AL-KAUNIYAH: Jurnal Biologi, 18(2), 2025, 285-293
Website: http://journal.uinjkt.ac.id/index.php/kauniyah
P-ISSN: 1978-3736, E-ISSN: 2502-6720

SPECIES DIVERSITY AND SECONDARY METABOLITE COMPOUNDS OF ORCHIDS IN MENOREH ECOTOURISM AREAS, KULON PROGO, SPECIAL REGION OF YOGYAKARTA

KEANEKARAGAMAN SPESIES DAN SENYAWA METABOLIT SEKUNDER ANGGREK DI KAWASAN EKOWISATA MENOREH, KULON PROGO, DAERAH ISTIMEWA YOGYAKARTA

Muhammad Fityatul Haq[1]*, Ratna Susandarini[2]
[1]*Faculty of Biology, Gadjah Mada University, Special Region of Yogyakarta 55281*
[2]*Laboratory of Plant Systematics, Faculty of Biology, Gadjah Mada University , Special Region of Yogyakarta 55281*
**Corresponding author:* ulhaq.fityatul@gmail.com

Submitted: 5 July 2024; Revised: 28 August 2024; Accepted: 22 November 2024

Abstract

Menoreh Hills is an area with high flora diversity. One of the plants reported as having high species diversity in Menoreh Hills is orchid. Most orchids species are known to be rich in secondary metabolites. This study aims to explore orchids diversity in Menoreh and to identify their secondary metabolites. Orchid data collection was carried out in Sidoharjo Waterfall and Mudal River, Kulon Progo. Inventory survey was carried out by explorative method without plot, and sample collection was done manually. Secondary metabolite identification was carried out by qualitatively on methanolic extract, and detected using thin layer chromatography (TLC). Identification of orchid species was done by comparing morphological characters with literature. Analysis of TLC was carried out by determining the Rf of sample extract spots. The result showed that there are 12 orchid species in Sidoharjo Waterfall and 11 orchid species in Mudal River Ecotourism. A total of 17 orchid species was found in this study, consisted of *Liparis condylobulbon*, *Peristylus goodyeroides*, *Nervilia plicata*, *Dendrobium crumenatum*, *Cymbidium* sp., *Acriopsis liliifolia*, *Eulophia cernua*, *Crepidium kobi*, *Spathoglottis plicata*, *Taeniophyllum* sp., *Liparis parviflora*, *Bryobium retusum*, *Zeuxine*, *Vanilla planifolia*, *Malaxis* sp., *Dienia ophrydis*, and *Phaius* sp. Results of secondary metabolite identification showed that most orchids species contain phenolic, flavonoid, and alkaloid compounds. TLC results indicated that the methanolic extract of orchid samples has high abundance of various secondary metabolites, including flavonoid, phenolic, and alkaloid compounds.

Keywords: Diversity; Inventory; Menoreh; Orchids; Secondary metabolite

Abstrak

Perbukitan Menoreh memiliki keanekaragaman tumbuhan yang tinggi. Salah satu tumbuhan dengan keanekaragaman yang tinggi di Menoreh adalah anggrek. Mayoritas anggrek memiliki metabolit sekunder yang melimpah. Penelitian ini bertujuan untuk menginventarisasi anggrek di Menoreh dan mengidentifikasi metabolit sekunder anggrek. Inventarisasi dan koleksi anggrek dilakukan di Air Terjun Sidoharjo dan Ekowisata Sungai Mudal, Kulon Progo. Inventarisasi dilakukan dengan metode eksploratif tanpa plot dan koleksi sampel secara langsung. Identifikasi metabolit sekunder dilakukan dengan uji kualitatif dan kromatografi lapis tipis (TLC). Identifikasi spesies dilakukan dengan membandingkan karakter morfologi sampel dengan literatur. Analisis TLC dilakukan dengan menentukan Rf bercak ekstrak sampel. Hasil penelitian menunjukkan bahwa terdapat 12 spesies anggrek di Air Terjun Sidoharjo dan 11 spesies anggrek di Ekowisata Sungai Mudal. Secara keseluruhan, terdapat 17 spesies anggrek di lokasi penelitian, yaitu Liparis condylobulbon, Peristylus goodyeroides, Nervilia plicata, Dendrobium crumenatum, Cymbidium sp., Acriopsis liliifolia, Eulophia cernua, Crepidium kobi, Spathoglottis plicata, Taeniophyllum sp., Liparis parviflora, Bryobium retusum, Zeuxine sp., Vanilla planifolia, Malaxis sp., Dienia ophrydis, dan Phaius sp. Identifikasi metabolit sekunder anggrek menunjukkan bahwa mayoritas anggrek memiliki profil senyawa flavonoid, fenolik, dan alkaloid yang beragam.

