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AL-KAUNIYAH JOURNAL TEMPLATE

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

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

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Abstract

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 pengamatan dan koleksi sampel secara langsung. Identifikasi metabolit sekunder dilakukan dengan uji kualitatif dan kromatografi lapis tipis (TLC). Hasil penelitian menunjukkan bahwa terdapat 12 spesies di Air Terjun Sidoharjo dan 11 spesies di Ekowisata Sungai Mudal. Secara keseluruhan, terdapat 17 spesies 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. Kedua lokasi memiliki tingkat keanekaragaman anggrek sedang. Identifikasi metabolit sekunder anggrek menunjukkan bahwa mayoritas anggrek memiliki profil senyawa flavonoid, fenolik, dan alkaloid yang beragam.

Kata kunci: Anggrek; Inventarisasi; Keanekaragaman spesies; Menoreh; Metabolit Sekunder

Abstract

Menoreh Hills harbor high flora diversity. One of the plants with high biodiversity in Menoreh Hills is orchid. Most orchids contain the richness of secondary metabolites. This research aims to inventory orchids in Menoreh and to identify the secondary metabolites of orchids. Orchids data collection were carried out in Sidoharjo Waterfall and Mudal River, Kulon Progo. Inventory were made by direct observation and sample collection by hands. Secondary metabolite identification were carried out by qualitative screening of orchids methanolic extract and Thin Layer Chromatography (TLC). The result shows that there are 12 orchid species in Sidoharjo Waterfall and 11 orchids species in Mudal River Ecotourism. There are 17 orchid species diverse in research areas, which are 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. The level of orchids diversity in the two areas are moderate. Secondary metabolite identification shows that most orchids contain phenolic, flavonoid, and alkaloid compounds. TLC results that the methanolic extract of orchid samples have high

abundance of various secondary metabolites, which are flavonoid, phenolic, and alkaloid compounds.

***Keywords**: Inventory; Menoreh; Orchids; Secondary metabolite; Species diversity*

INTRODUCTION

The astronomical position of Indonesia which is placed in the equatorial zone is one of the main factors of the high of biodiversity. Biodiversity will be higher as the transiton of region from polar zone to equatorial zone. The equatorial zone have the higher intensity of sunlight, abundant water, and the circumstances which is more consistent (Gizachew, 2022). Geological and geomorphological aspects influence the diversity of plant species as well, with the result that there are differences in vegetation in every ecosystems. Geological process will form type of rock in an ecosystem that affect in the formation of soil. Those process implicate on the differences of chemical components that are contain 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 (Portal Informasi Indonesia, 2020). Menoreh Karst Hills have a typical ecosystem (Kurniawan *et al*., 2018). The climate of Menoreh is relatively cool and moisture (Christianty & Widodo, 2022), the altitude is up to 700 m asl (Solekha *et al*., 2023), with a high density of vegetation coverage (Worosuprodjo, 2007). One of the plant s with the highest diversity is orchids. Orchidaceae is a member of Liliopsida which have the highest diversity in the world. The estimation is about 28000 species of orchid that widely spread in all continents. The species of orchid in Indonesia is estimated about 5000 species (Portal Informasi Indonesia, 2019). This number indicates that Indonesia is one of the hotspots of orchid in the world. The astronomical position of Indonesia which is passed over by equatorial line cause high diversity of orchids. Orchids are pantropical so the diversity of orchids will be higher in the equatorial zone and get lower as the place far from the equator. Biodiversity and systematics research of orchids has been continuously developed. Recent biodiversity and phylogeny research of orchids have been done by Prayoga *et al*. (2022). The identical research have been conducted by Purba and Chasani (2019) as well in Gunung Gajah, Purworejo, with the result is 13 species have been found in Gunung Gajah.

Orchids have been known to contain secondary metabolites which have physiological and pharmacological activity. Several compounds that have been founded 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 which is potential to used as phytomedicine. *Coelogyne cristata* and *Pholidota imbricata* has been reported contain the strong antibacterial activity (Marasini & Joshi, 2012). Some genus such as *Ephemeranthalon*, *Eulophia*, *Dendrobium*, *Gastrodia*, *Pholidota*, *Spiranthes*, *Vanda*, *Bletilla*, *Anoectochilus*, and *Bulbophyllum* have good anticancer agent (Shukla *et al*., 2022). Various kinds of bioactive compounds in orchids is a reason of the application as traditional medicine in such place like China and India (Pant, 2013). Nevertheless, the research on secondary metabolites of native orchids in Indonesia is still inadequate. Therefore, this research aims to inventorize the species of orchids in ecotourisms area in Menoreh Karst Hills and to identify secondary metabolites which is contain in orchids.

