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Diversity of *Hoya* Species across Three Plantation Landscapes in Jember Regency and the Phytochemical and Antioxidant Potential of *Hoya lacunosa* Blume

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Abstract. Jember Regency encompasses several plantation areas characterized by diverse ecological conditions, notably the Gumitir, Garahan, and Tancak plantations. Among the flora in these regions, the genus *Hoya* is notable for its medicinal potential. This study aimed to characterize the diversity of Hoya species, provide detailed morphological documentation, and evaluate the phytochemical and antioxidant properties of a medicinally used species. Field exploration employed a systematic plot-based design consisting of 150 plots (5 × 5 m²) with 3 m spacing. The most vegetatively dominant species, *Hoya lacunosa*, was subjected to GC–MS phytochemical analysis and DPPH-based antioxidant evaluation. A total of eleven Hoya species were recorded across the three plantation sites. The Gumitir plantation exhibited the highest diversity, containing ten species., i.e. *Hoya burtoniae*, *H. camphorifolia*, *H. carnosa*, *H. cinnamomifolia*, *H. dennisii*, *H. fuscomarginata*, *H. lacunosa*, *H. micrantha*, *H. purpureofusca*, and two unidentified *Hoya* spp. In contrast, the Garahan plantation supported only *H. dennisii* and *H. fuscomarginata*, while *H. lacunosa* was exclusively found in Tancak indicating a highly localized distribution. GC-MS profiling on *H. lacunosa* leaves revealed 26 compounds, comprising 16 secondary metabolites (12 phenolics, 2 alkaloids, and 2 terpenes) and 5 primary metabolites. The methanol extract exhibited strong antioxidant activity (IC_{50} = 50.89 ppm), reflecting its potential as a natural source of bioactive compounds. These findings demonstrate substantial *Hoya* diversity in Jember Regency and underscore the potential of *H. lacunosa* as a valuable source of bioactive metabolites for pharmaceutical development.

Keywords: antioxidant; bioactive; GC-MS; *Hoya*; plantation

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INTRODUCTION

The genus *Hoya* represents one of the largest groups of flowering plants within the family Apocynaceae, comprising approximately 450 species distributed predominantly across the Indomalayan and Australasian regions, extending to Japan and the Himalayan foothills (Rumaling et al., 2025). *Hoya* species are primarily epiphytic and are valued for their unique morphology and ecological significance. Indonesia is a biodiversity hotspot for *Hoya*, with around 120 species recorded, including 22 species on the island of Java (Rahayu et al., 2018). Notable species include *H. carnosa* (L. f.) R. Br., *H. lacunosa* Blume, *H. multiflora* Blume, and *H. purpureofusca* Hook., with leaf morphological variation observed in *H. carnosa* across different habitats in Indonesia (Bermuli et al., 2019).

Commonly referred to as "wax plants," *Hoya* species exhibit morphological similarities with orchids but are distinguished by their thick, fleshy, oval to heart-shaped leaves and small, star-like flowers arranged in umbels. The wax-like appearance of the flowers, often fragrant at night, gives rise to the name "wax flower" (Medina et al., 2016; Pudjas et al., 2022; Robika et al., 2015). While *Hoya* is primarily cultivated as an ornamental plant, its medicinal value is increasingly recognized across Southeast Asia. In Indonesia, the Tengger community in East Java traditionally uses *H. lacunosa* to treat stomach ailments, while the Balinese utilize *H. carnosa* for earaches and *H. purpureofusca* as an antiseptic (Rahayu, 2012). Other ethnomedicinal applications include *H. parasitica* (Wall.) for rheumatism, kidney disorders, jaundice, urinary tract issues, fever, and pain; *H. diversifolia* for rheumatism; *H. coriacea* for asthma; and *H. latifolia* for stomach pain (Basir et al., 2022). Additionally, *Hoya* extracts have been reported as natural insecticides against caterpillars in rice fields (Robika et al., 2015).

The medicinal potential of *Hoya* is attributed to its diverse secondary metabolites, including alkaloids, flavonoids, terpenoids, and phenolic compounds. These bioactive constituents exhibit antimicrobial, anti-inflammatory, anticancer, and antioxidant properties (Rumaling et al., 2025). Secondary metabolites are biosynthetic products not essential for primary metabolic processes but play pivotal ecological roles, such as defense against pathogens and herbivores, and attracting pollinators (Bazaid et al., 2023; Sarkar et al., 2022). *Hoya* secretes latex rich in triterpenyl cinnamate, and GC–MS analysis of the latex from H. lacunosa revealed multiple triterpenoids, with 1-octan-3-ol identified as the most abundant compound (Basir et al., 2022).

Natural populations of *Hoya* are predominantly found in humid, forested environments with relative humidity levels between 20% and 84%, often growing as epiphytes on host trees (Hidayat et al., 2012; Obeña, 2019). Similar habitat conditions are observed in plantation areas, which also support *Hoya* growth. In Jember Regency, East Java, plantations such as Gumitir, Garahan, and Tancak are characterized by the presence of coffee, rubber, and pine trees, providing suitable hosts for *Hoya*. However, routine clearing of undergrowth and epiphytes poses a threat to *Hoya* populations, potentially endangering their genetic diversity and medicinal value.

