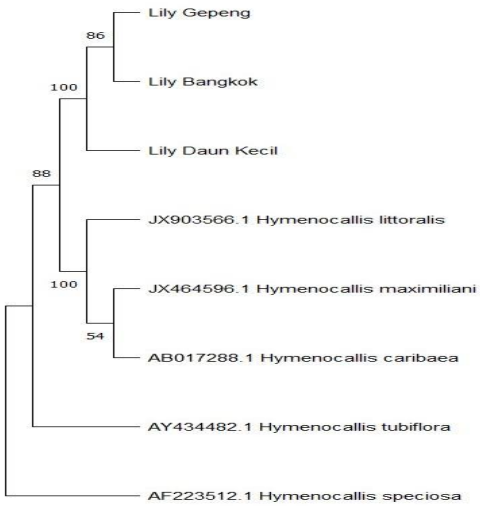

Figure 4: Phylogenetic Tree of spider lily collected in Denpasar (Bali) using the Maximum Likelihood

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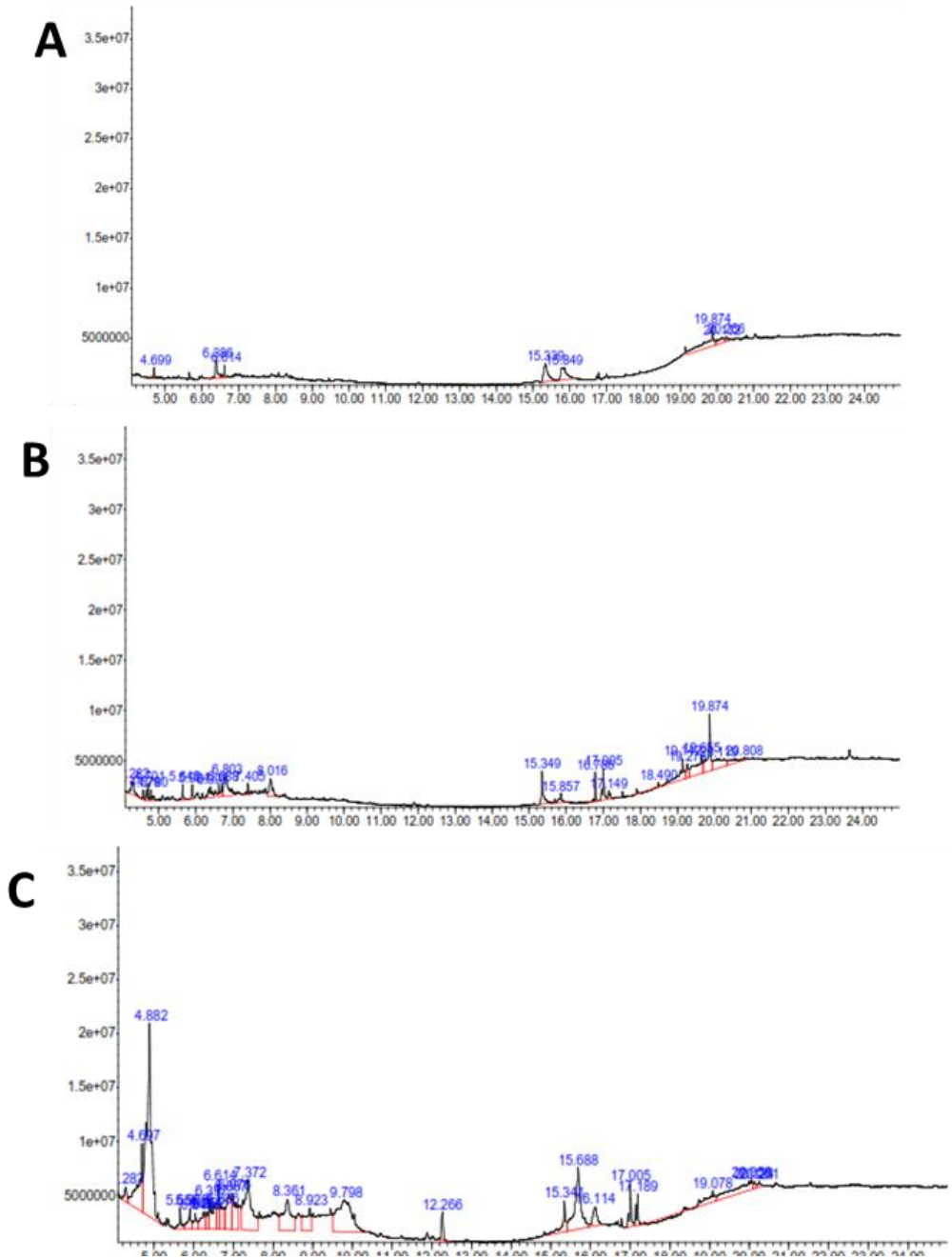