MATERIALS AND METHODS

This research 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 ecotourism area with contours of hills and cliffs. The altitude is about 400 m asl. The vegetation structure is composed of canopy with high coverage, bush, and floor vegetation structure that consist of grass and herbaceous. Mudal River is located in Jatimulyo Village, Girimulyo Subdistrict (7°45’45’’S, 110°06’58’’E). Mudal River is a river ecotourism. The water source flow

in Mudal River come from endokarst layer (Irsyad, 2020). The altitude is about 600 m asl. Mudal River has vegetatiton structure that composed of trees and canopy with high density.

Orchid Data Collection

Collection of orchid diversity data was carried out by tracking without plot in ecotourism area. The tracking line recorded by Geotracker 2.0. The species of orchid that have been found was recorded consist of the number of species, physicochemical parameters consist of humidity, air temperature, light intensity, altitude, and data of morphology. Morphological data was taken by photo and the description was compared with the book of identification of orchids, *Orchid of Java* (Comber, 1990). The orchids which have high abundance were collected to be extracted.

Orchid Extraction

The whole vegetative organ of each orchid was dried in 60°C. The dried sample was grinded to make a simplisia powder. As much as 10 g of simplisia of each orchids are macerated by 80% methanol with a proportion of 1:10. The solution was incubated as long as 24 hours and remacerated by 2 times. The macerates was evaporated by rotary vacuum evaporator.

Identification of Orchids Secondary Metabolite

Concentrated extract with the weight of 0.03 g 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 way.

Alkaloid test: 1 mL of sample solution was added by 10 drops of H_2SO_4 2 N and shaked well. The solution was added by some drops of Mayer, Dragendorff, and Wagner reagent. The positive of alkaloid in Mayer test will form a precipitate with the color of white, Dragendorff test will form a precipitate with reddish-brown color, and Wagner test will form a precipitate with brown color (Mailuhu *et al*., 2017).

Flavonoid test: 1 mL of sample solution was added by a few drops of NaOH 10%. The positive result of flavonoid will change the color of solution into orange color (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 solution into reddish-brown color (Mariyam *et al*., 2023).

Thin Layer Chromatography

The eluent system that be used in this research are aquades, methanol, ethyl acetate, and chloroform with proportion of 1:4:7:25. A few drops of glacial acetic acid was added to prevent the sample elongated. Silica plate GF_{254} (Merck) was activated in 120°C for 20 minutes. As much as 0.01 g of samples extract was dilluted with 1 mL of methanol 80%, then 1µL of each samples were transferred on the plate. The samples which have been prepared in silica plate were eluted in TLC chamber with the eluent system that have been prepared before as mobile phase. The plate was let dry after completing elution and was visualized under uv light 254 nm, 366 nm, and stained with $FeCl_3$ 10%, $AlCl_3$ 10%, and Dragendorff.

Orchid Diversity and Secondary Metabolite Data Analysis

The diversity of orchid in the research areas was analyzed by calculating the Shannon-Wiener Index (H') by the following equation (Barbour *et al*., 1999).

$$H' = -\sum_{i=1}^{k} pi \ln pi$$

$$Pi = ni/N$$

Note: Shannon-Wiener Index (H'); Proportional index of individuals (Pi); Total individual of *i* species (ni); Total species in the area (N)

The biodiversity grade was divided by following criteria (Indriani *et al*., 2009).

H'<1 : Low Biodiversity
1<H'<3 : Moderate Biodiversity
H>3 : High Biodiversity

TLC analysis was carried out by measure the retention factor of all spots of each samples by following equation.

$$Rf = \frac{distance\ of\ spot\ movement}{distance\ of\ eluent\ movement}$$

TLC staining with $FeCl_3$ solutions used to identify the secondary metabolites from phenolic compounds. The presece of phenolic compounds is indicated by yelow, orange, green, reddish violet, or reddish brown (Burman *et al*., 2019). $AlCl_3$ solutions is 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. The present of alkaloid compounds is indicated by orange spot after staining.

RESULTS

Inventory of orchids which have been done in Sidoharjo Waterfall show that there are 12 species were found which are shown in Table 1. The orchids in Sidoharjo Waterfall consist of 7 epiphytic orchids and 5 terrestrial orchids. The most abundance orchid in Sidoharjo Waterfall is *Bryobium retusum* with the total 64 individuals. *Crepidium kobi* and *Nervilia plicata* have high abundancy as well, but not as much as *Bryobium retusum*. The lowest presence species are *Liparis condylobulbon*.