Despite the increasing recognition of *Hoya* as a medicinal plant, comprehensive studies on species diversity and bioactive potential in plantation ecosystems remain limited. Therefore, this study aims to (1) assess the species diversity of *Hoya* in three plantation areas in Jember Regency based on morphological characteristics and (2) evaluate the medicinal potential of a selected species through phytochemical analysis and antioxidant activity assessment. Gas chromatography-mass spectrometry (GC-MS) is employed for the identification of volatile compounds, while antioxidant activity is measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a widely adopted technique for evaluating plant-based antioxidants (Gulcin & Alwasel, 2023). This research is expected to contribute to the conservation and sustainable utilization of *Hoya* species as a valuable genetic and medicinal resource in plantation landscapes.

METHODS

Study Area and Research Period

The fieldwork was conducted in two phases: the first from February 2020 to June 2021, and the second from September to October 2024, across three plantation areas in Jember Regency, East Java, Indonesia, namely the Gumitir, Garahan, and Tancak plantations. Morphological observations and phytochemical analyses were subsequently carried out at the Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Jember.

Sampling and Morphological Data Collection

Field sampling of *Hoya* species was conducted using a linear transect method covering 4.2 km within each plantation site. Along each transect, 150 plots measuring 5 × 5 m were established at 3 m intervals, with placement guided by the occurrence of phorophytes suitable for epiphytic *Hoya* growth. Within each plot, all *Hoya* individuals were recorded and georeferenced using a handheld GPS unit (Garmin eTrex 32x).

Representative specimens comprising both vegetative (leaves, stems, and roots) and reproductive (flowers, when available) structures were collected for morphological characterization and herbarium voucher preparation. Diagnostic morphological traits including root type, stem architecture, leaf arrangement, inflorescence structure, and corona morphology were documented in situ. The abiotic parameters such as light intensity, ambient temperature, and relative humidity were measured at each plot using calibrated portable instruments. Species identification was performed through comparative analysis with authoritative taxonomic literature and type descriptions. All verified specimens were pressed, dried, and deposited in the Herbarium of the Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Jember.

Figure 1. Map of the study locations for *Hoya* species across three plantation areas in Jember Regency: (a) Tancak, (b) Garahan, and (c) Gumitir

Extraction and Phytochemical Analysis of *Hoya lacunosa*

H. lacunosa was selected for phytochemical and antioxidant analysis due to its vegetative abundance in the Tancak plantation and its known traditional medicinal use (Basair et al., 2022). Fresh and healthy leaves (100 g) were collected, rinsed, and cut into small pieces. The material was then homogenized using a blender and subjected to maceration in 96% methanol at a 1:9 (w/v) ratio. The maceration process was carried out over 72 hours with intermittent stirring every 24 hours. The resulting solution was filtered using Whatman No. 1 filter paper, and the solvent was evaporated under reduced pressure using a rotary evaporator at 50°C for 15 minutes to obtain a crude methanolic extract (Setyati et al., 2020; Ulum et al., 2023).

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

The secondary metabolite profile of the methanol extract was analysed using a Shimadzu GC-MS-QP 2010 Plus system. The extract was first filtered through a 0.45 µm membrane syringe filter to remove particulates and prevent column blockage. For chromatographic separation, a 1 µL aliquot of the filtered extract was injected using a split mode. The analysis was performed on an Rtx-50 capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness) with helium as the carrier gas at a constant flow rate of 3 mL/min. The injector temperature was maintained at 290°C. The oven temperature was programmed to start at 80°C (held for 10 min), ramped to 230°C at a rate of 5°C/min, and held at 230°C for 10 minutes. The mass spectrometer was operated at 280°C. Detected compounds were identified by comparing their mass spectra to entries in the Wiley 9 library database (Ulum et al., 2023; Ulum et al., 2025).

DPPH Radical Scavenging Assay

The antioxidant capacity of *H. lacunosa* leaf extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a widely accepted method for screening the free radical neutralizing potential of natural products (Gulcin & Alwasel, 2023; Amalini et al., 2025; Farhan et al., 2025; Ulum et al., 2025). This colorimetric method relies on the reduction of the stable violet DPPH radical to a pale-yellow hydrazine derivative in the presence of hydrogen-donating antioxidants. For the extract assay, the crude methanolic extract was oven-dried at 40°C for 48 hours. Ten grams of the dried extract were re-dissolved in 10 mL of methanol to make a 1000 mg/ml stock solution. Serial dilutions were then prepared at concentrations of 80, 60, 40, and 20 mg/ml. For each concentration, 0.3 mL of sample or standard solution was mixed with 1.2 mL of DPPH solution and incubated in the dark at room temperature for 30 minutes to prevent light-induced degradation. Absorbance was measured at the previously determined λ_max, and the percentage of DPPH inhibition was calculated using the following formula:

$$\text{DPPH Scavenging (\%)} = \frac{\text{OD Control} - \text{OD Sample}}{\text{OD Control}} \, x \, 100$$

The IC_{50} value, defined as the concentration required to scavenge 50% of DPPH radicals, was determined by plotting the inhibition percentages against the respective extract concentrations and applying linear regression analysis. This parameter serves as a reliable quantitative indicator of antioxidant potency, with lower IC_{50} values indicating stronger radical scavenging activity (de Menezes et al., 2021; Amalini et al., 2025; Farhan et al., 2025; Ulum et al., 2025).

