Antimicrobial and Phytochemistry study of *Dendrobium linearifolium* Teijsm. & Binn. from Gumitir, Jember, Indonesia

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Abstract. *Dendrobium linearifolium* Teijsm. & Binn. is an epiphytic orchid growing in Mount Gumitir, Jember, Indonesia and had been used by indigenous people of Bali to treat earaches. Previous research has identified its DNA barcode and the presence of alkaloids and flavonoids with the highest concentration found in the leaves. However, an extensive analysis of their chemical constituents and their potential medicinal properties from this plant is not yet available. This study aims to determine the antimicrobial activity and phytochemical contents of the methanol extract of *D. linearifolium* leaves. The antimicrobial test was conducted using the agar diffusion method against two fungi: *Aspergillus niger* and *Aspergillus falvus* and three pathogenic bacteria i.e., *Streptococcus aureus*, *Salmonella typhi*, and *Escherichia coli*. Phytochemical analysis was performed using GC-MS. The results of the antimicrobial test indicate that the extract of *D. linearifolium* leaves possessed antimicrobial activity with the highest level against *A. niger* and the gram-positive bacteria *S. aureus*. The phytochemical analysis detected a total of 47 compounds with a total of 26 having medicinal potential. The five most dominant compounds with antimicrobial activity, i.e., 1-propanol, 2-(2-hydroxypropoxy)- (CAS) 2-(2-hydroxypropoxy)-1-propanol; 4-methyl-2,5-dimethoxybenzaldehyde; phenol, 2-methoxy- (CAS) guaiacol; 2-propanol, 1,1'-oxybis- (CAS) dipropylene glycol; and acetic acid (CAS) ethylic acid.

1 Introduction

Gumitir is a mountainous area located on the border between the Jember and Banyuwangi regions at an altitude of 440-625 m above sea level with an area of 1.2 thousand ha [1-2].

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This mountainous tropical forest area is home to a diverse range of flora. However, the economic potential of the area has led to the introduction of a coffee plantation under the forest canopy, which has had a negative impact on the diversity of flora [3]. One type of exotic plant that is still often found in Mount Gumitir is the orchid. Several types of orchids were identified with a total of 16 species of which 14 types were epiphytic orchids and 2 types of terrestrial orchids. One of the abundant epiphytic orchids in this area is the *Dendrobium linearifolium* Teijsm. & Binn. [4-6].

Dendrobium has been the most studied among the orchids in which dendrobium in China has been used as an herbal medicine which causes its availability in nature to be threatened [7-8]. Metabolomics studies using the GC-MS approach have been reported on two types of Dendrobium, namely *D. oficinale* and *D. huoshanense* for conservation purposes and reported that the highest production of metabolite compounds was obtained after the plants were three years old after relocation [8]. *D. officinale* extract contains 529 metabolites, and flavonoid compounds are the compounds most sensitive to changes in environmental conditions [9].

D. linearifolium distribution is spread around the western part of Indonesia from Bali, Java, and Sumatra Islands at an altitude of 800-2000 meters above sea level and is found in forests and mountains [10]. This type of orchid has stems that reach 70 cm, linear leaves grown along and at the base of the stem, there are oval pseudobulbs [11]. According to Sujarwo and Lestari (2018), the pseudobulb of *D. linearifolium* in the Hindu community in Bali has been used as a medicine for earache by dripping water stored in the pseudobulb and the orchid has been used as a medicinal ingredient to prevent infections in the ear canal [12]. The secondary metabolite content detected in all parts of this plant in the form of alkaloids and flavonoids has been reported with the highest content found in the leaves [13]. The DNA barcode with *ITS2* marker dah been reported elsewhere [6]. However, the content of the types of bioactive compounds contained in *D. Linearifolium* remains unknown.

This study aims to analyze the antimicrobial activity of *D. linearifolium* leaves against pathogenic fungi and bacteria and to determine the content of volatile and semi-volatile phytochemical compounds contained in leaf samples using GC-MS. In addition, the study will provide laboratory based information for the development of medicinal potential plant from Jember district forest in line with conservation of medicinal plants the habitat for sustainable utilisations.

2 Methods

2.1 Sample collection and extraction

The orchid sample of *Dendrobium linearifolium* was collected from Mount Gumitir in Jember Regency, Indonesia. The sample was then transported to the Laboratory of Botany-University of Jember for identification and a voucher The sample was planted at the Biology Botanical Garden of the Faculty of Mathematics and Sciences at the University of Jember [14]. Identification and DNA barcode of the specimen was published previously by a botanist, Su'udi, Ph.D [6]. For the leave extraction, the leaves sample were cleaned, dried, and ground into powder. The sample powder (10 g) was then macerated for 3x24 hours with methanol (50 mL). The filtrate was pooled and subjected to a rotary evaporator to obtain crude extract [15].

