**BIODIVERSITAS**
Volume 26, Number 6, June 2025
Pages: 2794-2805

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260624

# Morphological, molecular, and phytochemical characteristics of wild edible fern (*Diplazium esculentum*) from Jember, East Java, Indonesia

**YUSRINA RISKY AMALINI, KAHAR MUZAKHAR, MUKHAMAD SU'UDI, DWI SETYATI, EDIA FITRI DWINIANTI, FUAD BAHRUL ULUM♥**

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia.
Tel./fax.: +62-331-330225, ♥email: fuad.fmipa@unej.ac.id

Manuscript received: 30 July 2024. Revision accepted: 29 May 2025.

**Abstract.** *Amalini YR, Muzakhar K, Su'udi M, Setyati D, Dwinianti EF, Ulum FB. 2025. Morphological, molecular, and phytochemical characteristics of wild edible fern (*Diplazium esculentum*) from Jember, East Java, Indonesia. Biodiversitas 26: 2794-2805. Diplazium esculentum*, an edible pteridophyte with medicinal potential, exhibits morphological variation that risks improper medicinal use. This study aimed to comprehensively characterize *D. esculentum* from Jember, Indonesia, through an integrative approach, including morphological analysis, DNA barcoding, phytochemical profiling, and antioxidant activity assessment. Fifteen specimens, locally referred to as *pakis menir*, were collected from a single site within a rubber-mixed coffee plantation in Panti, Jember District, East Java, Indonesia. The examination of morphological traits, molecular identification using the rbcL marker, analysis of phytochemical profiles through GC-MS, prediction of biological activities using PASS online, and evaluation of antioxidant activity using the DPPH assay all pointed to the potential of *D. esculentum*. Morphological analysis confirmed *D. esculentum's* diagnostic traits, while rbcL DNA barcoding validated a 100% sequence match with *D. esculentum* (OL536867.1). GC-MS analysis of young fronds identified 25 bioactive metabolites, including 11 phenolics, 9 terpenoids, and 5 alkaloids, with 96% exhibiting high biological activity (Pa>0.7) according to PASS predictions. The dominant compound, 3,6-dimethylquinoline (12.38%), exhibited significant anticancer potential, while the frond extract showed strong antioxidant activity ($IC_{50}$: 91.25±7.60 ppm), reflecting its phenolic richness. These results highlight the potential of *D. esculentum*'s phytochemical wealth and pharmacological promise, supporting further exploration in pharmacognosy, nutraceuticals, and bioactive compounds.

**Keywords:** Antioxidant, biological activity, *Diplazium esculentum*, DNA barcoding, GC-MS

## INTRODUCTION

*Diplazium esculentum* (Retz.) Sw., commonly known as a wild edible fern, is highly valued for its tender young fronds, which are widely consumed as a traditional vegetable across various regions of Asia (Alamsjah et al. 2024; Kartikasari et al. 2024; Kongsung et al. 2024; Saha et al. 2024). As a key species within the genus *Diplazium*, which comprises 466 accepted species (POWO 2025), *D. esculentum* thrives in diverse habitats, such as riverbanks, highlands, volcanic craters, and forest floors (Tongco et al. 2014; Semwal et al. 2021; Watanabe et al. 2021; Hadi et al. 2022). The species is widely distributed across tropical Asia and Oceania (Takamiya et al. 1999) and is valued for its rich nutritional composition, including carbohydrates, proteins, and essential minerals (Tongco et al. 2014; Alamsjah et al. 2024; Kartikasari et al. 2024).

Beyond its nutritional benefits, *D. esculentum* also exhibits considerable medicinal potential. The fern is traditionally used to manage conditions such as hypertension, constipation, hemoptysis, and dysentery in different regions of Asia, including the Philippines, India, and Indonesia (Batoro and Siswanto 2017; Semwal et al. 2021). These ethnomedicinal uses are linked to its rich content of secondary metabolites, including alkaloids, flavonoids, phenolics, terpenoids, and tannins, which possess strong antioxidant and antimicrobial activities (Tongco et al. 2014; Junejo et al. 2018; Saha et al. 2024). In addition, pharmacological studies have shown that *D. esculentum* can inhibit acetylcholinesterase and butyrylcholinesterase enzymes, suggesting its therapeutic potential in managing neurodegenerative disorders such as Alzheimer's disease. These findings underscore the value of this fern not only as a functional food but also as a promising candidate for drug discovery and natural product development (Kunkeaw et al. 2021; Inthachat et al. 2024).

Accurate taxonomic identification of *D. esculentum* remains problematic due to its morphological resemblance to closely related congeners. Such phenotypic overlap frequently results in misidentification, particularly among regionally recognized variants like *mloko Jember* and *ayam Banyuwangi*, which differ subtly in crozier morphology and rhizome scale features (Kartikasari et al. 2024). These limitations underscore the need for integrative approaches incorporating molecular techniques to resolve taxonomic ambiguities. DNA barcoding has emerged as a powerful and indispensable tool for precise species authentication, particularly in ethnobotanical studies involving medicinal plants (Yu et al. 2021). Consortium for the Barcode of Life (CBOL) Plant Working Group recommended using the dual-locus chloroplast DNA barcoding system comprising *rbcL* (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) and *matK* (maturase K) as the standard approach

to achieve robust and reliable molecular identification of land plants. These markers are commonly used due to their universality, evolutionary conservation, and amplification efficiency (CBOL Plant Working Group 2009). In addition, the nuclear-encoded Internal Transcribed Spacer (ITS) region, particularly *ITS2*, is frequently employed as a supplementary marker due to its high sequence variability and superior discriminatory power at the species level, especially among closely related or morphologically cryptic taxa (Yao et al. 2010). Among these markers, *rbcL* has proven particularly effective for *D. esculentum*, given its high amplification success and consistent performance in distinguishing closely related taxa (Wang et al. 2016; Perwitasari et al. 2020; Su'udi et al. 2022; Rizqoni et al. 2024).

Phytochemical studies have further highlighted the bioactive potential of *D. esculentum*. Key secondary metabolites, such as phenolics, terpenoids, and alkaloids, are known to contribute to its antioxidant activity by effectively neutralizing free radicals and mitigating oxidative stress, which is closely linked to cellular damage and disease progression (Nurhasnawati et al. 2019; Choi et al. 2020; Praptiwi et al. 2021). These bioactive compounds have also been associated with a range of pharmacological effects, including anticancer and anti-inflammatory effects (Junejo et al. 2018; Kongsung et al. 2024). However, despite growing interest in its therapeutic potential, research on the phytochemical and medicinal properties of *D. esculentum,* specifically from East Java remains limited.. Therefore, this study aimed to conduct a comprehensive analysis of the morphological characteristics, molecular identity, phytochemical profile, and antioxidant activity of *D. esculentum* from Panti, Jember District, Indonesia, and to evaluate its medicinal potential using the Prediction of Activity Spectra for Substances (PASS) online tool.

## MATERIALS AND METHODS

### Study area

A total of 15 individual wild *D. esculentum* specimens were collected from one site of a ca. 500 $m^2$ area of a rubber-mixed coffee plantation in Suci Village, Panti Sub-district, Jember District, East Java, Indonesia (8°05'19"S 113°37'22"E) (Figure 1). The specimen collection was carried out together with local farmers who engaged in harvesting the species and introduced a local name of *pakis menir*. The specimens were identified, and three individuals were deposited in the Herbarium of the Botany Laboratory at the Universitas Jember, Jember, Indonesia, with the Voucher Codes: JR 0000001800, JR 0000001801, and JR 0000001803. Additionally, living specimens were cultivated in the Botany Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Jember.

### Procedures

*Morphological identification*

Three specimens of *D. esculentum* were observed and documented. Morphological description and identification were performed by examining vegetative and generative structures, including rhizomes, scales, stipes, frond architecture, sterile and fertile laminae, sorus, and spores (Sofiyanti et al. 2019). The identification process referred to several key reference works, including Cryptogams: Ferns and Fern Allies (de Winter and Amoroso 2003) and Biosystematic Study of the Fern Genus *Diplazium* in West Malesia (Praptosuwiryo 2008). Additionally, online resources, such as the Global Biodiversity Information Facility (GBIF) (https://www.gbif.org) and Pteridophyte Collections Consortium (PCC) (https://www.pteridoportal.org), were used for further verification.

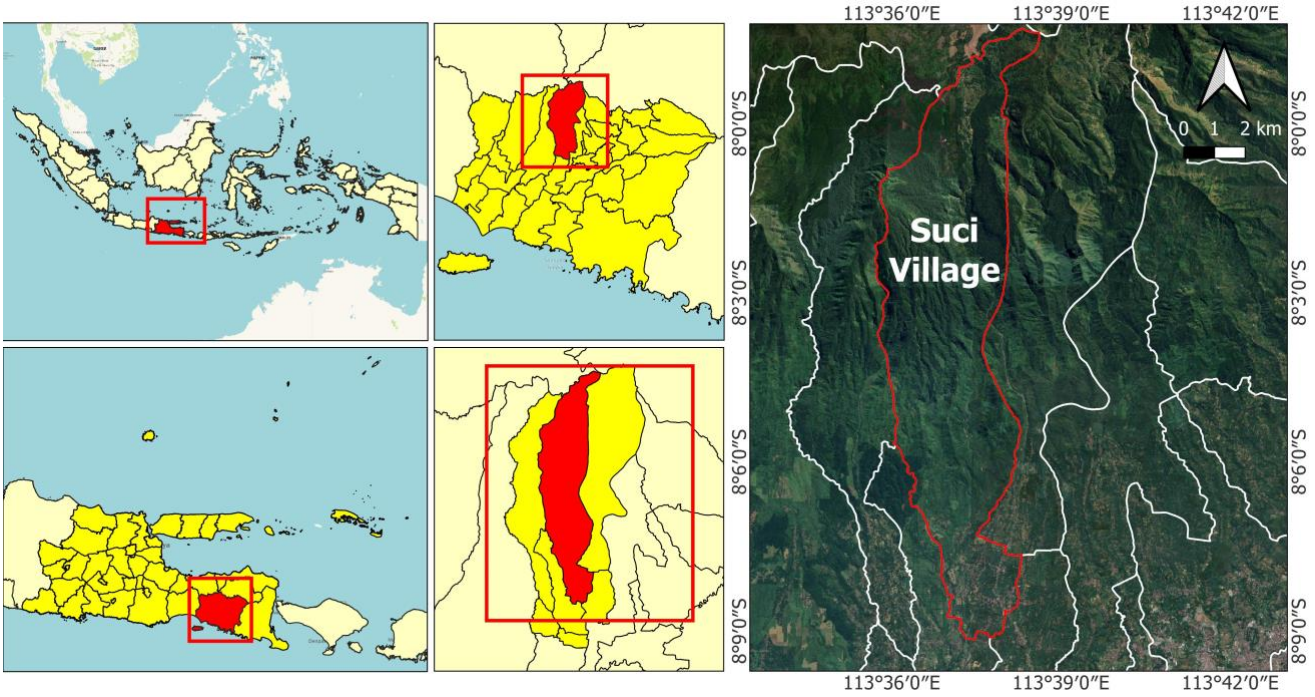


**Figure 1.** Research location at a rubber-mixed coffee plantation in Suci Village, Panti Sub-district, Jember District, East Java, Indonesia