Data Analysis

GC-MS output was analyzed by interpreting the chromatogram, where retention times (x-axis) corresponded to individual compounds, and peak intensities (y-axis) indicated relative abundance. Each peak was matched to compounds in the Wiley 9 database, and literature reviews were conducted to evaluate their potential pharmacological properties (Amalini et al., 2025; Farhan et al., 2025; Ulum et al., 2025).

RESULTS AND DISCUSSION

Species Richness and Distribution

Field surveys conducted in the Gumitir, Garahan, and Tancak plantations of Jember Regency documented a total of eleven *Hoya* species (Figure 4). Species richness differed markedly across the three sites, with the Gumitir plantation supporting ten species, Garahan two species, and Tancak only one (Table 1). The elevated diversity observed in Gumitir is likely attributed to its montane topography and the structural heterogeneity of its host tree community. Trees with rough, fissured bark and consistently high ambient humidity create optimal microhabitats for *Hoya* seed entrapment and germination. These conditions align with findings by (Rahayu et al., 2018), who highlighted that humid montane environments are particularly conducive to the proliferation of epiphytic *Hoya* species. Phylogenetic evidence further supports that epiphytism has played a critical role in the genus's diversification across the Indo-Australian Archipelago, with widespread radiation into tropical forests closely associated with this growth strategy (Wanntorp et al., 2014). This adaptation, likely driven by monsoonal climate shifts during the Himalayan uplift, has enabled *Hoya* to exploit canopy microhabitats and thrive in complex forest systems.

Species richness was highest in Gumitir, the greatest population density was recorded in Tancak, which hosted 46 individuals of *H. lacunosa* alone, followed by 39 individuals across multiple species in Gumitir, and only 6 in Garahan. The low density observed in Garahan may reflect suboptimal conditions for seedling establishment, likely due to a lack of suitable host trees and reduced microclimatic support. Hoya seeds exhibit slow germination and often depend on symbiotic relationships with ant nests, which provide fungistatic protection and a conducive microenvironment for early development (Rahayu et al., 2018; Rumaling et al., 2025; Wanntorp et al., 2014). The high abundance of *H. lacunosa* in Tancak may thus be attributed to the prevalence of such mutualistic ant interactions, compensating for the area's low species diversity by promoting successful colonization and persistence of a single dominant taxon.

Table 1. *Hoya* Species Found in Three Plantation Areas of Jember Regency

No	Species	Total individuals		
		Gumitir	Garahan	Tancak
1.	*Hoya burtoniae* Kloppenb.	4	-	-
2.	*Hoya camphorifolia* Warb.	3	-	-
3.	*Hoya carnosa* (L. f.) R. Br.	8	-	-
4.	*Hoya cinnamomifolia* Hook.	2	-	-
5.	*Hoya dennisii* P.I Forst. & Liddle	6	5	-
6.	*Hoya fuscomarginata* N.E. Br.	4	1	-
7.	*Hoya lacunosa* Blume	-	-	46
8.	*Hoya micrantha* Hook. f.	1	-	-
9.	*Hoya purpureofusca* Hook.	8	-	-
10.	*Hoya* sp. 1	2	-	-
11.	*Hoya* sp. 2	1	-	-
	Population	39	6	46

Table 2. Abiotic Factors of *Hoya* in Three Plantation Areas of Jember Regency

No	Parameters	Research locations		
		Gumitir	**Garahan**	**Tancak**
1.	Light Intensity (lux)	541 – 991	461 – 676	409 – 935
2.	Air Humidity (%)	48.8 – 69.0	67.8 – 72.5	59.6 – 68.5
3.	Temperature (°C)	23.4 – 28.1	24.7 – 28.9	25.9 – 29.0
4	Altitude (mdpl)	700-850	550-561	300-900

The abiotic parameters recorded across the three plantation sites are summarized in Table 2. Light intensity ranged from 409 to 991 lux, falling below the optimal threshold for *Hoya* growth as reported by (Bermuli et al., 2019), which lies between 723.7 and 1182.7 lux. This suboptimal light availability is likely due to the dense canopy structure of overstory species such as coffee, rubber, and pine, which restricts light penetration to the lower strata. Relative humidity across the sites ranged from 48.8% to 72.5%, aligning well with the documented optimal humidity range of 20%–84% for *Hoya* (Hidayat et al., 2012). Such humidity levels are essential for maintaining water balance and supporting nutrient cycling in epiphytic plants, which are particularly vulnerable to desiccation (Robika et al., 2015). The ambient temperature, recorded between 23.4°C and 29.0°C, also falls within the favourable thermal range of 22.8°C to 29.1°C reported by (Rahayu et al., 2018; Robika et al., 2015), supporting enzymatic activity and overall physiological stability. Elevational data indicated that *Hoya* species were distributed between 300 and 900 meters above sea level, consistent with (Wanntorp et al., 2014), who noted that species richness within the genus is highest in lowland and submontane habitats, typically declining beyond 1,000 meters elevation.