2.2 Antimicrobial assay

The antimicrobial test was conducted with agar diffusion methods against two fungi: *Aspergillus niger* and *Aspergillus falvus* and three bacteria i.e., *Streptococcus aureus*, *Salmonella typhi*, and *Escherichia coli*. The agar well method was with five treatments, including a positive control (0.1% chloramphenicol for antibacterial and 0.1 % Ketoconazole for antifungal), a negative control (sterile distilled water), and *D. linearifolium* leaf extract with varying concentrations of 25%, 50%, and 100%. Each treatment was repeated four times. The parameter measured was the inhibition zone formed around the well. The strength of antibacterial activity was categorized as low (<5 mm), medium (5-10 mm), strong (11-19 mm), or very strong (≥20 mm) [15-16].

2.3 Phytochemical analysis

Phytochemical analysis of D. linearifolium was conducted using GC-MS with a QP20010 Plus Shimizu, Japan in the Laboratory of Biosciences at Jember State Polytechnic, Indonesia. The methanol crude extract (1 µL in methanol) was injected into the GC-MS column with helium as the carrier gas. Phytochemical analysis and metabolite identification were conducted using the Wiley Library, following the method described by Ulum et al. [15].

3 Result and Discussion

3.1 Antimicrobial activities

The methanol extract of *Dendrobium linearifolium* leaves exhibits antifungal and antibacterial activities against the tested microbes. The antifungal activity test revealed that the methanol extract of *D. linearifolium* leaves produced inhibition zones against *Aspergillus niger* and *Aspergillus flavus* (Figure 1 f-g). The highest inhibition zone diameter was observed at 100% concentration (*A. niger* = 10.8 mm and *A. flavus* = 4.15 mm) (Table 1). The antibacterial activity test indicated that the methanol extract of *D. linearifolium* leaves produced inhibition zones against *E. coli*, *S. aureus*, and *S. typhi* (Figure 1 h-j). The antibacterial inhibition leaf extract of *D. linearifolium* in concentration 100 % was low against *E. coli* (4.05±0.67 mm) but moderate against *S. aureus* (8.25±0.72 mm) and *S. typhi* (4.45±0.58 mm). Table 1 displays the antifungal and antibacterial activities of the methanol extract of D. linearifolium leaves against five microbes.

The antifungal activity against *A. niger* varied among concentrations, with the strongest effect observed at a concentration of 100%, with an inhibition zone diameter of 10.8 ± 2.57 mm. At lower concentrations (25% and 50%), the antifungal activity was low, with inhibition zone diameters of 0.87 ± 1.75 mm and 3.28 ± 1.56 mm, respectively. On the other hand, the antifungal activity of the leaf extract from *D. linearifolium* was low against *A. flavus*. The inhibition zone was only detected at 50% and 100% extract concentrations, with inhibition zones of 2.2 ± 1.01 mm and 4.15 ± 0.38 mm, respectively (Table 1). The cloudy color of the inhibition zone in the fungi colonies indicated that there was still growth of *A. niger* and *A. flavus* colonies. However, the inhibition zone was not as clear as the positive control suggested that the methanol extract of *D. linearifolium* leaves is fungistatic rather than fungicidal. Therefore, fungal growth was still present around the inhibition zone as fungistatic substances only inhibit the fungi growth without killing the cells [17-18]. According to Setyati et al., *D. linearifoilum* leaves contain alkaloids and flavonoids [13]. Alkaloid compounds inhibit the function of the fungal cell's genetic material by inhibiting DNA formation, topoisomerase, and membrane synthesis, as explained by Sulaiman et al.

[19]. Meanwhile, the mechanism for inhibiting flavonoid compounds disrupts metabolism, damages cells, and inhibits the formation of fungal cell walls by destroying fungal protein compounds, thereby hampering hyphae growth [20].