Kata Kunci*: Anggrek; Inventarisasi; Keanekaragaman; Menoreh; Metabolit sekunder*

Permalink/DOI: http://dx.doi.org/10.15408/kauniyah.v18i2.39999

INTRODUCTION

The geographical position of Indonesia, which is located in the equatorial zone, is one of the main factors for the high biodiversity. Biodiversity will be higher as the transition region from the polar to the equatorial zone. The equatorial zone has higher intensity of sunlight, abundant water, and more mild climatic condituions (Gizachew, 2022). Geological and geomorphological aspects influence the diversity of plant species as well, resulting in differences in vegetation in every ecosystem. Geological processes will form various types of rocks that affects the formation of soil in an ecosystem. Those processes cause the differences in chemical components in the soil or rocks. Consequently, the distribution pattern of plants in the ecosystem depends on the chemical content in the soil (Cottle, 2004).

Menoreh Karst Hills is one of the biosphere reserves in Indonesia, which is recognized by UNESCO (Hidranto, 2020). Menoreh Karst Hills have a typical ecosystem (Kurniawan et al., 2018). The climate of Menoreh is relatively cool with moderate moisture level (Christianty & Widodo, 2022), and the altitude is up to 700 m asl (Solekha et al., 2023), with a high density of vegetation coverage (Worosuprodjo, 2007). One of the plants with the highest diversity among Angiosperms is orchids. The orchids, belong to family Orchidaceae is a member of the Class Liliopsida, which has high diversity in the world. The estimated number of orchid species is about 28,000, and it is widely distributed across all continents. The number of orchid species in Indonesia is estimated to be about 5,000 species (Portal Informasi Indonesia, 2019). This number indicates that Indonesia is one of the hotspots of orchids in the world. Orchids are pantropical, so the diversity of orchids will be higher in the equatorial zone compred to higher latitude regions. In line to this situation, studies on orchids diversity and systematics are continuously developed, such as orchids biodiversity and phylogeny reported by Prayoga et al. (2022). Meanwhile, diveristy study on orchids in Menoreh Hills area, namely Gunung Gajah, Purworejo, was reported by Purba and Chasani (2019who found 13 species.

Orchids have been known to contain secondary metabolites that have physiological and pharmacological properties. Several compounds found in orchids are stilbenoids, phenanthrene, alkaloids, terpenoids, flavonoids, anthocyanin, and phenolics (Bazzicalupo et al., 2023). Certain compounds of secondary metabolites found in several orchids have bioactive properties, with the potential to be used as a phytomedicine. *Coelogyne cristata* and *Pholidota imbricata* have been reported to contain strong antibacterial activity (Marasini & Joshi, 2012). Some genera such as *Ephemeranthalon*, *Eulophia*, *Dendrobium*, *Gastrodia*, *Pholidota*, *Spiranthes*, *Vanda*, *Bletilla*, *Anoectochilus*, and *Bulbophyllum* have good anticancer agents (Shukla et al., 2022). Various bioactive compounds in orchids are a reason for their application as traditional medicine in many regions such as China and India (Pant, 2013). Nevertheless, studies on secondary metabolites of native orchids in Indonesia is still very limited. Therefore, this study aims to explore species diveristy and their secondary metabolites of orchids in the ecotourism area in Menoreh Karst Hills.

MATERIALS AND METHODS

This study was conducted in Sidoharjo Waterfall and Mudal River. Sidoharjo Waterfall is located in Sidoharjo Village, Samigaluh Subdistrict (7°40’10’’S, 110°12’7’’E). Sidoharjo Waterfall is an eco-tourism area with hills and cliffs contours, with the altitude is about 400 m asl. The vegetation structure is composed of trees with high canopy, bush, and understorey vegetation consists of grass and herbaceous plants. Mudal River, a popular river-based ecotourism, is located in Jatimulyo Village, Girimulyo Subdistrict (7°45’45’’S, 110°06’58’’E). The water source flow in the Mudal River comes from the endo karst layer (Irsyad, 2020). The altitude is about 600 m asl. Mudal River has a vegetation structure composed of trees with high density canopy.

Orchid Data Collection

The collection of orchid diversity data was carried out using explorative method without a plot in Sidoharjo Waterfall and Mudal River. The tracking line was recorded by Geotracker 2.0. The tracking distance covered in this study was 1.2 km in Sidoharjo Waterfall and 1.04 km in Mudal River (Figure 1). Records ons orchid species consisted on locations, species name, and habitat. The number of individual and physicochemical parameters, such as humidity, air temperature, light intensity, altitude, and morphological characters, were also recorded. The orchid species with more than three individuals were collected to be preserved as herbarium specimens and extracted for secondary metabolites analysis.