Morphological Characteristics

The morphological assessment of the *Hoya* species revealed notable variability in root systems, stem structure, and leaf characteristics (Table 3). All eleven species possessed fibrous roots, with eight exhibiting woody stems and three having herbaceous stems. Leaf succulence was a prominent feature, with ten species displaying succulent leaves and only one (*Hoya* sp. 2) having non-succulent leaves (Figure 2).

These observations are consistent with (Rahayu et al., 2018), who reported that most *Hoya* species possess woody stems, although herbaceous stems are also common (Jumawan & Buot, 2016). Leaf succulence is a characteristic adaptation to water storage, often observed in epiphytic species (Medina et al., 2016). However, certain species, such as *Hoya* sp. 2, exhibit non-succulent leaves, demonstrating the genus's morphological plasticity (Jumawan & Buot, 2016; Medina et al., 2016).

Table 3. Morphological Characteristics of *Hoya* Found in Three Plantation Areas of Jember Regency

No	Hoya Species	Fibrous Rooting	Trunk		Leaf		Leaf Shape				
			Woody	Herb	Succulents	Non-Succulents	Ovoid	Aisle	Rhombus	Heart	Lancet
1	*H. burtoniae*	✓	✓		✓						✓
2	*H. camphorifolia*	✓	✓		✓		✓		✓		
3	*H. carnosa*	✓		✓	✓		✓				
4	*H. cinnamomifolia*	✓	✓	-	✓		✓				
5	*H. dennisii*	✓	✓	-	✓		✓				
6	*H. fuscomarginata*	✓	✓	-	✓		✓				
7	*H. lacunosa*	✓		✓	✓					✓	
8	*H. micrantha*	✓	✓		✓		✓				
9	*H. purpureofusca*	✓	✓		✓			✓			
10	*Hoya* sp. 1	✓	✓		✓		✓				
11	*Hoya* sp. 2	✓		✓		✓	✓				

Leaf morphology among the observed *Hoya* species exhibited notable variation, serving as a useful, though not definitive, taxonomic trait. Ovoid leaf forms were the most prevalent, occurring in seven species, while lanceolate, rhombic, and cordate (heart-shaped) types were less commonly represented. Although leaf shape provides valuable taxonomic cues, it must be interpreted alongside other vegetative and reproductive features for accurate species identification, as emphasized by traits (Robika et al., 2015). In this study, we provide concise morphological summaries of selected *Hoya* specimens to illustrate this diversity:

Hoya burtoniae **Kloppenb**

A liana plant that has a fibrous root system. Monopodial stem growth, cylindrical stem shape with an internodus length of 9 cm. Woody stem, smooth stem surface, yellow colored stem. Leaf shape is lanceolate, leaves are succulent, leaves are stiff, ventral and dorsal surfaces of leaves are smooth. Leaf veins are not visible, leaves are nested, and leaves are green. The base and tip of the leaf are pointed, the leaf edge is flat, 6.5 cm long, and 2.5 cm wide. Flat seed shape, black seed

Hoya camphorifolia **Warb**

A liana plant that has a fibrous root system. Monopodial stem growth, cylindrical stem shape with an internodus length of 6.5 cm. Woody stem, smooth stem surface, brown stem. Leaf shape is rhombic, leaves are succulent, leaves are stiff, ventral and dorsal surfaces of leaves are smooth. Leaf repetition is pinnate, leaves are arranged crosswise, and leaves are dark green in color. The base of the leaf is pointed, the tip of the leaf is tapered, the leaf edge is flat and black, 17.2 cm long and 8.2 cm wide.

Hoya carnosa **(L. f.) R. Br.**

A liana plant that has a fibrous root system. Monopodial stem growth, cylindrical stem shape with an internodus length of 7 cm. Herbaceous stem, smooth stem surface, green stem. Ovoid shape, succulent leaves, leaves are stiff, the ventral and dorsal surfaces of the leaves are smooth. Leaf repetition is pinnate, leaves are arranged alternately, and leaves are green. The base of the leaf is pointed, the tip of the leaf is tapered, the leaf edge is flat, 9 cm long and 3.5 cm wide.

Hoya cinnamomifolia **Hook.**

A liana plant that has a fibrous root system. Monopodial stem growth, cylindrical stem shape with an internodus length of 11.5 cm. Woody stem, smooth stem surface, brown stem. Ovoid shape, succulent leaves, stiff leaves, smooth ventral and dorsal surfaces. Leaf veins are curved, leaves are arranged alternately, and leaves are green. The base of the leaf is pointed, the tip of the leaf is tapered, the leaf edge is flat, 13.5 cm long and 8 cm wide.