*Molecular identification*

DNA isolation: The molecular identification based on DNA barcode was performed on one specimen of *D. esculentum* (JR 00000001801) for confirmation of the species identity, as determined through morphological analysis. DNA isolation was carried out using the standard CTAB method (Doyle 1991). Fresh leaves of 0.2 g were ground into a fine powder with liquid nitrogen using a mortar and pestle. Subsequently, 3 mL of CTAB buffer was added to the crushed sample. The mixture was homogenized, and 1 mL of the homogenate was transferred to a 1.5 mL microtube. The sample was vortexed and incubated in a thermo shaker at 65°C for 60 minutes. After incubation, 500 µL of chloroform was added, the sample was homogenized and then incubated at room temperature for 5 minutes. The mixture was centrifuged at 25°C at 10,000 rpm for 15 minutes. After centrifugation, 600 µL of the supernatant was collected and transferred to a new 1.5 mL microtube. Next, 5 µL of RNase was added to the supernatant, which was then incubated in a thermos shaker at 37°C for 30 minutes. After incubation, 600 µL of cold isopropanol was added. The sample was centrifuged at 4°C at 10,000 rpm for 11 minutes, repeated twice. Following centrifugation, the supernatant was discarded, and the pellet was washed with 500 µL of 70% ethanol. The mixture was then centrifuged again at 4°C at 10,000 rpm for 10 minutes. After the second centrifugation, the supernatant was discarded, and the pellet was air-dried in a desiccator for 20 minutes to remove any remaining ethanol. Finally, 44 µL of TE buffer was added to the dried pellet, and the sample was incubated in a thermos shaker at 55°C for 10 minutes to resuspend the DNA in the TE buffer fully.

DNA amplification and sequencing: The regions *rbcL* were amplified using universal primers: *rbcL*_F (5'-ATGTCACCACAAACAGAGACTAAAGC-3') and *rbcL*_R (5'-GTAAAATCAAGTCCACCGCG-3') (Bafeel et al. 2011). PCR (Polymerase Chain Reaction) analysis was conducted in a 20 µL reaction mixture containing 10 µL GreenMix, 6 µL nuclease-free water, 2 µL DNA template, and 1 µL each of forward and reverse primers. The PCR steps included pre-denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and a final extension at 72°C for 5 minutes. The next step involved confirming the PCR product using 1.25% agarose gel electrophoresis and visualization under a UV transilluminator. Next, to ensure the reliability of amplification results, duplicate DNA samples were loaded for each reaction. This was particularly important for the *matK* and *ITS2* markers, which exhibited poor amplification. The PCR products were then sent to the 1st BASE (Singapore) for purification and sequencing.

*Phytochemical identification*

Metabolite extraction: The crude extract of *D. esculentum* was prepared by drying young fronds collected from 10 individual plants in an oven at 40°C for two days. The dried samples were then pulverized using a commercial spice grinder. A total of 60 g of the leaf powder was macerated in methanol at a ratio of 1:5 for 72 hours, with the process repeated once. The liquid extract was filtered through the Whatman filter paper, and the filtrate was concentrated using a rotary evaporator, yielding *D. esculentum* crude extract (Setyati et al. 2023; Ulum et al. 2023). The crude extract was stored in a refrigerator for further analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) identification: GC-MS analysis of the crude extract of *D. esculentum* was conducted at the Biosciences Laboratory of the Politeknik Negeri Jember. The analysis was conducted using GC-MS, a hybrid analytical technique for compound identification. Gas chromatography separates volatile and semi-volatile compounds, while mass spectrometry provides detailed structural information about the detected compounds. GC-MS analysis was performed on a Shimadzu QP2010 Plus system (Japan). A sample of 1 µL was injected into the column under the following conditions: injector temperature set at 290°C, MS detector at 280°C, and the column used was an Rtx-50 (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness). The initial oven temperature was held at 80°C for 10 minutes, then increased to 230°C at a rate of 5°C/min and held for 10 minutes. Helium was used as the carrier gas, flowing at 3 mL/min. Metabolite identification was based on mass spectra comparison using the Wiley Library 9 database (Uraku 2015; Ulum et al. 2023; Setyati et al. 2024).

Metabolite potencies based on PASS online: The GC-MS-detected metabolites of *D. esculentum* were identified through a comprehensive review of scientific literature, with an emphasis on those demonstrating potential medicinal applications. Following identification, these metabolites underwent further analysis to predict their bioactivity (Abdul-Hammed et al. 2022; Amalini and Afidah 2023; Sururin and Khafiyya 2024). This involved comparing the Pa (probability of being active) and Pi (probability of being inactive) values of the metabolites.

Antioxidant activity measurement: The antioxidant activity of *D. esculentum* leaves was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, following the method of Baliyan et al. (2022). A total of 10 mg of *D. esculentum* crude extract was dissolved in 10 mL of methanol. Ascorbic acid (1 mg/mL) was used as the standard. Methanol was employed as the solvent to prepare a series of concentrations of 20, 40, 60, 80, and 100 ppm. DPPH solution (0.1 mM) was prepared by dissolving 2 mg of DPPH in 50 mL of methanol. The assay was performed using a 96-well plate, where 100 µL of each test sample was added to 100 µL of DPPH solution. The mixture was incubated for 30 minutes at room temperature in the dark. After incubation, absorbance was measured at 517 nm using a microplate reader. Methanol was used as the blank control. The percentage of scavenging activity was calculated. All tests were conducted in triplicate. The concentration of the samples required to achieve 50% inhibition of DPPH ($IC_{50}$ value) was calculated (Praptiwi et al. 2021; Manurung et al. 2022; Kurniawan et al. 2023).

**Data analysis**

Morphological characters were assessed based on organ shape, dimensions, coloration, and organ count. DNA sequencing data were processed using BioEdit and aligned through the Basic Local Alignment Search Tool (BLAST)

to confirm species identity. Verified sequences were submitted to GenBank. Phylogenetic analysis was conducted using MEGA XI software (Tamura et al. 2021), applying the Neighbor-Joining method with 1000 bootstrap replicates. Phytochemical structures were retrieved in Canonical Simplified Molecular Input Line Entry System (SMILES) format from the PubChem database (http://pubchem.ncbi.nlm.nih.gov/) and SpectraBase (https://spectrabase.com/). These SMILES strings were then analyzed using the Prediction of Activity Spectra for Substances (PASS) online platform to estimate the bioactivity profiles of each compound. Predicted activities were represented by Pa (probability of activity) and Pi (probability of inactivity) values, which served as the basis for bioactivity interpretation in subsequent analyses.

## RESULTS AND DISCUSSION

### Morphological characteristics

The specimens of *D. esculentum* were confirmed based on diagnostic morphological characters consistent with the taxonomic treatment by de Winter and Amoroso (2003). Field observations revealed that the species occurred in a wild state, with no evidence of cultivation, indicating its native occurrence in the region. The population was moderately abundant, primarily distributed along shaded streambanks in secondary forest fragments. The examined specimens were terrestrial in habit, with frond lengths ranging from 51 to 68 cm (Figure 2.A). The rhizome is erect with a diameter of ca. 40.06 cm. The stipe is brown to green, 14 cm long. Leaves were clustered at the apex of the rhizome. The petiole is 50-70 cm long, black, with brown scales at the base. Lamina are ovate-lanceolate, 54 cm long and 20.5 cm wide (Figure 2.B-C). The pinnae are green, 1-pinnate-bipinnate. The 1-pinnate type consists of 15-16 pairs, and the bipinnate type consists of 12-13 pairs. At the apex is a terminal pinna. The terminal pinna has an acute apex. The pinnules are linear-lanceolate and gradually smaller toward the base, having serrated edges, 11 cm long and 2.2 cm wide. The pinule consists of 11-12 pairs (Figure 2.C-D). The sori are elongated and occupy almost the whole length of the veins and are protected by a narrow scarious indusium (Figure 2.E). Scales on the stipe are dark brown latticed, 3.3 mm long and 0.7 mm wide, long, acuminate tip, and denticulate edge (Figure 2.F). Spores are monolate with a size of 30 µm x 20 µm (Figure 2.G).