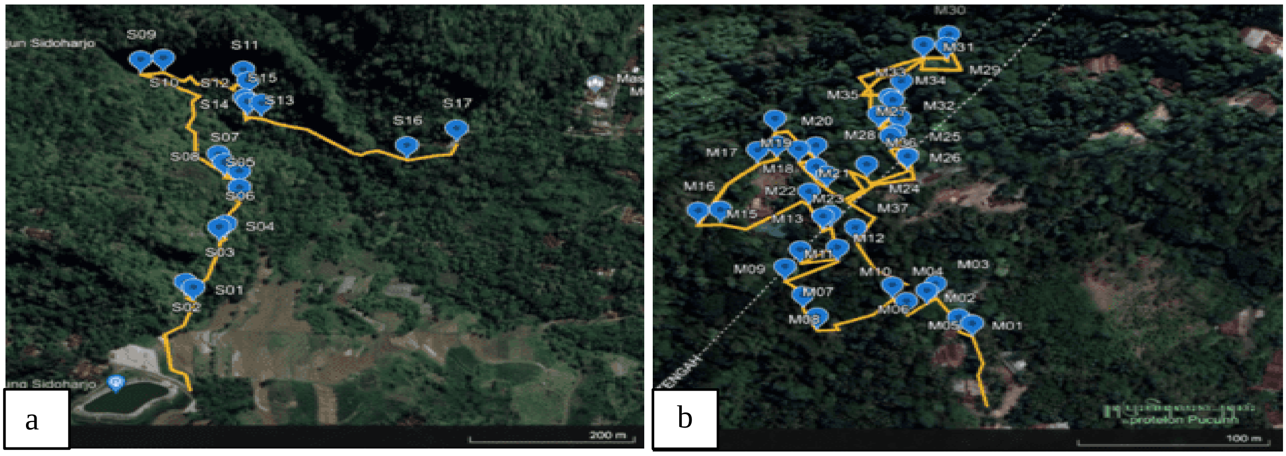

Figure 1. Track of exploration in study areas, Sidoharjo Waterfall (a) and Mudal River (b)

Extraction of Secondary Metabolites

The whole vegetative organ of each orchid samples was dried using electric oven at 60 °C. The dried sample was manually ground using mortar and pestle to fine powder. As much as 10 g of powder was macerated using 80% methanol with a proportion of 1:10 v/v. The solution was incubated for 24 hours and the maceration procedure was repeated twice. The resulted extract was evaporated by a rotary vacuum evaporator.

Identification of Orchids Secondary Metabolites

Concentrated extract with a weight of 0.03 g was dissolved in 6 mL of methanol 80%. The solution was ready to be used for coloration test. The coloration test was carried out in the following procedures.

Alkaloid test: 1 mL of sample solution was added by ten drops of H_2SO_4 2 N and shaken well. The solution was added by some drops of Mayer, Dragendorff, and Wagner reagent. The positive alkaloid in the Mayer test will form a precipitate with the white color, the Dragendorff test will form a precipitate with a reddish-brown color, and the Wagner test will form a precipitate with a brown color (Mailuhu et al., 2017).

Flavonoid test: 1 mL of sample solution was added with a few drops of NaOH 10%. The positive result of the flavonoid will change the color of the solution to orange (Mailuhu et al., 2017).

Phenolic test: 1 mL of sample solution was added by a few drops of $FeCl_3$ 10%. The positive result of phenolic compounds will change the color of solution into dark blue or green (Dauda et al., 2020).

Terpenoid test: 1 mL of sample solution was added by 1 mL of glacial acetic acid and 1 mL of H_2SO_4 2 N. The positive test of terpenoid compounds will change the color of the solution into a reddish-brown color (Mariyam et al., 2023).

Thin Layer Chromatography

The eluent used in this study are aquadest, methanol, ethyl acetate, and chloroform with the proportion of 1:4:7:25. A few drops of glacial acetic acid were added, and silica plate GF254 (Merck) was activated at 120 °C for 20 minutes. As much as 0.01 g of sample extract was diluted with 1 mL of methanol 80%, and then 1 µL of each sample was transferred to the plate. The samples prepared in silica plates were eluted in a Thin Layer Chromatography (TLC) chamber with the eluent system prepared before as the mobile phase. The plate was let to dry after completing elution and was visualized under UV light at 254 nm, 366 nm, and stained with $FeCl_3$ 10%, $AlCl_3$ 10%, and Dragendorff.

Identification of Orchids Species and Secondary Metabolites

Identification of orchids species was carried out by comparing the morphological characteristics of each species with the literature Orchid of Java (Comber, 1990). Data analysis on TLC results was carried out by measuring the retention factor of all spots of each sample by the following equation Rf= distance of spot movement/ distance of eluent movement.

TLC staining methods with $FeCl_3$ solutions was used to identify the secondary metabolites from phenolic compounds. The presence of phenolic compounds is indicated by yellow, orange, green, reddish violet, or reddish brown (Burman et al., 2019). $AlCl_3$ solutions are used to stain flavonoid compounds. The presence of flavonoids is indicated by yellow, green, or blue color under UV light 254 nm (Gwatidzo et al., 2018). Dragendorff reagent is used to stain alkaloid compounds, and the presence of alkaloid compounds is indicated by an orange spot after staining.

RESULTS

Result on inventory of orchids in Sidoharjo Waterfall showed that there are 12 orchid species (). The orchids species found in Sidoharjo Waterfall consist of 7 epiphytic and 5 terrestrial orchids. The most abundant orchid in Sidoharjo Waterfall is *Bryobium retusum* with a total of 64 individuals. *Crepidium kobi* and *Nervilia plicata* have high abundance as well, but not as much as *Bryobium retusum*. The lowest number of individuals found in this study is *Liparis condylobulbon*.