Hoya dennisii **P.I Forst. & Liddle**

A liana plant that has a fibrous root system. Monopodial stem growth, cylindrical stem shape with an internodus length of 5 cm. Woody stem, smooth stem surface, brown stem. Leaf shape ovate, succulent leaves, stiff leaves, smooth ventral and dorsal surfaces. Leaf repetition is pinnate, leaves are arranged alternately, and leaves are green. The base of the leaf is rounded, the tip of the leaf is pointed, the flat leaf edge is black, 7.9 cm long and 3.8 cm wide. The fruit is brown, with small flat seeds, and black seeds with fine hairs when the fruit is broken. The flower type is arranged in umbrella-like bunches forming a star formation of 4 flowers (Figure 3). The dark yellow crown consists of five petals. The orange corona is composed of five strands.

Hoya fuscomarginata **N.E. Br.**

A liana plant that has a fibrous root system. Monopodial stem growth, cylindrical stem shape with an internodus length of 9 cm. Woody stem, smooth stem surface, brown stem. Leaf shape is ovoid, leaves are succulent, leaves are stiff, ventral and dorsal surfaces of leaves are smooth. Leaf repetition is pinnate, leaves are arranged alternately, leaves are green. Blunt leaf base, pointed leaf tip, flat leaf edge, 19.6 cm long and 10.4 cm wide.

Hoya lacunosa **Blume**

A liana plant that has a fibrous root system. Monopodial stem growth, cylindrical stem shape with an internodus length of 2 cm. Herbaceous stem, smooth stem surface, green stem. The shape of the leaves when young is like a heart, the leaves are succulent, the leaves are stiff, and the ventral and dorsal surfaces of the leaves are smooth. Leaf repetition is pinnate, leaves are arranged crosswise, and leaves are dark green in color. Notched leaf base, tapered leaf tip, flat leaf edge, 3.1 cm long and 2.1 cm wide. Mature leaves are lanceolate, thin, and leaves are soft; the ventral and dorsal surfaces of the leaves are smooth. Having one leaf reinforcement, the leaves are arranged crosswise, the leaves are dark green in color. The base of the leaf is pointed, the tip of the leaf is tapered, the leaf edge is flat, 5.8 cm long, and 2 cm wide. The flower type is arranged in umbrella-like bunches forming a star formation of 21 flowers. The yellow crown consists of five petals. The corona is orange in color which is composed of five strands.

Figure 2. Epiphytic growth of *Hoya* on tree trunks in Jember plantations (a) *Hoya burtoniae* Kloppenb.; (b) *Hoya camphorifolia* Warb.; (c) *Hoya carnosa* (L. f.) R. Br.; (d) *Hoya cinnamomifolia* Hook.; (e) *Hoya dennisii* P.I Forst. & Liddle; (f) *Hoya fuscomarginata* N.E. Br.; (g) *Hoya lacunosa* Blume; (h) *Hoya micrantha* Hook. f.; (i) *Hoya purpureofusca* Hook.; (j) *Hoya* sp.1; (k) *Hoya* sp.2

***Hoya micrantha* Hook. f.**

A liana plant that has a fibrous root system. Monopodial stem growth, cylindrical stem shape with an internodus length of 5 cm. Woody stem, smooth stem surface, brown stem. The shape is ovoid, the leaves are succulent, the leaves are rigid, and the ventral and dorsal surfaces of the leaves are smooth. Leaf veins are not visible, leaves are arranged alternately, and leaves are yellow. The base of the leaf is pointed, the tip of the leaf is tapered, the leaf edge is flat, 6.5 cm long and 3.5 cm wide.

***Hoya purpureofusca* Hook.**

A liana plant that has a fibrous root system. Monopodial stem growth, cylindrical stem shape with an internodus length of 4 cm. Woody stem, smooth stem surface, brown stem. The shape of the leaves is jorong, the leaves are succulent, the leaves are rigid, and the ventral and dorsal surfaces of the leaves are smooth. Leaf veins are curved, leaves are arranged crosswise, and leaves are green. The base of the leaf is pointed, the tip of the leaf is tapered, the leaf edge is flat and black, 10 cm long and 4.1 cm wide.

***Hoya* sp.1**

A liana plant that has a fibrous root system. Monopodial stem growth, cylindrical stem shape with an internodus length of 5.5 cm. Woody stem, smooth stem surface, brown stem. Leaf shape is ovate, leaves are succulent, leaves are stiff, ventral and dorsal surfaces of leaves are smooth. Leaf repetition is pinnate, leaves are arranged alternately, leaves are dark green. The base of the leaf is rounded, the leaf tip is pointed, the leaf edge is flat, 10.2 cm long and 5.1 cm wide.

***Hoya* sp.2**

A liana plant that has a fibrous root system. Monopodial stem growth, cylindrical stem shape with an internodus length of 4 cm. Herbaceous stem, smooth stem surface, green stem. Ovoid shape, non-succulent leaves, soft leaves, smooth ventral and dorsal surfaces. Leaf veins are not visible, leaves are arranged alternately, leaves are green. The base of the leaf is pointed, the tip of the leaf is tapered, the leaf edge is flat, 5.5 cm long and 2.5 cm wide.