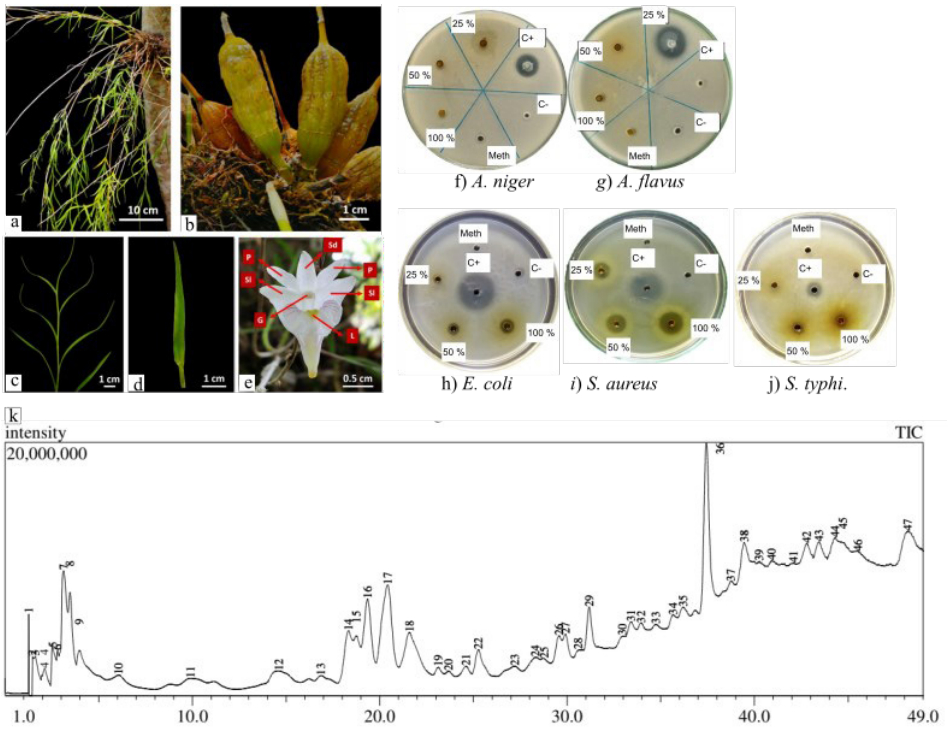

Fig. 1. *D. linearifolium* structure, antimicrobe activity and GC-MS profile; a-e) Morphological structure; f-j) inhibition zone of antimicrobial activity against fungi (f-g) and bacteria (h-j); k) GC-MS Chromatogram. G: gynoecium, L; labellum, P: Petal, Sd: Sepal dorsal, Sl: Sepal lateral, 25-100%: concentration of methanolic extract of the leaves, C+: positive control (antibiotic () /antifungal ()), C-: negative control (water), Meth: methanol

Table 1. Antimicrobial activity of leaf methanol extract *D. linearifolium* against two fungi and three bacteria. The antimicrobial activity was indicated by the star marker (*= low; **=medium, ***= strong).

Treatment	**Inhibition zone (mm)**				
	Antifungal		Antibacterial		
	A. niger	*A. flavus*	*S. aureus*	*S. typhi*	*E. coli*
25 %	0.87 ± 1.75*	0	5.42±0.49**	0	0.47±0.15*
50 %	3.28 ± 1.56*	2.2 ± 1.01*	6.95±0.51**	2.72±0.49*	2.35±1.03*
100 %	10.8 ± 2.57***	4.15 ± 0.38*	8.25±0.71**	4.45±0.58*	4.05±0.66*
Positive control	8.47 ± 0.80**	13.7 ± 1.81***	14±0.63***	4.9±1.04*	14.32±1.00***
Negative control	0	0	0	0	0
Blanko	0	0	-	-	-

The antibacterial activity of the methanol extract of *D. linearifolium* leaves was stronger against the positive Gramm bacteria, *S. aureus*. The inhibitory effect against *S. aureus* was at medium level presented at three concentrations 25%, 50%, and 100%, with inhibition zone diameters of 5.42 ± 0.486 mm, 6.95 ± 0.507 mm, and 8.25 ± 0.714 mm, respectively. The inhibitory activity against *E. coli* was low at all three concentrations of 25%, 50%, and 100%, with diameters size of 0.47 ± 0.15 mm, 2.35 ± 1.03 mm, and 4.05 ± 0.656 mm, respectively. Meanwhile, the inhibitory effect against *S. typhi* was only observed at higher concentrations of 50% and 100%, resulting in 2.72 ± 0.497 mm and 4.45 ± 0.58 mm, respectively. The inhibitory activity of phytochemical compounds against bacteria is generally lower against gram-negative bacteria than gram-positive bacteria [15]. This is due to the presence of the outer membrane structure of gram-negative bacteria, which has a hydrophobic structure that can inhibit the binding of hydrophilic structures such as phenolics from plant extracts [21].

3.2 Phytochemical of *D. linearifolium*

The results of the GC-MS analysis of the methanol extract of *D. linearifolium* were presented in the form of a chromatogram with 47 peaks of dominant compounds detected based on their area measured across the retention time (see Figure 1h). Phytochemical identification of each peak yielded 47 compounds (Table 2). The five most dominant compounds, with an area above 5% i.e., 1-propanol, 2-(2-hydroxypropoxy)- (CAS) 2-(2-hydroxypropoxy)-1-propanol; 4-methyl-2,5-dimethoxybenzaldehyde; phenol, 2-methoxy- (CAS) guaiacol; 2-propanol, 1,1'-oxybis- (CAS) dipropylene glycol; and acetic acid (CAS) ethylic acid.

Table 2. Phytochemical compound identified in methanolic extract of *D. linearifolium* leave.