Figure 5: Chromatogram of leaf extract of flat lily (A), Bangkok lily (B) and Small Leaf Lily (C).

Although it may not always provide the best discrimination at the species level compared to other markers, its high amplification success rate makes it a practical choice for many plant taxa.[17,18] The results of the identification of phytochemical compounds in the three spider lily samples using GC-MS are shown in Table 2. Each spider lily species (Flat Lily, Bangkok Lily, and Small Leaf Lily) showed various compounds with different retention times and AUCs, indicating a diverse chemical composition. Some compounds emerged as dominant compounds with high AUC values, such as Hydroxymethylfurfural in Flat Lily (AUC: 10.98) and Trehalose (AUC: 20.77) and Lactose (AUC: 18.47), indicating potentially higher concentrations in certain species. Each species has unique compounds that are not found in other species. For example, 1,2-Bis(trimethylsilyl)benzene with an AUC of 11.74 in Bangkok Lily and L-Arabinitol with an AUC of 11.21 in Small Leaf Lily indicate a particular specificity of chemical components within each species. The AUC value gives an idea of the relative concentration of the compounds. At the same time, the Rt (Retention Time) helps identify the chemical characteristics of each compound based on the elution time in the GC-MS column. High AUC values of certain compounds may indicate their essential role in the spider lily's pharmacological characteristics or specific aroma. Some compounds such as 9,12,15-Octadecatrienoic acid and N-Hydroxymethylacetamide have biological activities that have been investigated in other plant species and could have implications on the medical potential of spider lily. Some compounds common in flowering plants, such as Glycerin and Phytol, were found in these species; supporting literature reports that these compounds are widely found in flowering plants with biological or therapeutic activities. 2,4,6-Cycloheptatrien is one of the compounds identified with a high AUC value. It is commonly referred to as tropone, and it is a compound that has attracted interest in pharmacological research due to its unique structural properties and potential biological activities.

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Table 2: Phytochemical compounds in the ethanol extract of three types of spider lily from Bali based on GC-MS analysis