Table 1. Species diversity of orchids in Sidoharjo Waterfall

Species	Individuals	Life form
Liparis condylobulbon Rchb.f.	1	Epiphyte
Peristylus goodyeroides (D.Don) Lindl.	9	Terrestrial
Nervilia plicata (Andrews) Schltr.	28	Terrestrial
Dendrobium crumenatum Sw.	12	Epiphyte
Cymbidium sp.	3	Epiphyte
Acriopsis liliifolia (J.Koenig) Ormerod	4	Epiphyte
Eulophia cernua (Willd.) M.W.Chase, Kumar & Schuit	2	Terrestrial
Crepidium kobi (J.J.Sm.) M.A.Clem. & D.L.Jones	33	Terrestrial
Spathoglottis plicata Blume	6	Terrestrial
Taeniophyllum sp.	3	Epiphyte
Liparis parviflora (Blume) Lindl.	5	Epiphyte
Bryobium retusum (Blume) Y.P.Ng & P.J.Cribb	64	Epiphyte

The number of orchids species found in Mudal River are 11 species, consisting of 6 epiphytic and 5 terrestrial orchids (Table 2). *Bryobium retusum* is the most abundant species found in this location. The second-highest abundance in Mudal River is *V. planifolia* and the third-highest number of individulas is *D. crumenatum*. The species with lowest number of individuals in Mudal River are *Zeuxine* sp. and *Phaius* sp. which are only one individual.

Table 2. Species diversity of orchids in Mudal River

Species	Individuals	Life Form
Dendrobium crumenatum Sw.	35	Epiphyte
Vanilla planifolia Andrews	62	Epiphyte
Zeuxine sp.	1	Terrestrial
Acriopsis liliifolia (J.Koenig) Ormerod	15	Epiphyte
Liparis condylobulbon Rchb.f.	23	Epiphyte
Spathoglottis plicata Blume	11	Terrestrial
Malaxis sp.	16	Terrestrial
Dienia ophrydis (J.Koenig) Seidenf.	23	Terrestrial
Liparis parviflora (Blume) Lindl.	3	Epiphyte
Bryobium retusum (Blume) Y.P.Ng & P.J.Cribb	96	Epiphyte
Phaius sp.	1	Terrestrial

Results of secondary metabolites identification of orchid species are shown in Table 3. Secondary metabolite screening by color test showed that all orchids species found in this study contain phenolic and flavonoid compounds. Most orchids contain alkaloid compounds except some species such as *D. crumenatum* (SW), *N. plicata* (SW), and *A. liliifolia* (MR). Terpenoid compounds are not detected in all samples.

Table 3. Secondary metabolites identification of orchids in Menoreh Ecotourism Areas

Species	Alkaloid			Phenolics	Flavonoids	Terpenoids
	Mayer	Dragendorff	Wagner			
Liparis parviflora (SW)	+	+	+	+	+	-
Dendrobium crumenatum (SW)	-	-	-	+	+	-
Nervilia plicata (SW)	-	-	-	+	+	-
Crepidium kobi (SW)	+++	+++	+++	+	+	-
Bryobium retusum (SW)	+	++	+	+	+	-
Acriopsis liliifolia (SW)	++	++	++	+	+	-
Liparis condylobulbon (MR)	+++	+++	+++	+	+	-
Liparis parviflora (MR)	+++	+++	+++	+	+	-
Dendrobium crumenatum (MR)	+++	++	+++	+	+	-
Spathoglottis plicata (MR)	+	++	++	+	+	-
Dienia ophrydis (MR)	+	+	+	+	+	-
Bryobium retusum (MR)	+	+	+	+	+	-
Acriopsis liliifolia (MR)	-	-	-	+	+	-

Note: Sidoharjo Waterfall (SW); Mudal River (MR); negative result (-); color change (+); color change with little precipitate (++); color change with much precipitate (+++)

TLC chromatograms showed different fingerprints of secondary metabolites of orchids species (Figure 2a-e). The result showed that the orchids from Sidoharjo Waterfall and Mudal River have various compounds of phenolic and flavonoid (Figure 2d & e). TLC stain with Dragendorff reagent showed that alkaloid compounds do not eluted well by mobile phase (Figure 2c).