Peak	Retention Time	Area (%)	Name
1	1.265	0,21	ethyne, fluoro- (cas) fluoro acetylene
2	1.463	0,3	monomethyl ester of oxalic acid
3	1.611	1,92	Propanal, 2-methyl- (CAS) Isobutanal
4	2.103	0,97	butanal, 3-methyl- (CAS) 3-methylbutanal
5	2.553	1,06	Cyclopropyl carbinol
6	2.795	0,73	acetic acid (CAS) ethylic acid
7	3.132	6,2	Acetic acid (CAS) Ethylic acid
8	3.462	4,01	2-Propanone, 1-hydroxy- (CAS) Acetol
9	3.951	2,42	1,2-Ethanediamine, N-ethyl- (CAS) N-Ethylethylenediamine
10	6.050	1,31	Ethyl n-amyl disulfide
11	9.903	0,95	2-furan methanol (CAS) furfuryl alcohol
12	14.620	2,05	Hydrazine, (2-methyl-1-propenyl)- (CAS) Isocrotylhydrazine
13	16.872	0,23	1,2-cyclohexanedione (cas) 1,2-dioxocyclohexane
14	18.335	3,94	Ethanol, 2,2'-oxybis- (CAS) Diethylene glycol
15	18.754	2,91	Benzeneacetaldehyde (cas) hyacinthin

Peak	Retention Time	Area (%)	Name
16	19.356	6,71	2-Propanol, 1,1'-oxybis- (CAS) Dipropylene glycol
17	20.434	10,59	1-Propanol, 2-(2-hydroxypropoxy)- (CAS) 2-(2-Hydroxypropoxy)-1-propanol
18	21.584	6,91	Phenol, 2-methoxy- (CAS) Guaiacol
19	23.124	0,73	2-pyrrolidinone (cas) pyrrolidone
20	23.671	0,45	1H-4-azacycloprop[cd]indene, octahydro-4-methyl- (cas) 3-methyl-3-azatricyclo(6.1.0.0(5,9))nonane
21	24.603	0,81	trimethyl-tetrahydronaphthalene
22	25.270	2,05	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one
23	27.210	0,79	1H-azepine, hexahydro- (CAS) homopiperidine
24	28.308	0,94	3-oxo-.alpha.-damascone
25	28.787	0,4	2-furanmethanol, tetrahydro-5-methyl-, trans- (CAS)
26	29.572	1,35	2,3-dihydro-benzofuran
27	29.919	1,36	Phenol, 4-ethenyl-2-methoxy-
28	30.600	0,4	2-pyrrolidinone, 1-butyl- (CAS) N-n-Butyl-2-pyrrolidone
29	31.188	2,4	Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxyphenol
30	32.947	0,52	2,4,7-pteridinetriamine, 6-methyl-
31	33.410	1,27	5-oxo-pyrrolidine-2-carboxylic acid methyl ester
32	33.954	0,99	Silane, trimethyl(4-phenoxybutoxy)- (CAS)
33	34.746	0,55	hexanoic acid, 2,3-bis(acetyloxy)propyl ester (cas) 1-capro-2,3-diacetin
34	35.651	0,62	1-cyclohexanone, 2-formyl-6-isopropyl-3-methyl-
35	36.193	0,96	benzoic acid, 3-hydroxy-, methyl ester (CAS) methyl 3-hydroxybenzoate
36	37.444	9,14	4-methyl-2,5-dimethoxybenzaldehyde
37	38.740	1,23	N-(1-naphthyl)lauramide
38	39.463	3,9	1,6-anhydro-beta-d-glucopyranose (levoglucosan)
39	40.263	0,96	4-(4-hydroxy-2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-butan-2-one
40	40.920	0,63	phenol, 2,6-dimethoxy-4-(2-propenyl)- (CAS) 4-Allyl-2,6-dimethoxyphenol
41	42.091	0,12	cyclopentyl-methyl-phosphinic acid 2-isopropyl-5-methyl-cyclohexyl ester
42	42.785	1,7	2,4(1h,3h)-pyrimidinedione (cas) ura

Peak	Retention Time	Area (%)	Name
43	43.448	1,83	2,4(1H,3H)-Pyrimidinedione, 5-methyl- (CAS) Thymin
44	44.312	2,47	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy- (CAS) Coniferyl alcohol
45	44.733	2,01	1-tetradecanol (cas) alfol 14
46	45.533	1,68	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)- (CAS) Acetosyringone
47	48.191	4,31	Hexadecanoic acid (CAS) Palmitic acid