The local harvester introduced *D. esculentum* with the local name of *pakis menir*. It was distinguished by its smaller croziers and lighter frond color. A study on edible ferns from regions near Jember District reported putative *D. esculentum* under three local names: *mloko Jember*, *air Jember*, and *ayam Banyuwangi* (Kartikasari et al. 2024). The distinct characteristics of *D. esculentum pakis menir* set it apart from *D. esculentum* (*air Jember*), which possessed larger croziers and exhibited dark green young fronds. In contrast, *D. esculentum* (*ayam Banyuwangi*) was notable for the absence of rolled croziers. The morphology of *D. esculentum* (*mloko Jember*) showed high similarity to our sample. Further investigation with local harvesters and market suppliers confirmed that both terminologies referred to *D. esculentum,* which displayed the bright frond color observed in our research specimens. A comparison of the morphological characteristics of specimens collected from Panti reveals the bright green young fronds to those of *D. esculentum* from Japan (Watanabe et al. 2021). The spore structure of our specimen closely resembled the typical characteristics of spores in the genus *Diplazium*, displaying the ellipsoid monolete form described by Mynssen and Sylvestre (2019). The morphological identification of *D. esculentum* is further validated by molecular analysis, providing robust support for species confirmation.

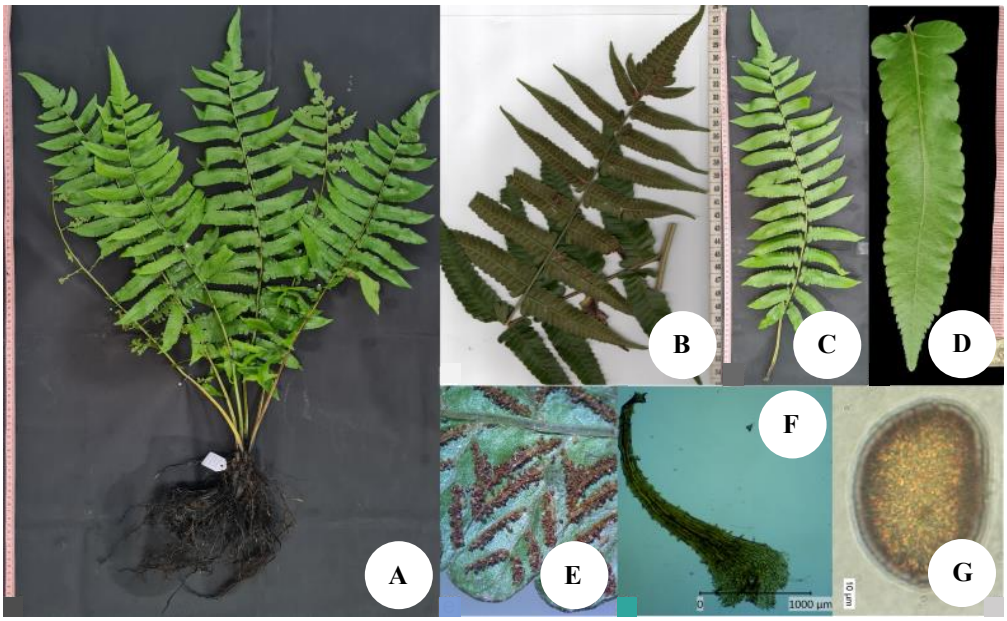


**Figure 2.** Morphology of *D. esculentum*: A. Habitus, B. Fertile fronds with sori, C. Adaxial fronds, D. Abaxial pinnules, E. Sori with inducium, F. Scale (40x magnification), G. Spore (400 x magnification)

**Molecular characteristics**

The PCR results revealed successful amplification only of the partial *rbcL* gene from the plastid DNA of *D. esculentum*, producing a band size of 514 bp. Of the three standard DNA barcoding markers (*rbcL*, *matK*, and *ITS2*) (CBOL Plant Working Group 2009), only rbcL was successfully amplified and sequenced in this study. Amplification attempts using *matK* and *ITS2* primers failed to produce viable PCR products, indicating their limited utility for this species under the current experimental conditions (Figure 3). This aligns with the findings that *rbcL* consistently exhibits the highest amplification success rate in ferns. For instance, studies by Wang et al. (2016) reported a 94.33% success rate in the genus *Adiantum* from China, and Trujillo-Argueta et al. (2021) observed a 96.77% success rate in tropical Mexican ferns. In contrast, *matK* demonstrated substantially lower amplification efficiency, with 0.00% success in Trujillo-Argueta et al. (2021) and only 33.33% in Wang et al. (2016), while *ITS2* amplification remained below 50% in both studies. The poor performance of *matK* in ferns is primarily attributed to structural rearrangements in the chloroplast genome, particularly the evolutionary loss of conserved *trnK* exons that in angiosperms flank *matK* and serve as primer binding sites (Nitta and Chambers 2022). The absence of these exons in ferns leads to primer mismatch and significantly limits the universality of standard *matK* primers across leptosporangiate fern lineages. Although lineage-specific primers have been developed to improve *matK* amplification in certain groups, their application remains narrow and not broadly transferable. Likewise, the use of *ITS2* in fern barcoding is hindered by the presence of multiple paralogous copies due to gene duplication, hybridization, and incomplete concerted evolution, often resulting in non-specific amplification and unreadable sequences (Wang et al. 2016). Thus, *rbcL* remains the most reliable marker for DNA barcoding in *D. esculentum* and other pteridophytes.

The BLAST analysis of the *rbcL* sequence from *D. esculentum* collected in Panti Sub-district confirmed a close genetic relationship with other accession *D. esculentum* sequences. We presented the BLAST sequence indicating the data of species name, accession number, percent identity, query cover, E-value, and sample origin (source) (Table 1). The highest sequence similarity (100% identity) was observed with accession OL536867.1 from the USA. Additionally, sequences from the Philippines (MZ501576.1) and Japan (AB042736.1, U05619.1) exhibited high homology, with percent identity values greater than 99%. The value of percent identity approaching 100% reflects the percentage similarity of the sequence between *D. esculentum* from Panti Sub-district and the four accessions of that *D. esculentum.* It should be regarded as the same species, as reported in the study of *Cosmos caudatus* (Purnobasuki et al. 2022). Moreover, the sequence similarity was also supported by the value of query cover and E-value. The query cover values among four accessions (>91%) indicated a high percentage of nucleotide sequence similarity between the sample *D. esculentum* Panti Sub-district and the sequences of four *D. esculentum* accessions in the GenBank database (Purnobasuki et al. 2022). An E-value of 0 indicated the similarity of the gene sequence of the sample and those from GenBank (Stover and Cavalcanti 2017). As no *rbcL* barcode data for *D. esculentum* from Indonesia is currently available (National Library of Medicine 2024), this study serves as a representative contribution to the DNA barcode research for this species using Indonesian samples.

The phylogenetic analysis using the Neighbor-Joining method based on *rbcL* sequences of *D. esculentum* samples from Panti and other *D. esculentum* accessions revealed the formation of a single clade. To further assess the robustness of the *rbcL* marker for phylogenetic studies of *Diplazium*, additional sequence data were incorporated, including more species from the genus (*Diplazium ceratolepis, Diplazium proliferum, Diplazium sandwichianum,* and *Diplazium dilatatum*) and an outgroup (*Athyrium microphyllum*) (Figure 4). The bootstrap value for the *D. esculentum* clade was 74, reflecting strong statistical support, as values above 70 are considered indicative of robust clade confidence (Russo and Selvatti 2018; Kapli et al. 2020). Additionally, the bootstrap analysis of six other *Diplazium* species and the outgroup demonstrated that the *rbcL* sequences corroborate the formation of distinct clades for each species.

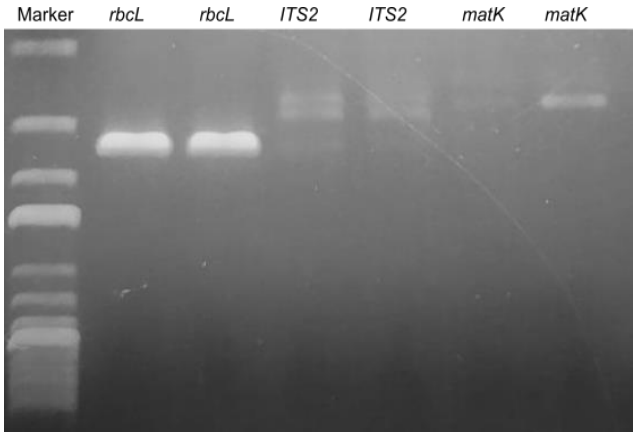


**Figure 3.** Amplification results of PCR products of *Diplazium esculentum* using primers *rbc*L, *mat*K, and *ITS*2

**Table 1.** BLAST result of *Diplazium esculentum* with *rbcL*

| Name | Accession number | Per. ident (%) | Query cover (%) | E-value | Source |
|---|---|---|---|---|---|
| *D. esculentum* | OL536867.1 | 100 | 96 | 0.0 | USA |
| *D. esculentum* | MZ501576.1 | 99.68 | 98 | 0.0 | Philippines |
| *D. esculentum* | AB042736.1 | 99.64 | 93 | 0.0 | Japan |
| *D. esculentum* | U05619.1 | 99.63 | 91 | 0 | Japan |