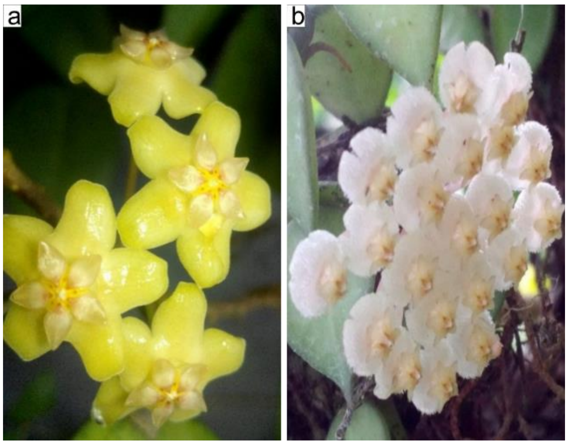

Figure 3. Inflorescence features of two *Hoya* species from Jember plantations (a) *Hoya dennisii* P.I Forst. & Liddle; (b) *Hoya lacunosa* Blume

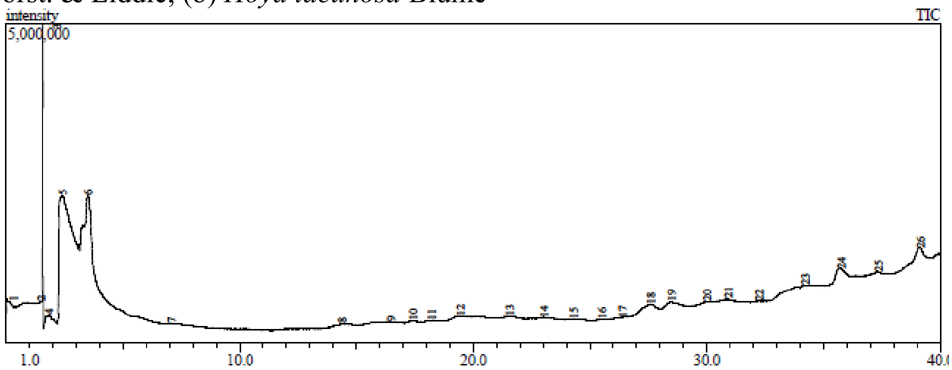

Figure 4. Chromatography GC-MS of Methanolic Leaf Extract of *Hoya lacunosa*

Secondary Metabolite of *Hoya lacunosa* Leaf Methanol Extract

GC-MS analysis of the methanol extract of *Hoya lacunosa* leaves revealed 26 chemical constituents, with retention times ranging from 0.153 to 39.165 (Figure 4). The identified compounds included 12 phenolic compounds, 2 alkaloids, 2 terpenoids, 7 fatty acids, 2 amino acids, and 1 monosaccharide (Figure 5; Table 4).

Phenolic Compound

Phenolics represented the dominant class of secondary metabolites in the methanol extract of *H. lacunosa* leaves, accounting for 46.2% of the total compounds detected. Major identified constituents included phenol derivatives such as 2-propanone and methyl benzoate. Phenolic compounds are widely recognized for their strong antioxidant potential, mainly due to hydroxyl-substituted aromatic rings that facilitate hydrogen donation and electron transfer, stabilizing free radicals (Goodarzi et al., 2018; Zhang et al., 2022). In this study, twelve phenolic compounds were identified (Table 4), many of which are associated with redox-based antioxidant activity. Beyond their antioxidant properties, phenolics also exhibit broad pharmacological effects, including antimicrobial, anti-inflammatory, and antifungal activities (Temiz & Tarakçı, 2017; Sarkar et al., 2022).

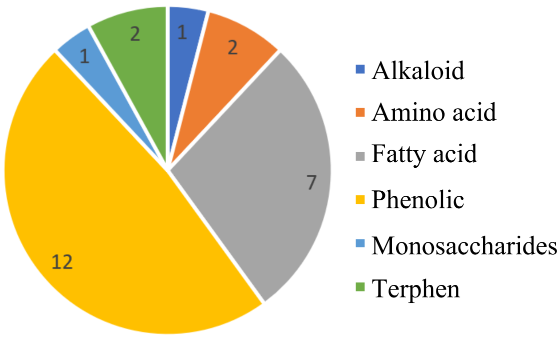

Figure 5. Classification of Compound Groups Identified in the GC-MS Profile of Methanolic Leaf Extract of *Hoya lacunosa*

Alkaloid Compound

Alkaloids constitute a large and structurally diverse class of nitrogen-containing secondary metabolites, encompassing indole, tropane, isoquinoline, pyridine, and other biosynthetic types (Mora-Vásquez et al., 2022). These compounds have long been recognized for their potent pharmacological activities, with notable examples including the antihypertensive indole alkaloid reserpine, the anticancer vinca alkaloids vinblastine and vincristine, and the tropane alkaloids hyoscyamine and scopolamine used as anticholinergics (Bhambhani et al., 2021). Several other plant-derived alkaloids such as morphine, codeine, berberine, and nicotine exert clinically relevant analgesic, anticancer, antimicrobial, and neurological effects (Bhambhani et al., 2021; Mora-Vásquez et al., 2022).