Chemical	Flat Lily		Bangkok Lily		Small Leaf Lily	
	Rt	AUC	Rt	AUC	Rt	AUC
3-{[(1,3-Dihydroxy-2-propanyl)oxy]methyl}-4-hydroxy-1H-pyrazole-5-carboxamide	-	-	4.282	3.69	-	-
Glyceraldehyde			-	-	4.283	0.85
n-Propyl Decyl Ether	-	-	4.579	0.57	-	-
Glycerin	-	-	-	-	4.697	6.38
Benzeneacetaldehyde	4.699	1.87	4.701	1.88	-	-
2-Methyl-1-ethylpyrrolidine	-	-	4.780	1.61	-	-
N-Hydroxymethylacetamide	-	-	-	-	4.882	19.94
4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	-	-	5.645	1.62	5.656	0.98
5-Methoxypyrrolidin-2-one	-	-	5.904	1.48	-	-
Aziridine, 2-methyl-3-(1-methylethyl)-, trans-	-	-	-	-	5.908	1.30
1-Tetradecyl acetate	-	-	-	-	6.04	1,16
1,2,3,4-Butanetetrol, [S-(R*,R*)]-	-	-	-	-	6.254	4.67
Propanamide	-	-	-	-	6.332	0.97
Hydroxymethylfurfural	6.386	10.98	-	-	-	-
Cyclopropanecarboxamide	-	-	-	-	6.397	3.48
3-Piperidinol	-	-	6.398	3.37	-	-
2,6-Octadienal,3,7-dimethyl-,(E)	6.614	2.42	-	-	6.614	1.54
3-Pentanone, dimethylhydrazone	-	-	6.688	2.36	-	-
d-Glycero-d-ido-heptose	-	-	6.803	9.01	-	-
3,4-Furandiol, tetrahydro-, trans-	-	-	-	-	6.686	6.04
N-Acetyl-l-methioninamide	-	-	-	-	6.976	3.81
Erythritol	-	-	-	-	7.372	8.72
2-Propanone, dimethylhydrazone	-	-	7.405	1.70	-	-
1,6-Anhydro-2,4-dideoxy-.beta.-D-ribo-hexopyranose	-	-	8.016	5.72	-	-
1-Deoxy-d-mannitol	-	-	-	-	8.361	6.17
L-Arabinitol	-	-	-	-	9.798	11.21
3-(3-Fluoroanilino)-1-(3-nitrophenyl)-1-propanone	-	-	-	-	12.266	1.23
Trehalose	15.339	20.77	-	-	-	-
n-Hexadecanoic acid	-	-	15.349	5.88	15.341	2.27
Lactose	15.849	18.47	-	-	-	-
1-Ethanone, 1-[4-acetyl-2,5-dimethyl-1-(8-quinolinyl)-1H-pyrrol-3-yl]-	-	-	15.857	2.59	-	-
Pentanoic acid, ethyl ester	-	-	-	-	15.688	8.35
Galactitol	-	-	-	-	16.114	2.00
Phytol	-	-	16.786	2.33	-	-
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	-	-	17.005	4.55	17.005	1.69
1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one	-	-	-	-	19.078	0.93
1,8-Octanediamine, N,N'-dimethyl-	-	-	17.149	1.31	-	-
Ethyl 9,12,15-octadecatrienoate	-	-	-	-	17.189	1.23
2-Ethylacridine	-	-	19.142	8.85	-	-
1,4-Bis(trimethylsilyl)benzene	-	-	19.273	2.86	-	-
1,2-Bis(trimethylsilyl)benzene	-	-	19.655	11.74	-	-
Tazettine	-	-	19.874	13.33	-	-

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Chemical	Flat Lily		Bangkok Lily		Small Leaf Lily	
	Rt	AUC	Rt	AUC	Rt	AUC
2,4,6-Cycloheptatrien-1-one,is-trimethylsilyl-	19.874	34.25	18.490	0.05	20.056	4.38
Arsenous acid, tris(trimethylsilyl) ester Tris(tert-butyldimethylsilyloxy)ar	20.132	8.30	-	-	-	-
4-(4-Hydroxyphenyl)-4-methyl-2-pentanone, TMS derivative	20.256	2.93	20.119	9.33	-	-
Tetrasiloxane, decamethyl-	-	-	20.808	4.18	-	-

AUC (area under curve), RT (retention time)