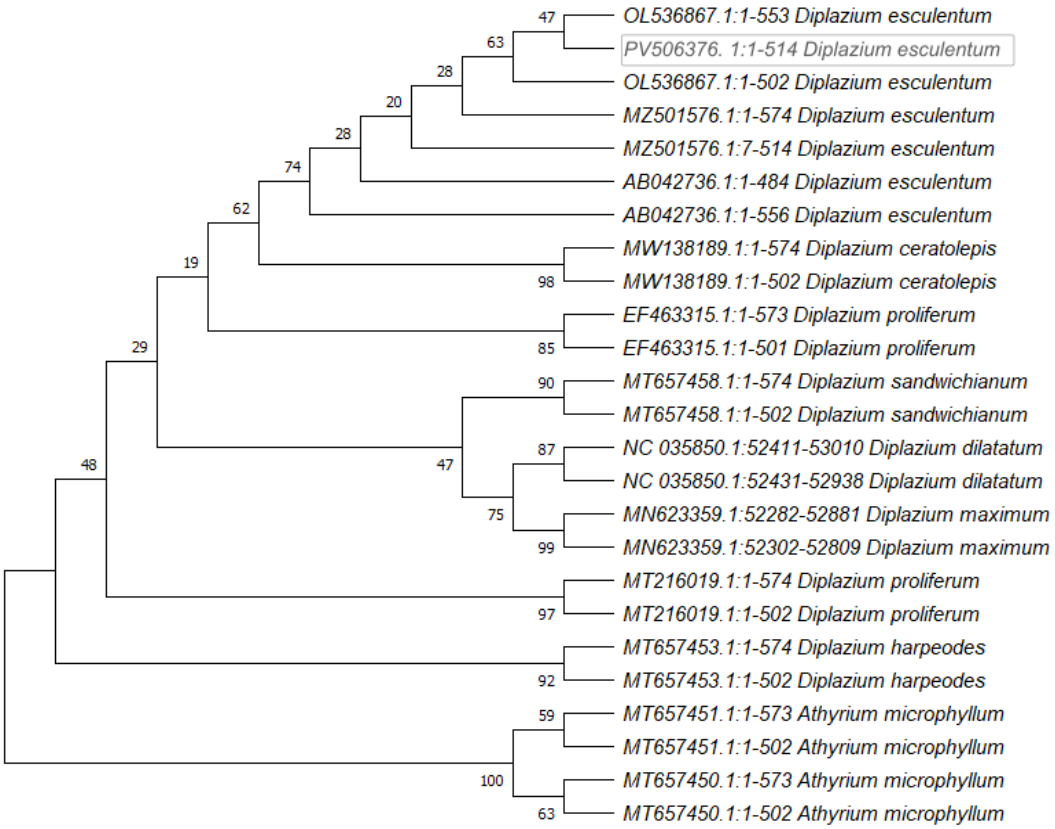


**Figure 4.** Phylogeny tree of *Diplazium esculentum* from *rbcL* sequences. The sample obtained in this study is highlighted in the box

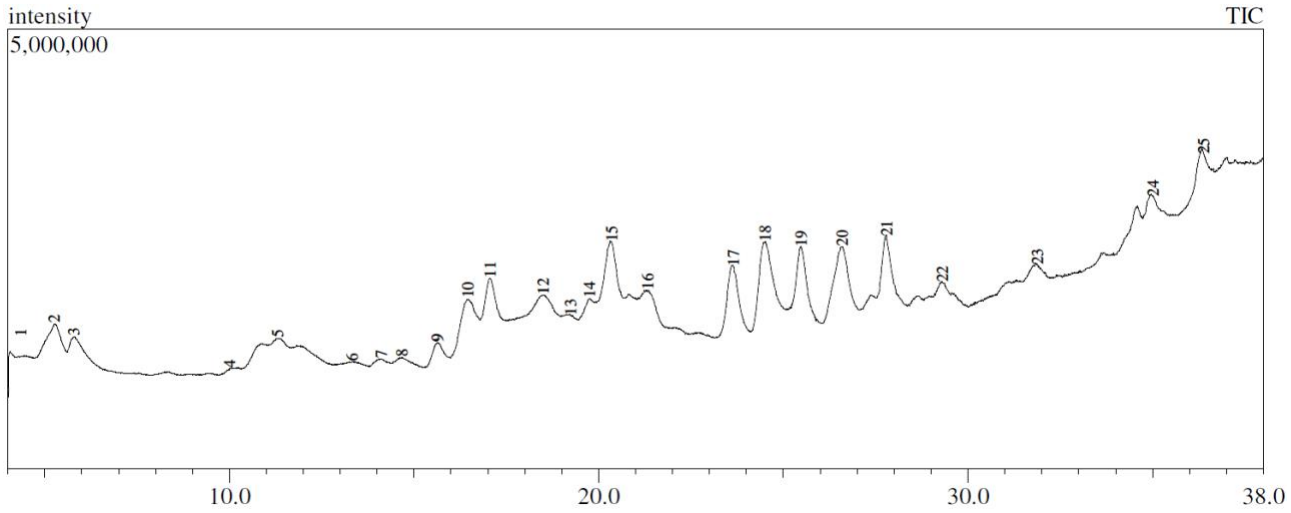


**Figure 5.** The GC-MS chromatogram illustrates the separation of phytochemical compounds from the methanolic leaf extract of *Diplazium esculentum*

## Phytochemical profile

*Gas Chromatography-Mass Spectrometry (GC-MS) identification*

The GC-MS analysis of the metabolites in the extract of *D. esculentum* yielded a chromatogram identifying a total of 25 compounds (Figure 5). These bioactive compounds were categorized according to their molecular weight (Table 2). The data outlined the names of the compounds, along with the percentage area they occupied, reflecting their relative concentrations in the sample. The predominant compound identified was 3,6-dimethylquinoline, accounting for the largest percentage area at 12.38%. This percentage area reflected the relative abundance of the compound in the sample. Consequently, the concentration of 3,6-dimethylquinoline in the young leaves exceeded 10%. The 25 compounds identified from the *D. esculentum* sample were grouped into three categories: phenols, terpenes, and alkaloids. The phenol group consists of 11 compounds (44%), terpenes 9 compounds (36%), and alkaloids 5 compounds (20%) (Table 2).

The plants synthesize phenols, terpenes, and alkaloids as key secondary metabolites, primarily as a defense mechanism against biotic and abiotic stress. These metabolites had been extracted by humans for medicinal purposes, owing to their potent biological activities. The study confirmed that these compounds had exhibited a range of biological properties, including antimicrobial, anticancer, antioxidant, and anti-inflammatory effects, all of which had been well-documented in previous studies, such as Sun and Shahrajabian (2023). The comparative analysis of the compounds from putative *D. esculentum* (*moko Jember*) (fresh, steamed, and dried) (Kartikasari et al. 2024) and Menir revealed several similarities in their chemical profiles. Notably, ethyl 1-hexyl-4-hydroxy-2(1h)-oxo-3-quinoline carboxylate was identified in both the fresh and dried *moko Jember* fern samples, as well as in the Menir sample, indicating its consistent presence across different processing stages. Additionally, acetic acid was found in all three threatened samples of *mLoko Jember* (fresh, steamed, and dried) and in *D. esculentum menir*, suggesting its stability and persistence regardless of treatment. Methyl-d3 1-Dideuterio-2-propenyl Ether, another shared compound, was present in both the dried *mLoko Jember* fern and *pakis menir* samples. At the same time, methoxy, phenyl-, oxime appeared in the dried putative *D. esculentum mLoko Jember* and *pakis menir*. These shared compounds point to a degree of chemical consistency between the two ferns, suggesting that despite differences in origin or processing, there are notable similarities in their metabolite profiles, which may be linked to comparable biological activities and therapeutic potentials.

*Metabolite potencies based on PASS online*

The biological activities of the identified compounds were evaluated using the structure-based bioactivity prediction tool, Prediction of Activity Spectra for Substances (PASS). The fresh leaf extract of *D. esculentum* in this study yielded over 96% of the metabolites (24 compounds). It demonstrated high biological activity (Pa > 0.7), while the remaining compound was not represented in the PASS database (Table 2). A Pa score exceeding 0.7 indicated strong biological activity (Zeb et al. 2021; Amalini and Afidah 2023).

*D. esculentum* contains high amounts of phenolic compounds. A study from the Philippines reported a total phenol content ranging from 11 to 125.6 mg per 100 g of the sample (Tongco et al. 2014). In this study, we present a total of 11 phenolic compounds identified through GC-MS analysis of *D. esculentum*. The leaf samples revealed a substantial presence of phenolic compounds, further highlighting the biological activities, yielding a total of 1,526 predicted activities. Ethyl benzoate exhibited the highest biological activity, with a total of 225, indicating its broad potential across various biological processes. Pyruvaldehyde and 1,2,3-Propanetriol monoacetate followed closely, displaying 176 and 172 activities, respectively, suggesting significant involvement in diverse bioactive pathways. 1,2-Cyclohexanedione and Benzeneacetaldehyde (Hyacinthin) demonstrated notable biological activities with totals of 162 and 123, respectively. Compounds such as 3,6-Dimethylquinoline and Tetradecamethylcyclo-heptasiloxane showed fewer predicted activities, with 49 and 76, but still contributed to the overall biological potential of the sample. As a member of the quinoline alkaloids group, 3,6-dimethylquinoline with the molecular formula $C_{11}H_{11}N$ has been recognized for its significance in medicinal research. Quinoline has gained considerable interest due to its diverse pharmacological activities, particularly for its potential as an anticancer agent, owing to its ability to target various cancer cell lines (Ilakiyalakshmi and Napoleon 2022). Matada et al. (2021) highlighted the diverse medicinal properties of quinoline, including its potent antibacterial, antifungal, antiviral, antimycobacterial, antiprotozoal, and antimalarial activities. Additionally, quinoline has demonstrated significant effects on cardiovascular health and the central nervous system, functioning as an antioxidant, anticonvulsant, analgesic, anti-inflammatory, and anthelmintic agent, further emphasizing its versatility in pharmaceutical applications.

The phenolic compound from *D. esculentum* primarily acted as an enzyme inhibitor across various metabolic pathways, suggesting potential therapeutic applications (Rahman et al. 2021). Antioxidant and anti-inflammatory properties were observed, with compounds inhibiting enzymes like 3-hydroxybenzoate 6-monooxygenase and superoxide dismutase, thus indicating capabilities for reducing oxidative stress and inflammation (Sun and Shahrajabian 2023). Additionally, the anticancer potential was highlighted through inhibitors targeting BRAF expression, enhancing TP53 expression, and modulating Myc activity, suggesting possible impacts on tumor suppression (Shan et al. 2024). The extract also demonstrated antimicrobial and antiviral properties, with inhibitors effective against enzymes such as arylsulfate sulfotransferase and choline dehydrogenase, as well as compounds active against picornavirus and rhinovirus (Bajaj and Sakhuja 2016). Neuroprotective and anxiolytic effects emerged through beta-adrenergic receptor kinase inhibitors and imidazoline receptor agonists, indicating potential benefits for neurotransmitter regulation and mood stability (Bousquet et al. 2020). Cardiovascular support and metabolic modulation were inferred from inhibitors of ADP-thymidine kinase and cholesterol antagonists, which may aid in cholesterol regulation and heart health (Michaeli et al. 2023). Additionally, the extract contained compounds with insecticidal and antiparasitic properties, suggesting utility in agricultural applications (Sridhar 2023). Overall, this analysis confirmed that the phenolic compounds in the plant extract exhibited a broad range of biological activities, positioning the extract as a promising candidate for pharmaceutical, nutraceutical, and agricultural uses.