In the present study, two alkaloid-type metabolites were detected in the methanolic leaf extract of *H. lacunosa* using GC–MS analysis (Table 4). These compounds were identified as 1-methyldodecylamine (peak no. 1) and N-methyl-2-aminopropanamide (peak no. 2), with retention times of 0.153 and 1.507 minutes, respectively. Both metabolites have been previously associated with antimicrobial activity, primarily through mechanisms involving cell-membrane disruption, interference with microbial enzymatic processes, and destabilization of metabolic pathways (Hamed et al., 2021; Oloyede et al., 2020). The presence of these alkaloids in *H. lacunosa* supports the plant's traditional medicinal use and highlights its potential as a source of antimicrobial agents. Alkaloids exhibit notable antibiotic potential, functioning as broad-spectrum antibacterial agents through multiple mechanisms, such as metal ion chelation, inhibition of key enzymatic pathways, disruption of transcriptional regulation, and inactivation of critical metabolic intermediates (Yan et al., 2021).

Table 4. Types of Compounds Identified in the GC-MS Profile of *Hoya lacunosa* Leaf Extract and Their Reported Medicinal Potencies

No	Retention Time	% Area	Compound Name	Groups	Medicine potential
1	0.153	0.2	1-Methyldodecylamine	Alkaloid	Antimicrobial (Hamed et al., 2021)
2	1.507	0.22	Propanamide, N-methyl-2-amino-	Alkaloid	Antimicrobial dan Antiviral (Oloyede et al., 2020)
3	3.505	16.89	2-Propanone, 1-hydroxy- (CAS)	Phenol	Antioxidants (Gündeşli et al., 2020)
4	7.098	3.54	2-Cyclopenten-1-one (CAS)	Terpen	Anti cancer, cardiovascular disorders, and neurodegenerative conditions (Bazaid et al., 2023)
5	14.393	0.54	Cyclopentanone, 2-methyl- (CAS)	Terpen	Antitumor (Liu et al., 2022)
6	18.207	0.14	t-Butyl-dimethylacetamide	Phenol	Liver therapy (Liu et al., 2016
7	19.439	1.52	Phenol (CAS)	Phenol	Antioxidants (Mathew et al., 2015
8					
9	23.027	0.25	Benzoic acid, 2-hydroxy-, methyl ester (CAS	Phenol	Anti cancer (Anantharaju et al., 2017
10	23.027	0.25	Benzoic acid, 2-hydroxy-, methyl ester (CAS	Phenol	Anti cancer (Anantharaju et al., 2017
11	25.513	0.06	Phenol, 3-ethyl- (CAS)	Phenol	Antioxidants (Mathew et al., 2015
12	26.393	0.1	2-Propanone, 1-(3,5,5-trimethyl-2-cyclohexe	Phenol	Antioxidants (Gündeşli et al., 2020
13	28.503	2.76	Phenol, 2-methyl-5-(1-methylethyl)- (CAS)	Phenol	Antioxidants (Mathew et al., 2015
14	30.02	2.39	Phenol, 3,4-dimethoxy- (CAS)	Phenol	Antioxidants (Mathew et al., 2015
15	32.26	0.89	Furane-2-carboxylic acid, 5-(4-allyl-2-metho	Phenol	Antioxidants (Li et al., 2020
16	34.233	8.74	1,4-Benzenediol	Phenol	Antimicrobial (Lana et al., 2019
17	35.761	7.01	Phenol, (1,1-dimethylethyl)-4-methoxy- (CA	Phenol	Antioxidants dan Antimicrobial (Jaradad et al., 2021
18	37.361	8.07	2-Pentadecanone, 6,10,14-trimethyl- (CAS)	Phenol	Antidiabetes (Siyumbwa et al., 2019

Terpenoid Compound

Terpenoids constitute a structurally diverse class of secondary metabolites derived from isoprene units via the mevalonic acid (MVA) and methylerythritol phosphate (MEP) pathways (Siddiqui et al., 2024; Câmara et al., 2024). GC–MS analysis of *H. lacunosa* leaf extract revealed two terpenoid constituents: 2-cyclopenten-1-one (peak 7) and 2-methylcyclopentanone (peak 8), eluting at 7.098 and 14.393 min, respectively. Both metabolites have been reported to exhibit antimicrobial and cytotoxic properties (Table 4), consistent with the well-known bioactivity of small terpenoid ketones.

The antimicrobial potency of terpenoids, particularly monoterpenes and low-molecular-weight cyclic ketones, is largely attributed to their lipophilic nature, which enables membrane destabilization and inhibition of microbial growth (Wright et al., 2017). Although only two terpenoids were detected in the leaves, previous analyses of *H. lacunosa* flowers reported substantially higher terpenoid richness linked primarily to floral scent production and pollinator attraction (Basir et al., 2022). This contrast indicates tissue-specific metabolic partitioning between reproductive and vegetative organs.