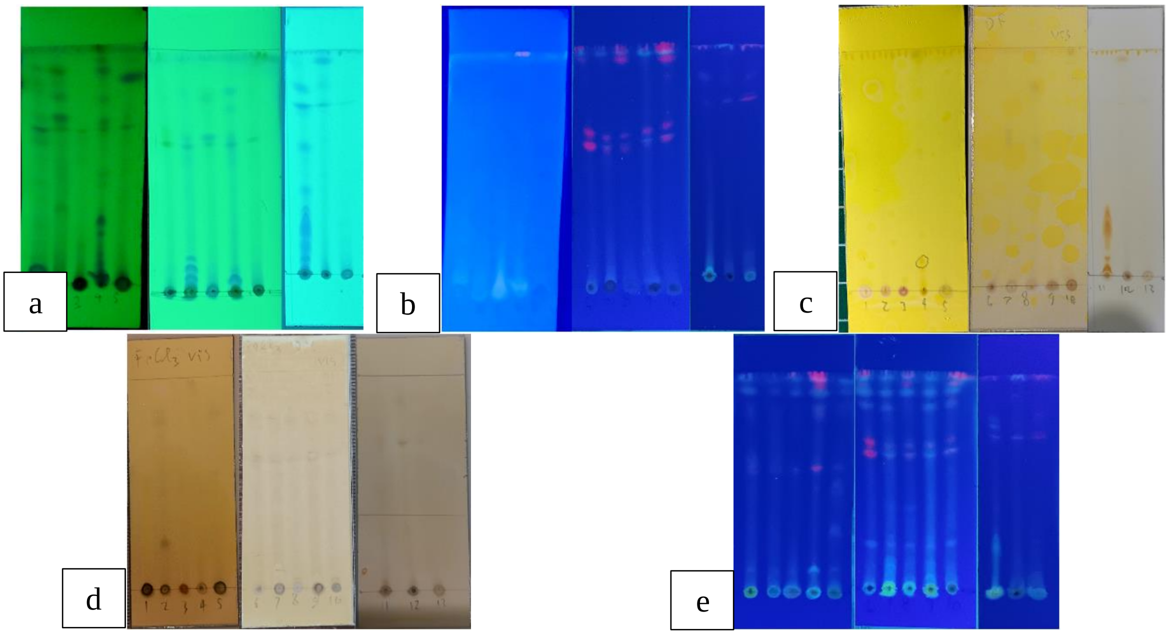

Figure 2. Thin layer chromatograms of methanolic extract of orchids, UV 254 nm (a); UV 366 nm (b); Dragendorff (c); $FeCl_3$ (d); and $AlCl_3$ (e)

The data showed that *A. liliifolia* (SW) and *S. plicata* (MR) contain various phenolic compounds which have 7 fractions of phenolic compounds (Table 4). *D. crumenatum* (SW) also has various of phenolic compounds which 6 fractions were identified (Table 4). Orchids with a high diversity of flavonoid compounds are *L. condylobulbon* (MR) and *D. crumenatum* (MR) with 8 fractions of flavonoids (Figure 1e; Table 4). Meanwhile, the alkaloid compounds were successfully fractionated from *C. kobi* (SW), *L. parviflora* (MR), *Dienia ophrydis* (MR), and *B. retusum* (MR). One orchid species, namely *Acriopsis liliifolia* (MR), contain the lowest number of secondary metabolites with only one fraction of flavonoid and phenolic were detected.

Table 4. Retention factor (Rf) of secondary metabolites of orchids in Menoreh Ecotourism Areas

Species	Alkaloid	Flavonoid	Phenolic
Liparis parviflora (SW)		0.10; 0.27; 0.57; 0.90; 0.97	0.08; 0.27; 0.34; 0.54
Dendrobium crumenatum (SW)		0.25; 0.58; 0.70; 0.80; 0.90; 0.97	0.22; 0.32; 0.47; 0.75; 0.80; 0.85
Nervilia plicata (SW)		0.90; 0.97	0.03; 0.07; 0.31
Crepidium kobi (SW)	0.12	0.10; 0.83; 0.90; 0.97	0.27; 0.31; 0.98
Bryobium retusum (SW)		0.10; 0.28; 0.47; 0.83; 0.90; 0.97	0.34; 0.42; 0.49; 0.54; 0.85
Acriopsis liliifolia (SW)		0.20; 0.83; 0.88; 0.95	0.15; 0.57; 0.59; 0.78; 0.88; 0.92; 0.97
Liparis condylobulbon (MR)		0.08; 0.19; 0.20; 0.59; 0.63; 0.80; 0.87; 0.97	0.08; 0.12; 0.16; 0.58; 0.78
Liparis parviflora (MR)	0.60; 0.83	0.19; 0.35; 0.88; 0.92; 0.95	0.04; 0.58; 0.75; 0.82;
Dendrobium crumenatum (MR)		0.03; 0.11; 0.18; 0.23; 0.59; 0.82; 0.88; 0.95	0.07; 0.15; 0.58; 0.62; 0.75
Spathoglottis plicata (MR)		0.19; 0.53; 0.82; 0.88; 0.94	0.04; 0.17; 0.60; 0.78; 0.88; 0.93; 0.96
Dienia ophrydis (MR)	0.02; 0.06; 0.21; 0.26	0.20; 0.27; 0.72; 0.77; 0.80	0.03; 0.23; 0.68; 0.70
Bryobium retusum (MR)	0.75; 0.96	0.72; 0.98	0.28; 0.68; 0.87
Acriopsis liliifolia (MR)		0.08	0.68

Note: Sidoharjo Waterfall (SW); Mudal River (MR)