**Table 2.** Phytochemical profile of *Diplazium esculentum* based on GC-MS and its medicinal potential based on the PASS website

| Peak | Retention time | % Area | Compound name | Group | Pa | Biological activity |
|---|---|---|---|---|---|---|
| 1 | 4.357 | 0.31 | Alpha-d4-hexamethylene oxide | Phenol | 0.924 | - |
| 2 | 5.269 | 5.26 | Propanal, 2-oxo- (CAS) Pyruvaldehyde | Phenol | 0.975 | Advanced glycation end products (AGEs) inhibitor |
| 3 | 5.792 | 313 | Ethanol, 2-(dimethylamino)- (CAS) N, N-Dimethylethanolamine | Alkaloids | 0.954 | Glycosylphosphatidylinositol phospholipase D inhibitor |
| 4 | 10.020 | 0.05 | 1,3,5,7-cyclooctatetraene (CAS) cyclooctatetraene | Terpene | 0.948 | Aspulvinone dimethylallyltransferase inhibitor |
| 5 | 11.313 | 2.02 | 1,5-heptadiene, 2-ethyl-6-methyl- | Terpene | 0.928 | Mucous membrane protector Aspulvinone |
| 6 | 13.329 | 0.38 | 2,4,6-tris(allyloxy)-s-triazine | Alkaloids | 0.888 | Aspulvinone dimethylallyltransferase inhibitor |
| 7 | 14.125 | 0.45 | Butanoyl chloride, 4-chloro- (CAS). Gamma. -Chlorobutyryl | Terpene | 0.864 | 2-Hydroxymuconate-semialdehyde hydrolase inhibitor |
| 8 | 14.676 | 0.63 | Pyrazine, trimethyl- (CAS) Trimethylpyrazine | Alkaloids | 0.843 | Taurine dehydrogenase inhibitor |
| 9 | 15.638 | 1.10 | 1,2-cyclohexanedione (CAS) 1,2-dioxocyclohexane | Phenol | 0.934 | Testosterone 17 beta-dehydrogenase (NADP+) inhibitor |
| 10 | 16.467 | 4.20 | 1-Hexadecanol, 2-methyl- (CAS) 2-methylhexadecanol | Terpene | 0.940 | Ubiquinol-cytochrome-c reductase inhibitor |
| 11 | 17.06 | 5.37 | Benzeneacetaldehyde (CAS) hyacinthin | Phenol | 0.937 | Breast cancer treatment |
| 12 | 18.505 | 8.46 | Benzoic acid, phenyl ester (CAS), Phenyl benzoate | Phenol | 0.919 | - |
| 13 | 19.233 | 1.93 | Phenol, 2-methoxy- (CAS) Guaiacol | Phenol | 0.965 | Bone loss disease inhibitor |
| 14 | 19.767 | 2.65 | Benzoic acid, ethyl ester (CAS), Ethyl benzoate | Phenol | 0.954 | Antibacterial and antifungal |
| 15 | 20.346 | 9.18 | Hexadecane, 1-chloro- (CAS) 1-Chlorohexadecane | Terpene | 0.943 | Polyporopepsin inhibitor |
| 16 | 21.339 | 4.79 | Tetradecamethylcycloheptasiloxane | Phenol | 0.998 | - |
| 17 | 23.644 | 3.20 | Trans-7-tetradecene | Terpene | 0.954 | Anti-eczema |
| 18 | 24.519 | 5.61 | 1,2,3-Propanetriol, monoacetate | Phenol | 0.943 | Cerebrovascular disease treatment |
| 19 | 25.495 | 3.74 | 1,2,3-Propanetriol, triacetate | Phenol | 0.953 | All-trans-retinyl-palmitate hydrolase inhibitor |
| 20 | 26.603 | 4.61 | Pyridinium, 1-hexadecyl-, chloride, monohydrate (CAS) Cetylpyridinium chloride monohydrate | Alkaloids | - | - |
| 21 | 27.799 | 4.77 | 1,1'-biphenyl (CAS) biphenyl | Terpene | 0.955 | Aspulvinone dimethylallyltransferase inhibitor |
| 22 | 29.319 | 2.08 | Hexadecane, 1-(ethenyloxy)- | Terpene | 0.964 | Alkanal monooxygenase inhibitor |
| 23 | 31.881 | 3.31 | Phosphonic acid, dioctadecyl ester (CAS) Di(n-octadecyl) phosphite | Phenol | - | - |
| 24 | 355.013 | 12.38 | 3,6-dimethylquinoline | Alkaloids | 0.875 | Anticancer, antimicobe, antioxidant, anticonvulsan |
| 25 | 36.385 | 10.4 | 2-Heptanone, 7-phenyl- (CAS) 7-Phenyl-2-heptanone | Terpene | 0.947 | Mucous membrane protector |

Note: A total of 2,897 biological activities were recorded in PASS online (supplementary data)

Propanal, 2-oxo- (CAS), also known as pyruvaldehyde or methylglyoxal, accounted for a percentage area of 5.26 and had a Pa-value of 0.975. This suggests its potential for tumor treatment through the inhibition of advanced glycation end products (AGEs) (Bellier et al. 2019). The compound phenol, 2-methoxy- (guaiacol), registered a percentage area of 1.93 with a Pa-value of 0.965, serving as an inhibited RANKL-induced osteoclastogenesis and prevented bone loss (Zhi et al. 2020) and potential medicine for Adult Polyglucosan Body Disease (APBD) (Kakhlon et al. 2018). Ethyl benzoate, identified as benzoic acid ethyl ester, showed a percentage area of 2.65 and a Pa-value of 0.954, acting as antibacterial and antifungal (Del Olmo et al. 2017). 1,2,3-propanetriol, monoacetate, or acetin was noted for its significant percentage area of 5.61 and a Pa-value of 0.943, marking it for the treatment of cerebrovascular disease (Durlak et al. 2019). Lastly, benzeneacetaldehyde exhibited a percentage area of 5.37 and a Pa-value of 0.937, which was reported for breast cancer treatment (Choi et al. 2020).

Terpenes include monoterpenes, sesquiterpenes, diterpenes, and isoprene. Isoprene is the basic structural unit of terpenoid hydrocarbons, which are widely found in plants (Fan et al. 2023). Terpenes in ferns exhibit significant medicinal potential, particularly for their antimicrobial, antioxidant, and anti-inflammatory activities (Lima et al. 2024). Among these, sesquiterpenes stand out for their pronounced therapeutic properties (Lima et al. 2024). In this study, *D. esculentum* was found to contain nine distinct terpenes. This aligns with the findings of Essien et al. (2019), who reported that terpenes constitute approximately 97.7% of the essential oil composition in *D. esculentum*, underscoring their dominant role in the plant's bioactive profile. The predicted biological activities of the terpenes in this study were evaluated, yielding a cumulative total of 1,142 activities. 1,3,5,7-Cyclooctatetraene demonstrated the

highest level of biological activity, with a total of 361, suggesting its potential significance across a wide range of bioactive processes. 1,1'-Biphenyl exhibited substantial biological activity with 194 predicted functions, further supporting its relevance in multiple biological roles. Similarly, trans-7-Tetradecene displayed notable bioactivity with 180 predicted activities. Moderate activity levels were observed for 1-Hexadecanol, 2-methyl-, and Hexadecane, 1-chloro-, with 111 and 101 activities, respectively, indicating their potential involvement in diverse biochemical pathways. In contrast, compounds such as 1,5-Heptadiene, 2-ethyl-6-methyl-, and Butanoyl chloride, 4-chloro- showed lower predicted biological activities, with 39 and 32, respectively.

The biological activities of the terpenes were thoroughly evaluated, revealing significant inhibitory effects on key enzymes, such as (R)-6-Hydroxynicotine oxidase, S-hydroxynicotine oxidase, Aldehyde dehydrogenase, and Alkanal monooxygenase. These enzymes are known to play crucial roles in metabolic pathways, including nicotine metabolism and detoxification processes (Yildiz and Yildiz 2021). Additionally, several compounds demonstrated inhibitory effects on enzymes such as Phospholipid-translocating ATPase, suggesting potential involvement in regulating lipid metabolism and membrane transport (Rosas-Rodríguez et al. 2017). The predicted bioactivities also suggested potential therapeutic applications of these terpenes as anesthetics, anti-inflammatory agents, and angiogenesis stimulants, underlining their relevance in the management of pain, inflammation, and wound healing. Moreover, several compounds were predicted to possess antineoplastic and antiviral activities, with specific efficacy against viruses such as rhinovirus, indicating their potential utility in cancer and antiviral treatment strategies. Furthermore, a subset of compounds was identified with potential therapeutic roles in addressing dermatological conditions (e.g., antiseborrheic), mucositis, and dyskinesia, further demonstrating a wide spectrum of medicinal applications (Mustafa et al. 2023). These findings emphasize the diverse biological activities of the terpenes, offering promising avenues for future research in drug development and therapeutic applications.

The PASS analysis of the alkaloid compounds of *D. esculentum* used in the study revealed a diverse range of biological activities. Among the compounds, ethanol, 2-(dimethylamino)- (CAS) N,N-Dimethylethanolamine exhibited the highest number of biological activities, with a total of 148. Pyrazine, trimethyl- (CAS) Trimethylpyrazine, followed with 57 biological activities, while 2,4,6-tris(allyloxy)-s-triazine demonstrated 23 activities. The compound Pyridinium, 1-hexadecyl-, chloride, monohydrate (CAS), Cetylpyridinium chloride monohydrate, was associated with just 1 biological activity. In total, these alkaloid compounds accounted for 229 distinct biological activities, indicating a broad spectrum of potential pharmacological applications.