Table 1. Diversity of Orchids in Sidoharjo Waterfall

Species	Individuals	Life Form
Liparis condylobulbon Rchb.f.	1	Epiphyte
Peristylus goodyeroides (D.Don) Lindl.	9	Terrestial
Nervilia plicata (Andrews) Schltr.	28	Terrestrial
Dendrobium crumenatum Sw.	12	Epiphyte
Cymbidium sp.	3	Epiphyte
Acriopsis liliifolia (J.Koenig) Ormerod	4	Epiphyte
Calanthe 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 orchids that have been found in Mudal River are 11 species, consist of 6 epiphytic orchids and 5 terrestrial orchids (Table 2). *Bryobium retusum* is the most abundant species that have been founded. The second highest abundancy in Mudal River is *V. planifolia* and the third highest one is *D. crumenatum*. The smallest number of individuals in Mudal River are *Zeuxine* sp. and *Phaius* sp. which are only one individual that have been found.

Table 2. 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

The species with high abundancy that have been collected and secondary metabolites identification are shown in Table 3. Secondary metabolite screening by color test shows that all orchids that have been collected contain phenolic and flavonoid compounds. Most of orchids contain alkaloid compounds except some orchids that are *D. crumenatum* (SW), *N. plicata* (SW), and *A. liliifolia* (MR). All of orchids are do not have terpenoid compounds.

Table 3. Secondary Metabolite 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 show the different fingerprint of secondary metabolites of orchids each others (Figure 1a-e). The result shows that the orchids in Sidoharjo Waterfall and Mudal River have various compounds of phenolic and flavonoid (Figure 1d, e). TLC stain with Dragendorff reagent shows that alkaloid compounds do not eluted well by mobile phase (Figure 1c).

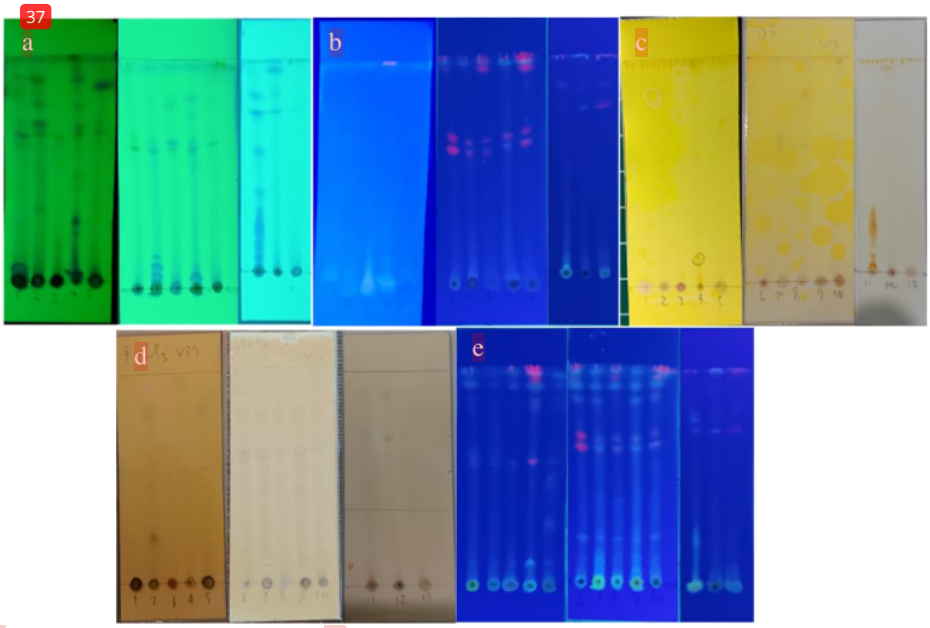

Figure 1. Thin Layer Chromatograms of Methanolic Extract of Orchids. UV 254 nm (a); UV 366 nm (b); Dragendorff (c); $FeCl_3$ (d); $AlCl_3$ (e)

The data shown that *A. liliifolia* (SW) and *S. plicata* (MR) contain various of phenolic compounds which are have 7 fraction of phenolic compounds (Table 4). *D. crumenatum* (SW) also has various of phenolic compounds which is 6 fraction has been identified (Table 4). Orchids with high diversity of flavonoid compounds are *L. condylobulbon* (MR) and *D. crumenatum* (MR) which have 8 fractions of flavonoids (Figure 1e; Table 4). The alkaloid compounds that have been successfully fractionated are in *C. kobi* (SW), *L. parviflora* (MR), *Dienia ophrydis* (MR), and *B. retusum* (MR). *Acriopsis liliifolia* (MR) contain poor of secondary metabolites which is only one fraction of flavonoid and phenolic has been found.