Terpenoids also function as phytoalexins in plant defense. In rice, diterpenoids such as momilactones and oryzalexins accumulate under UV or pathogen stress, enhancing antimicrobial and antioxidant protection (Park et al., 2014; Vaughan et al., 2015). The identification of 2-cyclopenten-1-one in *H. lacunosa*, a compound previously associated with anti-inflammatory and antioxidant activity in natural products such as honey (Bazaid et al., 2023), suggests a similar defensive or stress-responsive role. The detection of Cyclopentadecanone, a macrocyclic ketone commonly used in fragrance and pharmaceutical formulations, further supports the pharmacological potential of *Hoya* metabolites due to its non-genotoxic and bioactive properties (Liu et al., 2022).

Antioxidant Activity

The antioxidant potential of *H. lacunosa* leaf methanol extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a standard method for assessing free radical quenching capacity in plant extracts (Pudjas et al., 2022; Sarkar et al., 2022). This assay measures the ability of antioxidant molecules to reduce the purple DPPH radical to its yellow non-radical form, which is quantified by the decrease in absorbance (Chaves et al., 2020; Ermavitalini et al., 2025; Zahrina et al., 2024).

The percentage of DPPH inhibition was plotted against varying concentrations of the extract, yielding a linear regression equation: $y = 0.829x + 7.789$, with a high correlation coefficient ($R^2 = 0.988$), indicating strong model fit (Figure 6). Based on this regression, the IC_{50} value, defined as the concentration required to scavenge 50% of DPPH radicals (de Menezes et al., 2021), was determined to be 50.89 μg/mL. This places *H. lacunosa* within the "strong" antioxidant category, as per the classification by (Arsianti et al., 2020), which designates IC_{50} values < 50 μg/mL as very strong, 50–100 μg/mL as strong, 101–150 μg/mL as moderate, and >150 μg/mL as weak. Notably, the antioxidant potential of *H. lacunosa* surpasses that of *H. parasitica*, previously reported to exhibit an IC_{50} of 70.89 μg/mL under comparable assay conditions (Sarkar et al., 2022), further emphasizing the superior free radical scavenging ability of *H. lacunosa*. This activity is likely attributable to its rich content of phenolic compounds, as revealed in the GC-MS profile. Phenolics are known for their potent radical scavenging mechanisms, including hydrogen donation, electron transfer, and metal ion chelation (Chaves et al., 2020). The strong antioxidant capacity exhibited by *H. lacunosa* further supports its ethnomedicinal use and highlights its potential application in pharmaceutical or nutraceutical formulations targeting oxidative stress-related disorders.

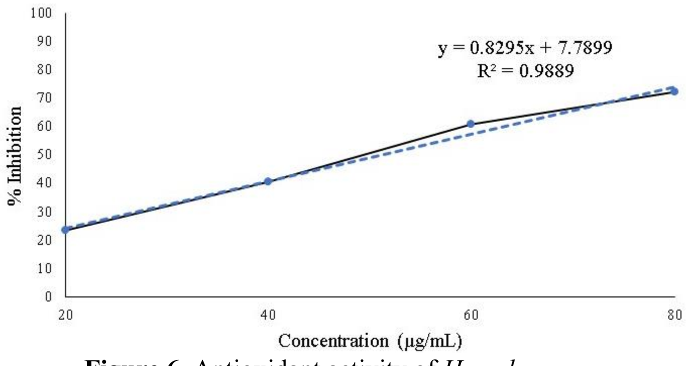

Figure 6. Antioxidant activity of *Hoya lacunosa*

CONCLUSION

This study identified 11 *Hoya* species distributed across three plantation areas in Jember Regency, with the highest species richness observed in the Gumitir site. Notably, *Hoya lacunosa* was found exclusively in the Tancak plantation, where it formed a dominant population. Phytochemical profiling of *H. lacunosa* leaf extract using GC–MS revealed 26 chemical constituents, including 16 secondary metabolites, predominantly phenolics, alkaloids, and terpenoids, known for their pharmacological relevance. Antioxidant activity analysis using the DPPH assay demonstrated strong radical scavenging capacity, with an IC_{50} value of 50.89 µg/mL. These findings underscore the significance of *H. lacunosa* as a promising source of bioactive compounds and highlight the conservation value of plantation habitats as reservoirs of medicinal epiphytic biodiversity.

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AUTHOR CONTRIBUTION STATEMENT

Fuad Bahrul Ulum was responsible for conceptualization, methodology design, metabolite profiling, data analysis, and writing of the original draft. Asa Hanifatul Hayyinah contributed to sample collection and morphological description, while Hari Sulistiyowati assisted in field sampling. Tri Ratnasari carried out plant descriptions. Mukhamad Suudi contributed to the original draft preparation and manuscript writing. Edia Fitri Dwinianti supported the preparation and editing of the manuscript. Dwi Setyati contributed to sample collection, manuscript preparation, and provided overall supervision of the research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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