Alkaloids are organic chemical compounds with cyclic ring structures containing one or more basic nitrogen atoms. Alkaloids can be found as secondary metabolites in plants and animals (Heinrich et al. 2021). They exhibit activities such as antihypertensive, analgesic, Alzheimer's, and malaria (Amirkia and Heinrich 2014), as well as antiviral, anticancer, and antioxidant properties (Dey et al. 2020). The leaf extract of *D. esculentum* used in the study revealed a complex array of alkaloid compounds, each associated with various biological activities. The identified compounds demonstrated inhibitory effects on numerous enzymatic pathways, with notable examples being (S)-6-hydroxynicotine oxidase and 2-hydroxy-3-oxoadipate synthase inhibitors, which suggested the potential to modulate oxidative stress-related processes (Boiangiu et al. 2023). Inhibition of metabolic enzymes such as aldose reductase, creatinine kinase, and beta-adrenergic receptor kinase pointed to possible applications in managing metabolic disorders like diabetes and cardiovascular conditions (Bellier et al. 2019). Additionally, the presence of betaine-aldehyde dehydrogenase and carnitine amidase inhibitors indicated potential roles in lipid metabolism and energy homeostasis (Rosas-Rodríguez et al. 2017). Several compounds exhibited anesthetic, antieczematic, antihypoxic, and antineurotic activities, suggesting the extract's broad therapeutic potential in pain management, dermatological conditions, respiratory issues, and neurological disorders. Furthermore, the identification of choline kinase and choline-phosphate cytidylyltransferase inhibitors highlighted the extract's potential to affect cellular signaling and membrane integrity, possibly impacting drug resistance mechanisms in cancer therapy (Inthachat et al. 2024). Alkaloids related to nervous system function, such as neurotransmitter uptake inhibitors and nicotinic receptor antagonists, suggested a role in neurodegenerative disease treatment and mood regulation (Wei et al. 2022). Antioxidant properties were also observed, with NADPH peroxidase inhibitors and oxygen scavenger compounds contributing to the protection against oxidative damage (Reis et al. 2020). Finally, the presence of vasoprotective and vasodilatory compounds, such as vasoprotective and testosterone 17 beta-dehydrogenase inhibitors, indicated potential applications in maintaining vascular health and managing hormonal imbalances (Poirier 2024). Overall, the alkaloid compounds identified in the plant extract demonstrated a wide range of biological activities, emphasizing their potential in pharmaceutical and therapeutic developments.

*Antioxidant activity*

The antioxidant activity test of *D. esculentum* methanol leaf extract yielded an average $IC_{50}$ value of 91.25±7.60 ppm, while Vitamin C, used as a control, exhibited an $IC_{50}$ value of 2.58±1.24 ppm. A lower $IC_{50}$ value indicates stronger antioxidant activity, and the concentration of 91.25 ppm falls within the strong antioxidant category. This result demonstrates that *D. esculentum* leaf extract at 91.25 ppm effectively neutralized 50% of DPPH free radicals. According to Molyneux (2004), an $IC_{50}$ value of between 50 and 100 ppm falls under the strong category, further highlighting the potent antioxidant activity of this extract. However, Junejo et al. (2018) reported that *D. esculentum* from India exhibited slightly lower antioxidant activity compared to ascorbic acid, as measured by the DPPH method. The $IC_{50}$ value for *D. esculentum* was 138.8

µg/mL, while ascorbic acid had an $IC_{50}$ of 125.2 µg/mL. Additionally, a comprehensive literature review indicated that the antioxidant activity of *D. esculentum* leaves varied from weak to strong, depending on the origin of the sample (Semwal et al. 2021). The high antioxidant activity is closely related to the presence of secondary metabolites, particularly phenolic compounds. Phenolic compounds are one of the primary contributors to the radical scavenging properties of plant extracts (Junejo et al. 2018; Nurhasnawati et al. 2019). Our findings, which identified 11 phenolic compounds, are consistent with several studies that demonstrate a strong positive correlation between high phenolic content and the antioxidant activity of plants (Tongco et al. 2014; Nurhasnawati et al. 2019; Praptiwi et al. 2021; Manurung et al. 2022). These phenolics are capable of donating hydrogen atoms to the DPPH solution, forming a stable reduced DPPH (DPPH-H), thereby confirming the extract's potential as a natural antioxidant source (Nurhasnawati et al. 2019).

The comparative significance of our research lies in its integrated approach, which combines morphological analysis, molecular validation via DNA barcoding, and a comprehensive phytochemical assessment supported by PASS online analysis. Unlike studies conducted in other regions that might focus on isolated aspects, such as morphology or molecular profiling alone (Junejo et al. 2018; Alamsjah et al. 2024), our study provides a holistic understanding of *D. esculentum* from the Jember region. This combined methodology not only validates the taxonomic identity of the species with precision but also reveals its rich phytochemical profile and associated bioactive potentials. The PASS online data offers extensive insight into possible pharmacological applications, significantly expanding the scope for its utilization in developing nutraceuticals and therapeutic agents. The combined morphological and molecular identification, utilizing the *rbcL* marker, confirmed that the edible fern sample *pakis menir* from Panti Sub-district, Jember District, was indeed *D. esculentum*, showing a 100% sequence identity with *D. esculentum* (OL536867.1) from the USA. GC-MS analysis of young fronds identified 25 bioactive metabolites, including 11 phenolics, 9 terpenoids, and 5 alkaloids, all contributing to a wide range of biological activities. The comprehensive PASS online analysis revealed a comprehensive spectrum of 2,897 predicted biological activities across the major classes of metabolites. Phenolic compounds accounted for the largest share, with 1,526 predicted activities, followed by 1,142 activities attributed to terpenoids and 229 to alkaloids. Key bioactive compounds included ethyl benzoate with 225 activities, pyruvaldehyde with 176, and 1,3,5,7-cyclooctatetraene with 361 activities, showcasing diverse biochemical potential. The alkaloid fraction further contributed 229 activities, led by N,N-Dimethylethanolamine (148 activities), supporting the pharmacological relevance of these compounds. The antioxidant assay classified the frond extract's activity as strong, with an $IC_{50}$ of 91.25±7.60 ppm, correlating well with the high bioactivity of its phenolic compounds. These findings collectively highlight the distinct morphological and molecular characteristics of *D. esculentum* from the Jember region, alongside its rich biochemical profile. This underscores the species' significant potential in pharmacology, nutraceuticals, and bioactive compound discovery, paving the way for further research into its therapeutic applications.

In conclusion, this study provides a comprehensive investigation into the morphological, molecular, and phytochemical characteristics of *D. esculentum*, solidifying its classification and emphasizing its pharmacological potential. The morphological analysis confirmed the species' diagnostic traits, while molecular analysis using the *rbcL* marker confirmed its taxonomic identity with 100% sequence similarity to reference data. Phytochemical profiling revealed 25 bioactive metabolites, including phenolics, terpenoids, and alkaloids, with the majority exhibiting high biological activity. The dominant compound, 3,6-dimethylquinoline, demonstrated significant anticancer potential. The frond extract, on the other hand, exhibited robust antioxidant activity, which could potentially lead to the development of new health supplements, further underscoring the species' medicinal and nutraceutical value.

## ACKNOWLEDGEMENTS

This research was funded by *Hibah Pascasarjana* No. 3391/UN25.3.1/LT/2024, year 2024, to Dr.rer.nat. Fuad Bahrul Ulum, from Symplast research group at Botany Laboratory, Biology Department, Faculty of Mathematics and Sciences, Universitas Jember, Jember, East Java, Indonesia.

## REFERENCES

Abdul-Hammed M, Adedotun IO, Olajide M, Irabor CO, Afolabi TI, Gbadebo IO, Rhyman L, Ramasami P. 2022. Virtual screening, ADMET profiling, PASS prediction, and bioactivity studies of potential inhibitory roles of alkaloids, phytosterols, and flavonoids against COVID-19 main protease ($M^{pro}$). Nat Prod Res 36 (12): 3110-3116. DOI: 10.1080/14786419.2021.1935933.

Alamsjah F, Fandini S, Mildawati M. 2024. Evaluation of antibacterial activities and phytochemical composition of ethanolic extract of *Diplazium esculentum*. Biodiversitas 25 (3): 937-941. DOI: 10.13057/biodiv/d250304.

Amalini YR, Afidah Y. 2023. In-silico PASS prediction for medicinal potency of antioxidant from *Ranunculus japonicus* Thunb. Life Sci Biotechnol 1 (2): 59-65. DOI: 10.19184/LSB.V1I2.45078.

Amirkia V, Heinrich M. 2014. Alkaloids as drug leads – A predictive structural and biodiversity-based analysis. Phytochem Lett 10: xlviii-liii. DOI: 10.1016/j.phytol.2014.06.015.

Bafeel SO, Arif IA, Bakir M, Khan H, Al Farhan AH, Al Homaidan AA, Ahamed A, Thomas J. 2011. Comparative evaluation of PCR success with universal primers of maturase K (*mat*K) and ribulose-1, 5-bisphosphate carboxylase oxygenase large subunit (*rbc*L) for barcoding of some arid plants. Plant Omics J 4 (4): 195-198.

Bajaj K, Sakhuja R. 2016. Benzotriazole: Much More Than Just Synthetic Heterocyclic Chemistry. In: Monbaliu JM (eds). The Chemistry of Benzotriazole Derivatives. Topics in Heterocyclic Chemistry. Springer, Cham. DOI: 10.1007/7081_2015_198.

Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang CM. 2022. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. Molecules 27: 1326. DOI: 10.3390/molecules27041326.

Batoro J, Siswanto D. 2017. Ethnomedicinal survey of plants used by local society in Poncokusumo District, Malang, East Java Province, Indonesia. Asian J Med Biol Res 3 (2): 158-167. DOI: 10.3329/ajmbr.v3i2.33563.