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

Recent research of orchids diversity in Curug Setawing ecotourism, Kulon Progo reported that there are 15 species have been found in the area consist 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* (Kurniawan *et al*., 2018). Orchids that have been identified in Ayunan Langit areas which located in Purwosari Village, Kulon Progo, are about 14 species, consist 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). The research that have been done in Sidoharjo Waterfall shows that several species such as *A. liliifolia*, *D. crumenatum*, *L. parviflora*, and *B. retusum* have wide distribution in Menoreh Karst Hills. This is proven by their presence in such region in Menoreh such as Curug Setawing (Kurniawan *et al*., 2018), Mudal River (Basri *et al*., 2019), and Ayunan Langit (Usmanti *et al*., 2022). This phenomenon indicates that these orchids have successfull to survive in environment and have high sustainability. The most abundant species both in Sidoharjo Waterfall and Mudal River is *B. retusum*. The same situation have been reported in Curug Setawing Ecotourism which is *B. retusum* has the largest number of individuals, to be specific is 99 (Kurniawan *et al*., 2018). The calculation of Shannon-Wiener Index in Sidoharjo Waterfall is 1.86. This number indicates that the diversity of orchids in Sidoharjo Waterfall classified as moderate.

The orchids inventory in Mudal River results that *B. retusum* is the highest number of individuals as well as in Sidoharjo Waterfall. The other species with high abundandy is *V. Planifolia* with the number of individuals is 62 and *D. crumenatum* with the number of individuals is 35. *Vanilla planifolia* has been found frequently in Mudal River. However, the distribution of *V. planifolia* is not occured naturally. The main reason of this phenomenon is caused by culture process that carried out by local people in Jatimulyo (Sudibyanung *et al*., 2023). The previous research in Mudal River reported that there are 15 species of orchid, which are *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, dan Zeuxine gracilis* (Basri *et al*., 2019). However, there are several species have not been found in this research, namely *Cymbidium bicolor*, *Crepidium ridleyi*, *Arundina graminifolia*, *Stereosandra javanica*, *Taeniophyllum* sp., and *Vanda* sp. The reason is the area of exploration in this research is different than the previous research (Basri *et al*., 2019). Diversity level of orchids in Mudal River based on Shannon-Wiener Index shows that the diversity level is moderate, proven by tha value of Shannon-Wiener Index is 1.89.

Secondary metabolites screening shows that only terpenoid compound are not present in all of methanolic extract of orchids. This might be caused by the low polarity of terpenoids so the compounds do not have good affinity with solvent. Secondly, several terpenoids can be highly volatile that can be easily vapour in room temperature. This problem can be solved by select the extraction method with low temperature or even distillation is needed. Identification of alkaloid result that most of orchids contain alkaloid. This is proven by the changing of samples color and the form 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 light shows the fingerprint of different metabolite fractions in each specimen (Figure 1a). The largest compound fractions are *Crepidium kobi* (SW) with 12 fractions and *Dienia ophrydis* (MR) with 10 fractions. The fewest compound fractions are *N. plicata* (SW) with 1 fraction and *A.liliifolia* (MR) with 1 fraction. The phenolic compounds of the orchids have different profile with respect to their habitat. This can be seen from the same orchids such as *D. crumenatum* in SW and MR have different profile of phenolic compounds (Table 4). *D. crumenatum* which are collected from SW have more fraction than MR with the different value of their Rf. The similar condition occurs on the phenolic profile of *B. retusum* and *A. liliifolia* as well. This can happen because the production of secondary metabolites occurs based on the plant's response to its environment (Nugroho, 2016). 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 above sea level with an average temperature of 29°C. The environmental conditions of Sidoharjo Waterfall, which are relatively hot and tend to be exposed to sunlight, 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 obtained by Sedjati *et al.* (2023) which shows that giving UV-B radiation to *Chlorella* sp. for 80 minutes/day for 10 days produced the highest total phenolic levels. Apart from that, increased phenolic levels were also experienced in horsewhip plants (*Stachytarpeta jamaicensis*) which were grown in relatively hot environmental conditions (Utomo *et al*., 2020). The flavonoid compounds that are contained in orchids also different relatively to their circumstances. It does not show a specific pattern such as the phenolic profile. Flavonoid profile of *D. crumenatum* MR is more various than in SW. Contrary, *B. retusum* located in SW has more various flavonoid fractions than in MR. The best results is the spot with Rf value between 0.2 and 0.8. This Rf range shows the specific fraction of all samples that relatively different each other. Rf values that are below or above will have interference in visualization (Muttaqin *et al*., 2016). The alkaloid compounds that are identified by TLC show that only *B. retusum* (SW and MR), *L. parviflora* (MR), and *Dienia ophrydis* have alkaloid fraction. Most of alkaloids are have high polarity, whereas the eluent system that used in this research are semipolar. The compound with the poor affinity to solvent cannot be eluted so they do not move on the plate.

CONCLUSION

Menoreh Ecotourism Areas to be specific in Sidoharjo Waterfall and Mudal River have a moderate level of orchid diversity. There are overall 17 species have been found in research areas. Sidoharjo Waterfall has total 12 species of orchid have been found and 11 species have been found in Mudal River. Secondary metabolites identification by TLC shows that most orchids have flavonoids, alkaloids, and phenolics with high variation. TLC chromatograms show that the profiles of secondary metabolite of orchids might be different with respect to their circumstances and the species.

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