Literature reviews were conducted to observe the medicinal potencies of phytochemical compounds identified in the methanolic extract of *D. linearifolium* leaves. A total of 26 phytochemicals had medicinal potencies (Table 3). This article describes the five most dominant phytochemicals, whereas the rest of the 21 compounds were presented in the table 3 with the references. The highest phytochemical detected was 1-propanol, 2-(2-hydroxypropoxy)- (CAS) 2-(2-Hydroxypropoxy)-1-propanol with 10.59 % of cover area among all detected molecules. It belongs to the group of secondary alcohols and has antimicrobial activity and humectant properties [18]. 4-methyl-2,5-dimethoxybenzaldehyde is the second highest phytochemical with 9.14 % of the cover area. This chemical compound belongs to the benzenes group and has antimicrobial activity against *E. coli* and *Salmonella enterica* [22]. Phenol, 2-methoxy- (CAS) guaiacol was used as an antiseptic and expectorant. It has an induction effect on cell proliferation due to its ability to scavenge reactive oxygen species (ROS) [23]. The last two compounds were 2-propanol, 1,1'-oxybis- (CAS) dipropylene glycol with a % area of 6.71 %, and acetic acid (CAS) ethylic acid with a % area of 6.2 %. 2-propanol, 1,1'-oxybis- (CAS) dipropylene glycol or commercial glycol was reported to inhibit the bacteria of *Streptococcus mutans* and *E. coli* [24], while Muriady et al. reported that acetic acid (CAS) ethyl acid has antimicrobial activities against *E. coli*, *S. aureus*, and *B. subtilis* [25]. Overall, free phenolics and acid groups present in the molecules might contribute to their anti-microbial activities.

Table 3. Medicinal potencies of phytochemical compound identified from methanolic extract of *D. linearifolium* leaves with references.

No.	Name	Antimicrobe	Other potencies
1	1-Propanol, 2-(2-hydroxypropoxy)- (CAS) 2-(2-Hydroxypropoxy)-1-propanol	Yes	Humectant [18]
2	4-methyl-2,5-dimethoxybenzaldehyde	Yes	[22]
3	Phenol, 2-methoxy- (CAS) Guaiacol	Yes	Antiseptic, expectorant, Antioxidant [23], [26]
4	2-Propanol, 1,1'-oxybis- (CAS) Dipropylene glycol	Yes	[24]
5	Acetic acid (CAS) Ethylic acid	Yes	[25]
6	Hexadecanoic acid (CAS) Palmitic acid	Yes	anti-inflammatory and

No.	Name	Antimicrobe	Other potencies
			hepatoprotective [27]
7	2-Propanone, 1-hydroxy- (CAS) Acetol	Yes	[25]
8	Ethanol, 2,2'-oxybis- (CAS) Diethylene glycol	Yes	[28]
9	1,6-anhydro-beta-D-glucopyranose (Levoglucosan)	Yes	[29]
10	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy- (CAS) Coniferyl alcohol	No	Anti-Quorum Sensing (Anti-QS) [30]
11	Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxyphenol	Yes	[31]
12	Hydrazine, (2-methyl-1-propenyl)- (CAS) Isocrotylhydrazine	Yes	anticancer, antipsychotic, and antidepressant drugs [32]
13	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	No	Antioxidant [33]
14	1-Tetradecanol (CAS) Alfol 14	Yes	[34]
15	5-oxo-pyrrolidine-2-carboxylic acid methyl ester		Anti alzheimer [35]
16	Cyclopropyl carbinol	Yes	[36]
17	Silane, trimethyl(4-phenoxybutoxy)- (CAS)	Yes	[37]
18	Butanal, 3-methyl- (CAS) 3-Methylbutanal	Yes	[38]
19	Benzoic acid, 3-hydroxy-, methyl ester (CAS) Methyl 3-hydroxybenzoate	Yes	anti-inflammatory [39]
20	4-(4-hydroxy-2,2,6-trimetthyl-7-oxa-bicyclo[4.1.0]hept-1-YL)-butan-2-one	No	Anticancer [40]
21	2-Furanmethanol (CAS) Furfuryl alcohol	No	Antioxidant [41]
22	3-oxo-.alpha.-damascone	No	Anticancer [42]
23	1H-Azepine, hexahydro- (CAS) Homopiperidine	No	Anticancer [43]
24	Acetic acid (CAS) Ethylic acid	Yes	[44]
25	2-Pyrrolidinone (CAS) Pyrrolidone	Yes	[45]
26	Phenol, 2,6-dimethoxy-4-(2-propenyl)- (CAS) 4-Allyl-2,6-dimethoxyphenol	No	Antioxidant [46]

4 Conclusion

The *D. linearifolium* leaves extract presented both antifungal and antibacterial properties. The highest level of antimicrobial activity was observed against the fungal *A. niger* and the

gram-positive bacteria *S. aureus*. The antifungal activity was found to be fungistatic, with a strong inhibitory effect observed only at a concentration of 100% against *A. niger* and low inhibitory effect against other fungi. The antibacterial activity of *D. linearifolium* was found to be moderately effective against *S. aureus* at concentrations of 25 % to 100 % while exhibiting low inhibitory activity against other bacteria. GC-MS analysis detected 47 phytochemical compounds in *D. linearifolium*, 26 of which have medicinal potential. The five most dominant compounds identified by GC-MS exhibit antimicrobial activity, i.e., 1-propanol, 2-(2-hydroxypropoxy)- (CAS) 2-(2-hydroxypropoxy)-1-propanol; 4-methyl-2,5-dimethoxybenzaldehyde; phenol, 2-methoxy- (CAS) guaiacol; 2-propanol, 1,1'-oxybis-(CAS) dipropylene glycol; and acetic acid (CAS) ethylic acid.