Bellier J, Nokin MJ, Lardé E, Karoyan P, Peulen O, Castronovo V, Bellahcène A. 2019. Methylglyoxal, a potent inducer of AGEs, connects between diabetes and cancer. Diabetes Res Clin Pract 148: 200-211. DOI: 10.1016/J.DIABRES.2019.01.002.

Boiangiu RS, Brinza I, Honceriu I, Mihasan M, Hritcu L. 2023. Insights into pharmacological activities of nicotine and 6-hydroxy-l-nicotine, a bacterial nicotine derivative: A systematic review. Biomolecules 14 (1): 23. DOI: 10.3390/biom14010023.

Bousquet P, Hudson A, García-Sevilla JA, Li JX, France CP. 2020. Imidazoline receptor system: The past, the present, and the future. Pharmacol Rev 72 (1): 50-79. DOI: 10.1124/pr.118.016311.

CBOL Plant Working Group. 2009. A DNA barcode for land plants. Proc Natl Acad Sci USA 106 (31): 12794-12797. DOI: 10.1073/pnas.0905845106.

Choi HS, Kim SL, Kim JH, Ko YC, Lee DS. 2020. Plant volatile, phenylacetaldehyde targets breast cancer stem cell by induction of ROS and regulation of stat3 signal. Antioxidants (Basel) 9 (11): 1119. DOI: 10.3390/ANTIOX9111119.

de Winter W, Amoroso V. 2003. Cryptogams: Ferns and Fern Allies. Backhuys, Leiden.

Del Olmo A, Calzada J, Nuñez M. 2017. Benzoic acid and its derivatives as naturally occurring compounds in foods and as additives: Uses, exposure, and controversy. Crit Rev Food Sci Nutr 57 (14): 3084-3103. DOI: 10.1080/10408398.2015.1087964.

Dey P, Kundu A, Kumar A, Gupta M, Lee BM, Bhakta T, Dash S, Kim HS. 2020. Analysis of Alkaloids (Indole Alkaloids, Isoquinoline Alkaloids, Tropane Alkaloids). In: Nabavi SM, Saeedi M, Nabavi SF, Silva AT (eds). Recent Advances in Natural Products Analysis. Elsevier, Amsterdam.

Doyle J. 1991. DNA Protocols for Plants. In: Hewitt G, Johnson AWB, Young JPW (eds). Molecular Techniques in Taxonomy. NATO ASI Series H Cell Biol 57: 283-293. DOI: 10.1007/978-3-642-83962-7_18.

Durlak P, Jerzykiewicz M, Ćwieląg-Piasecka I. 2019. 1,2,3-propanetriol radicals formed during oxidative stress. Magn Reson Chem 57 (4): S95-S100. DOI: 10.1002/mrc.4822.

Essien EE, Ascrizzi R, Flamini G. 2019. Characterization of volatile compounds of *Diplazium esculentum*. Chem Nat Compd 55: 958-959. DOI: 10.1007/s10600-019-02860-y.

Fan M, Yuan S, Li L, Zheng J, Zhao D, Wang C, Wang H, Liu X, Liu J. 2023. Application of terpenoid compounds in food and pharmaceutical products. Fermentation 9 (2): P119. DOI: 10.3390/fermentation9020119.

Hadi AA, Ng JY, Shamsuddin M, Matmin J, Malek NANN. 2022. Green synthesis of silver nanoparticles using *Diplazium esculentum* extract: Catalytic reduction of methylene blue and antibacterial activities. Chem Papers 76: 65-77. DOI: 10.1007/s11696-021-01835-0.

Heinrich M, Mah J, Amirkia V. 2021. Alkaloids used as medicines: Structural phytochemistry meets biodiversity-An update and forward look. Molecules 26 (7): 1836. DOI: 10.3390/molecules26071836.

Ilakiyalakshmi M, Napoleon AA. 2022. Review on recent development of quinoline for anticancer activities. Arab J Chem 15 (11): 104168. DOI: 10.1016/j.arabjc.2022.104168.

Inthachat W, Chantong B, Pitchakarn P, Takoon C, Karinchai J, Suttisansanee U, Temviriyanukul P. 2024. Enhancing therapeutic efficacy of donepezil, an alzheimer's disease drug, by *Diplazium esculentum* (Retz.) Sw. and its phytochemicals. Pharmaceuticals 17 (3): 341. DOI: 10.3390/ph17030341/s1.

Junejo JA, Gogoi G, Islam J, Rudrapal M, Mondal P, Hazarika H, Zaman K. 2018. Exploration of antioxidant, antidiabetic and hepatoprotective activity of *Diplazium esculentum* - A wild edible plant from North Eastern India. Futur J Pharm Sci 4 (1): 93-101. DOI: 10.1016/j.fjps.2017.10.005.

Kakhlon O, Ferreira I, Solmesky LJ, Khazanov N, Lossos A, Alvarez R, Yetil D, Pampou S, Weil M, Senderowitz H, Escriba P, Yue WW, Akman HO. 2018. Guaiacol as a drug candidate for treating adult polyglucosan body disease. JCI Insight 3: e99694. DOI: 10.1172/jci.insight.99694.

Kapli P, Yang Z, Telford MJ. 2020. Phylogenetic tree building in the genomic age. Nat Rev Genet 21: 428-444. DOI: 10.1038/s41576-020-0233-0.

Kartikasari SN, Witono Y, Handoyo T, Santoso U. 2024. Hydrolysis with two enzymes in edible fern (*Diplazium esculentum*) as a source of flavours. Indonesian Food Sci Technol J 7 (2): 148-159. DOI: 10.22437/ifstj.v7I2.30960.

Kongsung S, Inthachat W, Chantong B, Suttisansanee U, On-Nom N, Chupeerach C, Thangsiri S, Pitchakarn P, Temviriyanukul P. 2024. Box–behnken design-based optimization of phytochemical extraction from *Diplazium esculentum* (Retz.) Sw. associated with its antioxidant and anti-alzheimer's properties. Molecules 29 (10): 2204. DOI: 10.3390/molecules29102204.

Kunkeaw T, Suttisansanee U, Trachootham D, Karinchai J, Chantong B, Potikanond S, Inthachat W, Pitchakarn P, Temviriyanukul P. 2021. *Diplazium esculentum* (Retz.) Sw. reduces BACE-1 activities and amyloid peptides accumulation in *Drosophila* models of Alzheimer's disease. Sci Rep 11: 23796. DOI: 10.1038/s41598-021-03142-w.

Kurniawan L, Laili AN, Anggraeni DS, Qurrotu'ain S, Wulandari DR, Ulum FB. 2023. Poiploidy induction of Indonesian black rice *Oryza sativa* L. var. Cempo Ireng with bio-catharantine. Life Sci Biotechnol 1 (2): 41-47. DOI: 10.19184/lsb.v1i2.43753.

Lima GP, Santos MG, de Paiva SR. 2024. Terpenoid composition of fern and lycophyte essential oils: A chemosystematic approach. NZ J Bot 1-24. DOI: 10.1080/0028825X.2024.2323505.

Manurung H, Susanto D, Kusumawati E, Aryani R, Nugroho RA, Kusuma R, Rahmawati Z, Sari RD. 2022. Phytochemical, GC-MS analysis and antioxidant activities of leaf methanolic extract of lai (*Durio kutejensis*), the endemic plant of Kalimantan, Indonesia. Biodiversitas 23 (11): 5566-5573. DOI: 10.13057/biodiv/d231104.

Matada BS, Pattanashettar R, Yernale NG. 2021. A comprehensive review on the biological interest of quinoline and its derivatives. Bioorg Med Chem 32: 115973. DOI: 10.1016/j.bmc.2020.115973.

Michaeli DT, Michaeli JC, Albers S, Boch T, Michaeli T. 2023. Established and emerging lipid-lowering drugs for primary and secondary cardiovascular prevention. Am J Cardiovasc Drugs 23 (5): 477-495. DOI: 10.1007/s40256-023-00594-5.

Molyneux P. 2004. The use of the stable free radical Diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 26 (2): 211-219.

Mustafa A, El-Kashef DH, Abdelwahab MF, Gomaa AAR, Mustafa M, Abdel-Wahab NM, Ibrahim AH. 2023. Investigation of Antiviral Effects of Essential Oils. In: Inamuddin (ed) Essential Oils: Extraction Methods and Applications. John Wiley & Sons, Inc., New Jersey. DOI: 10.1002/9781119829614.ch5.

Mynssen CM, Sylvestre LdS. 2019. Synopsis of *Diplazium* (Athyriaceae) from Brazil. American Fern J 109 (4): 283-342. DOI: 10.1640/0002-8444-109.4.283.

National Library of Medicine. 2024. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information www.ncbi.nlm.nih.gov/nuccore/?term=diplazium+esculentum.

Nitta JH, Chambers SM. 2022. Identifying cryptic fern gametophytes using DNA barcoding: A review. Appl Plant Sci 10 (2): e11465. DOI: 10.1002/aps3.11465.

Nurhasnawati H, Sundu R, Sapri, Supriningrum R, Kuspradini H, Arung ET. 2019. Antioxidant activity, total phenolic and flavonoid content of several indigenous species of ferns in East Kalimantan, Indonesia. Biodiversitas 20 (2): 576-580. DOI: 10.13057/biodiv/d200238.

Perwitasari DAG, Rohimah S, Ratnasari T, Sugiharto B, Su'udi M. 2020. DNA barcoding of medicinal orchid *Dendrobium discolor* Lindl. Tanimbar using *rbcL* and ITS genes. Buletin Penelitian Tanaman Rempah dan Obat 31 (1): 8-20. DOI: 10.21082/bullittro.v31n1.2020.8-20. [Indonesian]

Poirier D. 2024. Recent advances in the development of 17beta-hydroxysteroid dehydrogenase inhibitors. Steroids 213: 109529. DOI: 10.1016/j.steroids.2024.109529.

POWO [Plants of the World Online Kew Science]. 2025. *Diplazium* Sw. Plants of the World Online Kew Science. Retrieved April 15, 2025, from https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:328225-2.