Its derivatives, particularly those modified with trimethylsilyl groups, have been investigated for various pharmacological applications, including antioxidant, antimicrobial, and anticancer activities. One of the notable pharmacological activities of 2,4,6-cycloheptatrien-1-one is its antioxidant capability. Research indicates that tropone and its derivatives can scavenge free radicals, thereby mitigating oxidative stress, which is implicated in numerous diseases, including cancer and neurodegenerative disorders.[19,20] The presence of 2,4,6-cycloheptatrien-1-one in certain plant extracts has been associated with antioxidant properties, contributing to the overall health benefits of these extracts.[20] 2,4,6-cycloheptatrien-1-one also exhibits significant antimicrobial activity against various pathogens.[20,21] Moreover, tropone and its derivatives have shown promise in cancer research. The compound's ability to inhibit cell proliferation has been documented, with studies indicating that it may interfere with critical cellular pathways involved in cancer progression.[21,22] Hydroxymethylfurfural was the compound found with the high AUC value on Flat Lily. Hydroxymethylfurfural has garnered significant attention in pharmacological research due to its diverse biological activities. It is primarily recognised for its antioxidant, anti-inflammatory, and cardioprotective properties, contributing to its potential therapeutic applications in various diseases, including neurodegenerative disorders and cardiovascular diseases. One of the notable pharmacological activities of Hydroxymethylfurfural is its neuroprotective effect. Research shows that Hydroxymethylfurfural can activate N-methyl-d-aspartate (NMDA) receptor signalling, which is crucial for cognitive function and memory, suggesting its potential role in treating Alzheimer's disease (AD) by protecting against cognitive impairment.[23] Furthermore, hydroxymethylfurfural has been shown to exert antioxidant effects, which are vital in mitigating oxidative stress associated with neurodegenerative diseases.[24] Hydroxymethylfurfural is also reported to inhibit L-type calcium currents, which are implicated in various cardiac pathologies, thereby protecting against ischemia/reperfusion injury in cardiac tissues which is believed to be mediated through its antioxidant properties, which help reduce oxidative damage in cardiac cells.[25] Hydroxymethylfurfural is a multifaceted compound with significant pharmacological activities, including neuroprotection, cardioprotection, antimicrobial effects, and potential anticancer properties. These diverse activities suggest that hydroxymethylfurfural could be a valuable therapeutic agent in various medical applications, warranting further research to fully elucidate its mechanisms and clinical potential. Trehalose was found with the high AUC compounds in Flat Lily. Trehalose is a non-reducing disaccharide that has emerged as a promising pharmacological agent due to its multifaceted biological activities, particularly in neurodegenerative diseases, metabolic disorders, and cellular stress responses. Its pharmacological effects are primarily attributed to its ability to induce autophagy, stabilise proteins, and exert neuroprotective and anti-inflammatory actions. One of trehalose's most significant pharmacological activities is its role as an autophagy inducer. Trehalose activates the transcription factor EB, which is crucial for lysosomal biogenesis and autophagy regulation through the inhibition of the AKT pathway, leading to enhanced clearance of aggregated proteins and dysfunctional organelles in various cellular models.[26,27] Studies have shown that trehalose treatment can ameliorate atherosclerosis by promoting macrophage autophagy and lysosomal function, thereby reducing plaque formation.[28] Trehalose has also been shown to protect neurons from oxidative stress and neurotoxicity, as evidenced by its ability to suppress the activation of stress-related signalling pathways in models of neurodegeneration.[29] In addition to its neuroprotective effects, trehalose exhibits antioxidant, antiinflammatory, and anticancer properties.[30–32] The development of trehalose-based analogs aims to improve its bioavailability and therapeutic potential in cancer therapy and other diseases.[31,32]

For future studies, research could be expanded to investigate deeper genetic variations using other molecular markers besides the matK gene, such as rbcL or ITS, to further clarify the evolutionary relationships among species. Follow-up studies could also focus on how environmental factors (e.g., humidity, soil conditions, sunlight exposure) influence the phytochemical profiles of *Hymenocallis spp.*. Understanding these variations is crucial for identifying phytochemical properties that may be relevant for pharmaceutical applications. Additionally, further experiments could be conducted to evaluate the biological mechanisms of key compounds identified, such as Trehalose and Hydroxymethylfurfural, particularly their antioxidant, anti-inflammatory, and neuroprotective activities.

Conclusion

This study identified three species of spider lily in Denpasar (Flat Lily, Bangkok Lily, and Small Leaf Lily) through morphological and molecular approaches using DNA barcoding with the matK gene marker and phytochemical analysis through GC-MS. The study showed that the three species have genetic similarities with *Hymenocallis littoralis*, although there are differences in genetic distance and percentage identity. Morphological analysis revealed variations in the shape of stems, bulbs, leaves, and flowers, indicating genetic variation. Through GC-MS, several different bioactive compounds were identified in each species, with high AUC for compounds such as Trehalose and Hydroxymethylfurfural in Flat Lily. This study highlights the pharmacological potential of spider lily. It proposes that a combination of morphological and molecular approaches can improve the identification of species with high morphological similarity accuracy.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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