References

1. D. Setyati, H. Sulistiyowati, M.-P. Erizcy, T. Ratnasari, The Flavonoid and Alkaloid Content of *Cyclosorus parasiticus* (linn.) farwell ferns at the Plantation Areas of Jember Regency, *BIOLINK (Jurnal Biologi Lingkungan Industri Kesehatan)*, **7**, 1 (2020), doi: 10.31289/BIOLINK.V7I1.3026.
2. D. Setyati, H. Sulistyowati, R. Rahmawati, T. Ratnasari, The spores structure of ferns growing in mountain Gumitir coffee plantation area Jember Regency, *IOP Conf Ser Earth Environ Sci*, vol. **743**, 1 (2021), doi: 10.1088/1755-1315/743/1/012089.
3. S. Murdiyah, P. Pujiastuti, R.-T. Prasetyo, Diversity and Frequency of The Kind of Lichen in Gumitir Mountain area of Jember Regency," *Pancaran Pendidikan*, **8**, 1, (2019), doi: 10.25037/PANCARAN.V8I1.215.
4. L. Susilowati, Inventarisasi Jenis Anggrek di Gumitir, Thesis, Universtiy of Jember, Jember, 2018.
5. D. Setyati, D. Lutfianasari, T. Ratnasari, "Kandungan alkaloid dan flavonoid tiga spesies anggrek berpotensi obat dari Kawasan Gunung Gumitir Kabupaten Jember," *Jurnal Biologi Udayana*, **26**, 1, pp. 11–20, (2022), doi: 10.24843/JBIOUNUD.2022.V26.I01.P02.
6. M. Su'udi, D.-W. Budyartini, Z. Ramadany, DNA Barcoding Anggrek *Dendrobium linearifolium* Teijsm. & Binn. Berdasarkan Penanda Molekuler ITS2, *Al-Kauniyah: Jurnal Biologi*, **15**, 1, (2022), doi: 10.15408/KAUNIYAH.V15I1.16710.
7. A.-S. Nugraha, B. Triatmoko, P. Wangchuk, P.-A. Keller, Vascular Epiphytic Medicinal Plants as Sources of Therapeutic Agents: Their Ethnopharmacological Uses, Chemical Composition, and Biological Activities, *Biomolecules*, **10**, 2, (2020), doi: 10.3390/BIOM10020181.
8. Q. Jin, C. Jiao, S. Sun, C. Song, Y. Cai, Y. Lin, H. Fan, Y. Zhu, Metabolic Analysis of Medicinal *Dendrobium officinale* and *Dendrobium huoshanense* during Different Growth Years, *PLoS One*, **11**, 1, (2016), doi: 10.1371/journal.pone.0146607.
9. S.-M., Zuo, H.-D. Yu, W. Zhang, Q. Zhong, W. Chen, W. Chen, Y.-H. Yun, H. Chen, "Comparative Metabolomic Analysis of Dendrobium officinale under Different Cultivation Substrates," *Metabolites*, **10**, 8, p. 325, (2020), doi: 10.3390/metabo10080325.
10. J. B. Comber, *Anggrek Jawa*. (London: Royal Botanic Gardens Kew, 1990).
11. E.-S. Teoh, *Medicinal Orchids of Asia*, pp. (2016), doi: 10.1007/978-3-319-24274-3/COVER.