DISCUSSION

The number of orchids species in this study is comparable to those in other areas. A study on orchids diversity in Curug Setawing ecotourism, Kulon Progo by Kurniawan et al. (2018) reported 15 species, consisted of *A. liliifolia*, *D. crumenatum*, *N. aragoana*, *Cymbidium bicolor*, *Dienia ophrydis*, *B. retusum*, *Geodorum densiflorum*, *L. parviflora*, *L. condylobulbon*, *Habenaria reflexa*, *P. goodyeroides*, *N. punctata*, *Taeniophyllum* sp., *Zeuxine* sp., and *V. planifolia.* Orchids inventory study in another tourism area in Kulon Progo, the Ayunan Langit in Purwosari Villagefound 14 species, consisted of *A. liliifolia*, *Appendicula ramosa*, *Appendicula* sp., *B. retusum*, *D. crumenatum*, *D. plicatile*, *L. parviflora*, *Oberonia similis*, *Polystachya concreta*, *Spathoglottis plicata*, *Thrixspermum* sp., *Trichoglottis* sp., *V. planifolia*, and *Zeuxine gracilis* (Usmanti et al., 2022). Previous study in Sidoharjo Waterfall showed that several species, such as *A. liliifolia*, *D. crumenatum*, *L. parviflora*, and *B. retusum* were reported to have a wide distribution in Menoreh Karst Hills, indicated by their presence in Curug Setawing (Kurniawan et al., 2018), Mudal River (Basri et al., 2019), and Ayunan Langit (Usmanti et al., 2022). These results indicated that these orchids species have been successful in surviving in the environment and have high sustainability. The most abundant species in Sidoharjo Waterfall and Mudal River is *B. retusum*. The same result was reported in Curug Setawing Ecotourism, in which *B. retusum* has the largest population, reaching 99 individuals (Kurniawan et al., 2018).

The orchids inventory in Mudal River showed that *B. retusum* has the highest number of individuals as well as in Sidoharjo Waterfall. The other species with a high number of individuals is *V. planifolia* with 62 individuals, and *D. crumenatum,* with 35 individuals. The high number of *Vanilla planifolia* in the Mudal River area is due to the cultivation of this species by local people in Jatimulyo (Sudibyanung et al., 2023). Previous study in Mudal River by Basri et al. (2019) reported 15 species of orchid, consisted of *Acriopsis liliifolia*, *Arundina graminifolia*, *Bryobium retusum*, *Cymbidium bicolor*, *Crepidium ridleyi*, *Dendrobium crumenatum*, *Dienia* sp., *Liparis condylobulbon*, *Liparis parviflora*, *Spathoglottis plicata*, *Stereosandra javanica*, *Taeniophyllum* sp., *Vanda* sp., *Vanilla planifolia*, and *Zeuxine gracilis*. There are several species that was not found in this study, namely *Cymbidium bicolor*, *Crepidium ridleyi*, *Arundina graminifolia*, *Stereosandra javanica*, *Taeniophyllum* sp., and *Vanda* sp. This different result was due to different areas covered in this study compared to previous study carried out by Basri et al. (2019).

Secondary metabolites screening showed that only terpenoid compounds are not present in all of the methanolic extracts of orchids samples. This might be caused by the low polarity of terpenoids, so the compounds do not have good affinity with the solvent. In addition, several terpenoids can be highly volatile and can easily evaporate at room temperature. This problem can be solved by selecting the extraction method with low temperature, or extraction using distillation method. Most orchids samples were known to be positive in alkaloids test, as indicated by the changing of sample color and the formation of alkaloid salt precipitate. The principle of alkaloid screening is the formation of salt deposits caused by the acidic conditions of sulfuric acid (Wijayanti et al., 2013). The tertiary amine group found in alkaloid compounds will react with Dragendorff's reagent to form a brick-red precipitate that is insoluble in water (Raal et al., 2020). Other alkaloid tests such as Mayer and Wagner are the same as the Dragendorff test, in that the alkaloid is precipitated into an alkaloid salt by acidification, then reacted with a complex ion salt compound.