Praptiwi, Sulistiarini D, Qodrie ENP, Sahroni D. 2021. Antibacterial activity, antioxidant potential, total phenolic and flavonoids of three plant species of Rubiaceae from Banggai Island, Indonesia. Biodiversitas 22 (5): 2773-2778. DOI: 10.13057/biodiv/d220540.

Praptosuwiryo TN. 2008. Biosystematic Study of The Fern Genus *Diplazium* in West Malesia. [Dissertation]. IPB University, Bogor. [Indonesian]

Purnobasuki H, Rakhashiwi GA, Junairiah, Wahyuni DK, Putra RE, Raffiudin R, Soessilohadi RH. 2022. Morpho-anatomical characterization and DNA barcode of *Cosmos caudatus* Kunth. Biodiversitas 23 (8): 4097-4108. DOI: 10.13057/biodiv/d230830.

Rahman MM, Rahaman MS, Islam MR, Rahman F, Mithi FM, Alqahtani T, Almikhlafi MA, Alghamdi SQ, Alruwaili AS, Hossain MS, Ahmed M, Das R, Emran TB, Uddin MS. 2021. Role of phenolic compounds

in human disease: Current knowledge and future prospects. Molecules 27 (1): 233. DOI: 10.3390/molecules27010233.

Reis J, Massari M, Marchese S, Ceccon M, Aalbers FS, Corana F, Valente S, Mai A, Magnani F, Mattevi A. 2020. A closer look into NADPH oxidase inhibitors: Validation and insight into their mechanism of action. Redox Biol 32: 101466. DOI: 10.1016/j.redox.2020.101466.

Rizqoni MIA, Setyati D, Su'udi M, Dwinianti EF, Ulum FB. 2024. Molecular identification of *Cyclosorus parasiticus* (L.) Farw. from Gumitir, Jember Indonesia. Bioedukasi 22 (2): 218-223. DOI: 10.19184/bioedu.v22i2.45484.

Rosas-Rodríguez JA, Soñanez-Organis JG, Godoy-Lugo JA, Espinoza-Salazar JA, López-Jacobo CJ, Stephens-Camacho NA, González-Ochoa G. 2017. Betaine aldehyde dehydrogenase expression during physiological cardiac hypertrophy induced by pregnancy. Biochem Biophys Res Commun 490: 623-628. DOI: 10.1016/j.bbrc.2017.06.087.

Russo CAM, Selvatti AP. 2018. Bootstrap and rogue identification tests for phylogenetic analyses. Mol Biol Evol 35 (9): 2327-2333. DOI: 10.1093/molbev/msy118.

Saha J, Kumari T, Nickhil C, Deka SC. 2024. Studies on the cytotoxicity of leaf protein concentrate from *Diplazium esculentum*, pasta preparation and assessment of its quality during storage. Food Chem Adv 4: 100667. DOI: 10.1016/j.focha.2024.100667.

Semwal P, Painuli S, Painuli KM, Antika G, Tumer TB, Thapliyal A, Setzer WN, Martorell M, Alshehri MM, Taheri Y, Daştan SD, Ayatollahi SA, Petkoska AT, Sharifi-Rad J, Cho WC. 2021. *Diplazium esculentum* (Retz.) Sw.: Ethnomedicinal, phytochemical, and pharmacological overview of the Himalayan Ferns. Oxid Med Cell Longev 2021: 1917890. DOI: 10.1155/2021/1917890.

Setyati D, Adawiyah RA, Ratnasari T, Su'udi M, Ulum FB. 2023. Phenolic profile and antimicrobe of the *Asplenium Nidus* L. from Mount Gumitir, Jember, East Java, Indonesia. Bioedukasi 21 (3): 189-193. DOI: 10.19184/bioedu.v21i3.43368.

Setyati D, Su'udi M, Ravitamala ES, Miladina FF, Babudin B, Utarti E, Arimurti S, Nugraha AS, Putri YA, Farhan AM, Ulum FB. 2024. Antimicrobial and phytochemistry study of *Dendrobium linearifolium* Teijsm. & Binn. from Gumitir, Jember, Indonesia. BIO Web Conf 01001: 12. DOI: 10.1051/bioconf/202410101001.

Shan KS, Rehman TU, Ivanov S, Domingo G, Raez LE. 2024. Molecular targeting of the BRAF proto-oncogene/mitogen-activated protein kinase (MAPK) pathway across cancers. Intl J Mol Sci 25 (1): 624. DOI: 10.3390/ijms25010624.

Sofiyanti N, Iriani D, Fitmawati F, Marpaung AA. 2019. Morphology, palynology, and stipe anatomy of four common ferns from Pekanbaru, Riau Province, Indonesia. Biodiversitas 20 (1): 327-336. DOI: 10.13057/biodiv/d200138.

Sridhar KR. 2023. On the Bioactive Potential of Ferns: An Overview. In: Murthy HN (eds). Bioactive Compounds in Bryophytes and Pteridophytes. Reference Series in Phytochemistry. Springer, Cham. DOI: 10.1007/978-3-031-23243-5_11.

Stover NA, Cavalcanti ARO. 2017. Using NCBI BLAST. Curr Protoc Essent Lab Tech 14 (1): 1-11. DOI: 10.1002/cpet.8.

Su'udi M, Budyartini DW, Ramadany Z. 2022. DNA Barcoding of *Dendrobium linearifolium* Teijsm. & Binn. Orchid based on ITS2 molecular marker. Al-Kauniyah 15 (1): 53-61. DOI: 10.15408/kauniyah.v15I1.16710. [Indonesian]

Sun W, Shahrajabian MH. 2023. Therapeutic potential of phenolic compounds in medicinal plants-Natural health products for human health. Molecules 28 (4): 1845. DOI: 10.3390/molecules28041845.

Sururin FW, Khafiyya NA. 2024. Prediction prediction of the insecticidal potency of biduri plants (*Calotropis gigantea*) using the PASS online web resource. Life Sci Biotechnol 2 (1): 13-19. DOI: 10.19184/LSB.V2I1.47444.

Takamiya M, Takaoka C, Ohta N. 1999. Cytological and reproductive studies on Japanese *Diplazium* (Woodsiaceae; Pteridophyta): Apomictic reproduction in *Diplazium* with evergreen bi- to tripinnate leaves. J Plant Res 112: 419-436. DOI: 10.1007/PL00013897.

Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol 38 (7): 3022-3027. DOI: 10.1093/molbev/msab120.

Tongco JV, Villaber RA, Aguda RM, Razal RA. 2014. Nutritional and phytochemical screening, and total phenolic and flavonoid content of *Diplazium esculentum* (Retz.) Sw. from Philippines. J Chem Pharm Res 6 (8): 238-242.

Trujillo-Argueta S, del Castillo RF, Tejero-Diez D, Matias-Cervantes CA, Velasco-Murguía A. 2021. DNA barcoding ferns in an unexplored tropical montane cloud forest area of southeast Oaxaca, Mexico. Sci Rep 11: 22837. DOI: 10.1038/s41598-021-02237-8.

Ulum FB, Setyati D, Rizqoni A, Su'udi M. 2023. Antimicrobial and phytochemistry study of *Liparis resupinata* Ridl. from Mount Gumitir, East Java, Indonesia. J Pharm Sci Clinic Res 8 (3): 373-384. DOI: 10.20961/jpscr.v8I3.70856.

Uraku AJ. 2015. Leaves by Gas Chromatography-Mass Spectrometry (GC-MS) method. Res J Phytochem 9 (4): 175-187. DOI: 10.3923/rjphyto.2015.175.187.

Wang FH, Lu JM, Wen J, Ebihara A, Li DZ. 2016. Applying DNA barcodes to identify closely related species of ferns: A case study of the Chinese *Adiantum* (Pteridaceae). PLoS One 11 (9): e0160611. DOI: 10.1371/journal.pone.0160611.

Watanabe M, Miyashita T, Devkota HP. 2021. Phenolic compounds and ecdysteroids of *Diplazium esculentum* (Retz.) Sw. (Athyriaceae) from Japan and their chemotaxonomic significance. Biochem Syst Ecol 94: 104211. DOI: 10.1016/j.bse.2020.104211.

Wei Z, Wei M, Yang X, Xu Y, Gao S, Ren K. 2022. Synaptic secretion and beyond: Targeting synapse and neurotransmitters to treat neurodegenerative diseases. Oxid Med Cell Longev 2022: 9176923. DOI: 10.1155/2022/9176923.

Yao H, Song J, Liu C, Luo K, Han J, Li Y, Pang X, Xu H, Zhu Y, Xiao P, Chen S. 2010. Use of ITS2 region as the universal DNA barcode for plants and animals. PLoS One 5 (10): e13102. DOI: 10.1371/journal.pone.0013102.

Yildiz I, Yildiz BS. 2021. Mechanistic study of L-6-hydroxynicotine oxidase by DFT and ONIOM methods. J Mol Model 27 (2): 53. DOI: 10.1007/s00894-020-04646-4.

Yu J, Wu X, Liu C, Newmaster S, Ragupathy S, Kress WJ. 2021. Progress in the use of DNA barcodes in the identification and classification of medicinal plants. Ecotoxicol Environ Saf 208: 111691. DOI: 10.1016/j.ecoenv.2020.111691.

Zeb MA, Rahman TU, Sajid M, Xiao W, Musharraf SG, Bibi S, Akitsu T, Liaqat W. 2021. GC-MS analysis and in silico approaches of *Indigofera heterantha* root oil chemical constituents. Compounds 1 (3): 116-124. DOI: 10.3390/compounds1030010.

Zhi X, Fang C, Gu Y, Chen H, Chen X, Cui J, Hu Y, Weng W, Zhou Q, Wang Y, Wang Y, Jiang H, Li X, Cao L, Chen X, Su J. 2020. Guaiacol suppresses osteoclastogenesis by blocking interactions of RANK with TRAF6 and C-Src and inhibiting NF-κB, MAPK and AKT pathways. J Cell Mol Med 24 (9): 5122-5134. DOI: 10.1111/jcmm.15153.