12. A.-I. Puji, S. Hidayat, I. Bagus, D. Darnaedi, K. Arinasa, B. Powell, *Traditional Plant Usage in Four Villages of Bali Aga Tenganan Sepang Tigawasa and Sembiran Bali Indonesia*. (Bogor Indonesia: John D. and Catherine T. MacArthur Foundation, 2000).
13. D. Setyati, D. Lutfianasari, T. Ratnasari, Kandungan alkaloid dan flavonoid tiga spesies anggrek berpotensi obat dari Kawasan Gunung Gumitir Kabupaten Jember, *Jurnal Biologi Udayana*, **26**, 1, (2022), doi: 10.24843/JBIOUNUD.2022.v26.i01.p02.
14. F.-B. Ulum, D. Setyati, M. Su'udi, Epiphytic ferns and orchids adaptation mechanism based on stomatal structure and chlorophyll content in Urban Forest of Jember University, Indonesia, *Life Science and Biotechnology*, **1**, 1(2023), doi: 10.19184/LSB.V1I1.39678.
15. F.-B. Ulum, D. Setyati, M.-I.-A. Rizqoni, M. Su'udi, Antimicrobial and Phytochemistry study of *Liparis resupinata* Ridl. from Mount Gumitir, East Java, Indonesia," *JPSCR: Journal of Pharmaceutical Science and Clinical Research*, **8**, 3, (2023), doi: 10.20961/JPSCR.V8I3.70856.
16. D. Setyati, R. Adawiyah, T. Ratnasari, M. Su'udi, F.-B. Ulum, Phenolic Profile and Antimicrobe of the *Asplenium Nidus* L. from Mount Gumitir, Jember, East Java, Indonesia," *BIOEDUKASI*, **21**, (2023), doi: 10.19184/BIOEDU.V21I3.43368.
17. J.-R. Graybill, D.-S. Burgess, T.-C. Hardin, Key issues concerning fungistatic versus fungicidal drugs, *European Journal of Clinical Microbiology & Infectious Diseases*, **16**, 1, (1997), doi: 10.1007/BF01575120.
18. F. Shalihah, A. Saputra, Y. Sidiq, Antibacterial activity of emulgel essential oil from scented lemongrass (*Cymbopogon nardus* (L.) Rendle) against *Staphylococcus aureus* ATCC-29213," *Biogenesis: Jurnal Ilmiah Biologi*, **11**, 1, (2023), doi: 10.24252/bio.v11i1.35836.
19. M. Sulaiman, K. Jannat, V. Nissapatorn, M. Rahmatullah, A.-K. Paul, de L.-M. Pereira, M. Rajagopal, M. Suleiman, M.-S. Butler, M.-K-B. Break, Antibacterial and Antifungal Alkaloids from Asian Angiosperms: Distribution, Mechanisms of Action, Structure-Activity, and Clinical Potentials, *Antibiotics*, **11**, 9 (2022), doi: 10.3390/antibiotics11091146.
20. M.-S. Al Aboody and S. Mickymaray, Anti-Fungal Efficacy and Mechanisms of Flavonoids, *Antibiotics*, **9**, 2, (2020), doi: 10.3390/antibiotics9020045.
21. U. Anand, N. Jacobo-Herrera, A. Altemimi, and N. Lakhssassi, A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery, *Metabolites,* **9,** 11 (2019), doi: 10.3390/METABO9110258.
22. M. Friedman, P.-R. Henika, and R.-E. Mandrell, Antibacterial Activities of Phenolic Benzaldehydes and Benzoic Acids against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*," *J Food Prot*, **66**, 10, (2003), doi: 10.4315/0362-028X-66.10.1811.
23. C. Kantar, B. Kaya, M. Türk, and S. Şaşmaz, Novel Phthalocyanines Containing Guaiacol Azo Dyes: Synhthesis, Antioxidant, Antibacterial, and Anticancer Activity, *Journal of Structural Chemistry*, **59**, 5, (2018), doi: 10.1134/S0022476618050335.
24. T. Nalawade, K. Bhat, and SumaH. P. Sogi, Bactericidal activity of propylene glycol, glycerine, polyethylene glycol 400, and polyethylene glycol 1000 against selected microorganisms," *J Int Soc Prev Community Dent*, **5**, 2 (2015), doi: 10.4103/2231-0762.155736.
25. Muriady, H. Meilina, and M. Faisal, Antibacterial Activity of Liquid Smoke Powder from Rice Husk, *GEOMATE Journal*, **23**, 95, (2022), doi: 10.21660/2022.95.7522.