Thin Layer Chromatogram under UV 254 nm light showed the fingerprint of different metabolite fractions in each orchid extracts (Figure 1a). Species showing the highest number of compounds is *Crepidium kobi* (SW) with 12 fractions, and *Dienia ophrydis* (MR) with 10 fractions. The lowest number of compounds was detected in *N. plicata* (SW) and *A.liliifolia* (MR) with ony 1 fraction. The phenolic compounds of the orchids have different profiles with respect to their habitat. This can be seen from different profiles of phenolic compounds identified in two samples of *D. crumenatum* (Table 4). In this case, *D.* collected from SW have more fractions than MR with the different values of their Rf. A similar condition occurs on the phenolic profile of *B. retusum* and *A. liliifolia*. This phenomenon occur because the production of secondary metabolites reflects the plant's response to its environment (Nugroho, 2018). Sidoharjo Waterfall has an altitude range of 300–400 m asl with an average air temperature of 31 °C, while the Mudal River Ecotourism has an altitude of 500–600 meters asl with an average temperature of 29 °C. The environmental conditions of Sidoharjo Waterfall, which are relatively hot and tend to be exposed to sunlight, might cause an increase in the production of phenolic compounds in several types of orchids, namely *D. crumenatum*, *B. retusum*, and *L. parviflora*. The same phenomenon was reported by Sedjati et al. (2023) which showed that giving UV-B radiation to *Chlorella* sp. for 80 minutes/day for 10 days resulted in the highest total phenolic levels. Apart from that, increased phenolic levels were also found in *Stachytarpeta jamaicensis* which were grown in relatively hot environmental conditions (Utomo et al., 2020). The flavonoid compounds detected in orchids also showed differences related to the environmental conditions, since the resulted profles did not show any specific pattern. The flavonoid profile of *D. crumenatum* from Mudal River is more varied than in Sidoharjo Waterfall. Contrary, *B. retusum* from Sidoharjo Waterfall has more various flavonoid fractions than those from Mudal River. Analysis on TLC results showed that the best compound spots are those with Rf values between 0.2 and 0.8. This Rf range indicated the specific fraction of all samples that are relatively different from each other. Rf values that are below or above this range will have interfere in visualization (Muttaqin et al., 2016). The alkaloid compounds identified by TLC showed that only *B. retusum* (SW and MR), *L. parviflora* (MR), and *Dienia ophrydis* have an alkaloid fractions. Most of alkaloids have high polarity, whereas the eluent system used in this study is semipolar, and therefore compounds with poor affinity to solvent cannot be eluted, and consequently they did not move on the plate.

CONCLUSION

Sidoharjo Waterfall and Mudal River in the Menoreh Ecotourism areas have a moderate level of orchid diversity. There are overall 17 species found in these two areas. There are 12 orchid species found in Sidoharjo Waterfall, and 11 species in Mudal River. Secondary metabolites identification by TLC showed that most orchids have flavonoids, alkaloids, and phenolics with high variation among species. The conservation of orchid biodiversity in Menoreh needs to be carried out by modern approaches such as *in vitro* propagation to accelerate the cultivation process. The inventory and reintroduction of native orchids to their natural habitats will help maintain their abundance in nature. The utilization of secondary metabolites of orchids must consider their sustainability and occurrence in the wild.

ACKNOWLEDGMENTS

The author gives thanks and appreciation to Balai Konservasi dan Sumber Daya Alam (BKSDA) Yogyakarta for the permission to collect of orchids samples from Menoreh ecotourism areas.

REFERENCES

Basri, A. M., Putri, F., Kurniawan, F. Y., Mustika, N. D., & Semiarti, E. (2019). Diversity and conservation strategy of orchid species on karst land in Mudal River Park Ecotourism, Kulonprogo, Yogyakarta. *International Journal of Advances in Science Engineering and Technology*, *7*(3), 6-10.

Bazzicalupo, M., Calevo, J., Smeriglio, A., & Cornara, L. (2023). Traditional, therapeutic uses and phytochemistry of terrestrial European orchids and implications for conservation. *Plants*, *12*, 257.

Burman, V., Kanaujia, H., Lehari, K., Aastha., Singh, N. P., & Vaishali. (2019). Characterization of phenolic compounds of turmeric using TLC. *Journal of Pharmacognosy and Phytochemistry*, *8*(2S), 994-998.

Christianty, A. Y., & Widodo. (2022). Identifikasi jenis lumut di pekarangan rumah Dusun Puyang Purwoharjo Samigaluh Kulon Progo Yogyakarta. *Jurnal Tropika Mozaika*, *1*(1), 1-10.

Comber, J. B. (1990). *Orchid of Java*. Surrey: The Bentham-Moxon Trust.

Cottle, R. (2004). *Linking geology and biodiversity*. Peterborough: English Nature.

Dauda, H., Uba, G., & Ali, U. (2020). Preliminary phytochemical screening, quantitative analysis of flavonoids from the stem bark extract of *Commiphora africana* (*Burseraceae*). *Bulletin of Environmental Science and Sustainable Management*, *4*(1), 25-27.

Gizachew, G. (2022). Spatial-temporal and factors influencing the distribution of biodiversity: A review. *ASEAN Journal of Science and Engineering*, *2*(3), 273-284.

Gwatidzo, L., Dzomba, P., & Mangena, M. (2018). TLC separation and antioxidant activity of flavonoids from *Carissa bispinosa*, *Ficus sycomorus*, and *Grewia bicolor* fruits. *Nutrire*, *43*, 3.

Irsyad, M. (2020). Kondisi potensi wisata di ekowisata Sungai Mudal Kabupaten Kulon Progo. *Jurnal Kepariwisataan:Destinasi, Hospitalitas, dan Perjalanan*, *4*(1), 29-39.

Kurniawan, F. Y., Setiaji, A., Putri, F., Suyoko, A., & Semiarti, E. (2018). Diversity and conservation strategy of orchids under anthropogenic influence in Taman Wisata Alam Curug Setawing, Yogyakarta. *Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia*, Indonesia, *4*(2), 173-177.

Mailuhu, M., Runtuwene, M. R. J., & Koleangan, H. S. J. (2017). Skrining fitokimia dan aktivitas antioksidan ekstrak metanol kulit batang soyogik (*Saurauia bracteosa* DC). *Chemistry Progress*, *10*(1), 1-6.

Marasini, R., & Joshi, S. (2012). Antibacterial and antifungal activity of medicinal orchids growing in Nepal. *Journal of Nepal Chemical Society*, *29*, 104-109.

Mariyam, M., Anggraini, Y., & Suhartati, T. (2023). Identification of secondary metabolites and ft-ir analysis of getih-getihan fruit extract (*Rivina humilis* L.). *Jurnal Riset Kimia*, *14*(1), 35-42.

Muttaqin, F. Z., Yuliantini, A., Fitriawati, A., & Asnawi, A. (2016). Penetapan kadar senyawa metampiron dan diazepam dalam sediaan kombinasi obat menggunakan metode klt video densitometri. *Pharmacy*, *13*(2), 127-136.

Nugroho, L. H. (2018). *Struktur dan produk jaringan sekretori tumbuhan*. Yogyakarta: Gadjah Mada University Press.

Pant, B. (2013). Medicinal orchid and their uses: Tissue culture a potential alternative for conservation. *African Journal of Plant Science*, *7*(10), 448-467.

Portal Informasi Indonesia. (2019). Anggrek Indonesia. (2023, March 30). Retrieved from https://indonesia.go.id/kategori/seni/864/anggrek-indonesia?lang=1.

Hidranto, F. (2020). Pengakuan unesco untuk tiga cagar biosfer Indonesia. (2023, March 30). Retrieved from https://indonesia.go.id/kategori/seni/2166/pengakuan-unesco-untuk-tiga-cagar-biosfer-indonesia?lang=1.

Prayoga, G. I., Henri., Mustikarini, E. D., & Anggyansyah. (2022). Diversity and morphological relationship of orchid species (*Orchidaceae*) in Bangka Island, Indonesia. *Biodiversitas*, *23*(10), 5323-5332.

Purba, T. H. P., & Chasani, A. R. (2021). Phenetic analysis and habitat preferences of wild orchids in Gunung Gajah, Purworejo, Indonesia. *Biodiversitas*, *22*(3), 1371-1377.

Raal, A., Meos, A., Hinrikus, T., Heinämäki, J., Romāne, E., Gudienė, V., … Nguyen, H. T. (2020). Dragendorff's reagent: Historical perspectives and current status of a versatile reagent introduced over 150 years ago at the University of Dorpat, Tartu, Estonia. *Pharmazie*, *75*(7), 299-306.

Sedjati S., Supriyantini, E., Wulandari, S. Y., & Sulastri, N. I. (2023). Peningkatan kadar fenolik total dari *Chlorella* sp. menggunakan cekaman radiasi ultraviolet-b. *Jurnal Kelautan Tropis*, *26*(1), 49-58.

Shukla, M. K., Monika., Thakur, A., Verma, R., Lalhlenmawia, H., Bhattacharyya, S., … Kumar, D. (2022). Unraveling the therapeutic potential of orchid plant against cancer. *South African Journal of Botany*, *150*, 69-79.

Solekha, A. M., Yulia, I. T., Hanun, Z., Perwitasari, I. G., Cahyaningsih, A. P., Sunarto., … Setyawan, A. D. (2023). Local knowledge and the utilization of non-medicinal plants in the home garden by the people of Donorejo Village in the Menoreh Karst Area, Purworejo, Central Java, Indonesia. *Biodiversitas*, *24*(1), 645-657.

Sudibyanung., Prasetyo, P. K., & Rahmadi, A. (2023). Peluang penataan akses berdasarkan potensi wilayah (studi kasus di Kalurahan Jatimulyo) Kapanewon Girimulyo Kabupaten Kulon Progo). *Jurnal Pertanahan*, *13*(2), 85-100.

Usmanti, E., Kurniawan, F. E., Meidianing, M. I., Basri, A. R., & Semiarti, E. (2022). Biodiversitas dan kekerabatan fenetik spesies anggrek alam di kawasan ekowisata Ayunan Langit, kulonprogo. *Al-Kauniyah: Jurnal Biologi*, *15*(2), 277-289.

Utomo, D. S., Kristianti, E. B. E., & Mahardika, A. (2020). Pengaruh lokasi tumbuh terhadap kadar flavonoid, fenolik, klorofil, karotenoid dan aktivitas antioksidan pada tumbuhan pecut kuda (*Stachytarpheta Jamaicensis*). *Bioma*, *22*(2), 143-149.

Wijayanti, T. Y., Harlia., & Rudiyansyah. (2013). Pengaruh asam terhadap kandungan alkaloid pada ekstrak daun salam (*Syzigium polyanthum*). *Jurnal Kimia Khatulistiwa*, *2*(3), 138-141.

Worosuprodjo, S. (2007). Analisis spasial ekologikal sumberdaya lahan di Provinsi Daerah Istimewa Yogyakarta. *Forum Geografi*, *21*(2), 95-103.