26. E. Orlo, C. Russo, R. Nugnes, M. Lavorgna, M. Isidori, Natural Methoxyphenol Compounds: Antimicrobial Activity against Foodborne Pathogens and Food Spoilage Bacteria, and Role in Antioxidant Processes,"*Foods*, **10**, 8, (2021), doi: 10.3390/foods10081807.
27. U.-A. Fadzir, K.-I. Mokhtar, B.-E. Mustafa, D.-S. Darnis, Evaluation of Bioactive Compounds on Different Extracts of Linum Usitatissimum and Its Antimicrobial Properties against Selected Oral Pathogens, *Makara Journal of Health Research*, **22**, 3, (2018), doi: 10.7454/msk.v22i3.10181.
28. A.-V. Bailey, G.-J. Boudreaux, G. Sumrell, A. F. Novak, Antimicrobial properties of some mixed diesters of aliphatic diols, *J Am Oil Chem Soc*, **53**, 10, (1976), doi: 10.1007/BF02586276.
29. S. Vijayarathna, Z. Zakaria, Y. Chen, L.-Y. Latha, J.-R. Kanwar, S. Sasidharan, The Antimicrobial Efficacy of *Elaeis guineensis*: Characterization, in Vitro and in Vivo Studies, *Molecules*, **17**, 5, (2012), doi: 10.3390/molecules17054860.
30. D. Deryabin, A. Tolmacheva, Antibacterial and Anti-Quorum Sensing Molecular Composition Derived from *Quercus cortex* (Oak bark) Extract, *Molecules*, **20**, 9, (2015), doi: 10.3390/molecules200917093.
31. H. Rao, P. Li, H. Wu, C. Liu, W. Peng, W. Su, Simultaneous Determination of Six Compounds in Destructive Distillation Extracts of Hawthorn Seed by GC-MS and Evaluation of Their Antimicrobial Activity, *Molecules*, **24**, 23, (2019), doi: 10.3390/molecules24234328.
32. M.-D. Jalageri, A. Nagaraja, a Y.-M. Puttaiahgowda, Piperazine based antimicrobial polymers: a review, *RSC Adv*, **11**, 25, (2021), doi: 10.1039/D1RA00341K.
33. X. Yu, M. Zhao, F. Liu, S. Zeng, J. Hu, Identification of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one as a strong antioxidant in glucose–histidine Maillard reaction products, *Food Research International*, **51**, 1, (2013), doi: 10.1016/j.foodres.2012.12.044.
34. Y. Iriani, Y. Ramona, aN. P. A. Astiti, Efektifitas daun salam dalam mengurangi cemaran mikroba penyebab busuk telur itik, *Intisari Sains Medis*, **13**, 2, (2022), doi: 10.15562/ism.v13i2.1321.
35. S. Sansenya, C. Winyakul, K. Nanok, W. Phutdhawong, Synthesis and inhibitory activity of N-acetylpyrrolidine derivatives on α-glucosidase and α-amylase, *Res Pharm Sci*, **15**, 1, (2020), doi: 10.4103/1735-5362.278711.
36. M. Akbarizare, GC-MS Analysis and Antimicrobial Activity of an Iranian Traditional Medicinal Smoke (Anbarnasara), *Journal of Medical Microbiology and Infectious Diseases*, **9**, 3, (2021), doi: 10.52547/JoMMID.9.3.148.
37. A. Adawy, S. El-Mesallamy, E. Badr, Z. Abdeen, Surface Properties and Biological Activity of the Synthesized Polymeric Surfactant and its Complexes, *Letters in Applied NanoBioScience*, **11**, 3, (2021), doi: 10.33263/LIANBS113.36783688.
38. S. Xiao, J. Xu, X. Chen, X. Li, Y. Zhang, Z. Yuan, 3-Methyl-1-butanol Biosynthesis in an Engineered Corynebacterium glutamicum, *Mol Biotechnol*, **58**, 5, (2016), doi: 10.1007/s12033-016-9929-y.
39. K. Krishnan, A. Mani, Structural Elucidation and Identification of 2-Hydroxy Benzoic Acid: An Antibacterial and Cytotoxic Compound from *Streptomyces* sp. VITHM1 Isolated from Marine Sediment Sample of Alappuzha Beach, Kerala, India, *Arab J Sci Eng*, **43**, 7, (2018), doi: 10.1007/s13369-017-2953-9.

40. K. Sun, X. Li, W. Li, J. Wang, J. Liu, Y. Sha, Two New Lactones and One New Aryl-8-oxa-bicyclo[3,2,1]oct-3-en-2-one from Descurainia sophia, *Chem Pharm Bull (Tokyo)*, **52**, 12, (2004), doi: 10.1248/cpb.52.1483.
41. K. Sharma, V. Pasricha, G. Satpathy, R. K. Gupta, Evaluation of phytochemical and antioxidant activity of raw Pyrus communis (l), an underexploited fruit,"*J Pharmacogn Phytochem*, **3**, 5, (2015).
42. E.-D. Babot, C. Aranda, R.-O.-J.-C. Del, R. Ullrich, J. Kiebist, K. Scheibner, M. Hofrichter, A.-T. Martınez, A. Gutiérrez, Selective Oxygenation of Ionones and Damascones by Fungal Peroxygenases, *J Agric Food Chem*, **68**, 19, (2020), doi: 10.1021/acs.jafc.0c01019.
43. S. Teimoori, K. Panjamurthy, K. Vinaya, D. S. Prasanna, S.-C. Raghavan, K. S. Rangappa, Synthesis and Biological Evaluation of Novel Homopiperazine Derivatives as Anticancer Agents, *J Cancer Ther*, **02**, 04, (2011), doi: 10.4236/jct.2011.24069.
44. Y. Goudjal, O. Toumatia, N. Sabaou, M. Barakate, F. Mathieu, A. Zitouni, Endophytic actinomycetes from spontaneous plants of Algerian Sahara: indole-3-acetic acid production and tomato plants growth promoting activity, *World J Microbiol Biotechnol*, **29**, 10, (2013), doi: 10.1007/s11274-013-1344-y.
45. G. Sathiyanarayanan, R. Gandhimathi, B. Sabarathnam, G. Seghal Kiran, J. Selvin, Optimization and production of pyrrolidone antimicrobial agent from marine sponge-associated Streptomyces sp. MAPS15, *Bioprocess Biosyst Eng*, **37**, 3 (2014), doi: 10.1007/s00449-013-1023-2.
46. G. Gusmailina, S. Komarayati, Exploration of organic compounds strychnine bush (*Strychnos lucida*) as source of medicines. In Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia **1**, 7, (2015) doi: 10.13057/psnmbi/m010738.