BIODIVERSITAS
Volume 27, Number 2, February 2026
Pages: 11

ISSN: 1412-033X
E-ISSN: 2085-4722
https://doi.org/10.13057/biodiv/d270234

Bioactivities and phytochemical profile of *Cyclosorus parasiticus* from East Java, Indonesia via GC-MS and LC-MS

FUAD BAHRUL ULUM[1,♥], DWI SETYATI[1], IKA OKTAVIANAWATI[2], MOH. IKMAL ALFI RIZQONI[1], ERLIN NOVITA SARI[1], KAHAR MUZAKHAR[1], MUKHAMAD SU'UDI[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Jember. Jl. Kalimantan 37, Jember 68121, East Java, Indonesia. Tel./fax.: +62-331-330225, ♥email: fuad.fmipa@unej.ac.id

[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Jember. Jl. Kalimantan 37, Jember 68121, East Java, Indonesia

Manuscript received: 12 September 2025. Revision accepted: 27 February 2026.

Abstract. *Ulum FB, Setyati D, Oktavianawati I, Rizqoni MIA, Sari EN, Muzakhar K, Su'udi M. 2026. Bioactivities and phytochemical profile of* Cyclosorus parasiticus *from East Java, Indonesia via GC-MS and LC-MS. Biodiversitas 27 (2): d270234. https://doi.org/10.13057/biodiv/d270234*. *Cyclosorus parasiticus* is a terrestrial fern abundantly distributed in the area of Gumitir Mountain, East Java, Indonesia. Despite its ecological abundance, its pharmacological potential remains undercharacterised, although leaf tissues from Gumitir populations accumulate relatively high levels of flavonoids and alkaloids. Through an integrative bioassay–metabolomics approach, this study presents the first systematic evaluation of the antibacterial and antioxidant activities, together with comprehensive chemical profiling, of *C. parasiticus* from the Gumitir region. Metabolite annotation revealed pronounced chemical diversity, with 39 compounds identified by GC-MS and six dominant LC-MS features. Importantly, the diterpene euphornin and the alkaloids canthiumine and haplophytine were detected and are reported here for the first time in a fern, substantially expanding current knowledge of pteridophyte secondary metabolism and highlighting previously overlooked alkaloid diversity in fern taxa. The methanolic extract exhibited concentration-dependent antibacterial inhibition against *Staphylococcus aureus*, *Kocuria rosea*, *Pseudomonas aeruginosa*, and *Escherichia coli*, with greater susceptibility observed in Gram-positive bacteria. Antioxidant activity measured by the DPPH radical-scavenging assay yielded an IC_{50} value of 176.66±14.64 ppm, indicating moderate radical-scavenging capacity. Collectively, these findings establish *C. parasiticus* (Gumitir population) as a chemically rich fern with measurable antibacterial and antioxidant potential, supporting its promise as a natural source of pharmacologically relevant metabolites.

Keywords: Alkaloids, antibacterial, antioxidant, *Cyclosorus parasiticus*, metabolite profiling

INTRODUCTION

Indonesia is recognised as a global centre of plant diversity and represents a major reservoir of untapped natural products. Within this flora, ferns (pteridophytes) constitute an important but comparatively underexplored lineage, despite long-standing ethnomedicinal use for conditions such as fever, gastrointestinal disorders, wound healing, and respiratory infections (Singh et al. 2021; Setiawan 2022). Increasing evidence indicates that medicinally relevant pteridophytes possess diverse bioactivities, including antioxidant, antimicrobial, anti-inflammatory, and anticancer effects, driven by secondary metabolites such as flavonoids, alkaloids, terpenoids, and phenolics (Bandyopadhyay and Dey 2022). However, for most species, pharmacological validation remains limited, and their metabolite composition remains poorly resolved, underscoring the need for deeper phytochemical and metabolomics-based investigations to support standardisation and bioprospecting (Sun et al. 2024).

Among these species, *Cyclosorus parasiticus* (Linn.) Farwell emerges as a fern of notable medicinal potential, widely distributed in Jember, East Java, particularly in the Gumitir Mountain region where favourable ecological conditions support its growth (Rizqoni et al. 2024). Leaves from this population exhibit elevated flavonoid and alkaloid contents compared with those from other plantation areas, indicating locally enriched reservoir of secondary metabolites (Setyati et al. 2020). Phytochemical investigations have identified alkaloids, flavonoids, and chalcone derivatives, including parasitin A-C, associated with antioxidant, antimicrobial, immunosuppressive, and anti-inflammatory activities (Rudrapal et al. 2021). Bioassays further demonstrate significant anti-inflammatory activity in acetone extracts, while acute toxicity tests confirm safety at doses up to 2000 mg/kg (Tangavelou and Viswanathan 2017). Nevertheless, its pharmacological profile remains insufficiently characterised, particularly regarding metabolite diversity and functional relevance.

The need for deeper investigation is further justified by global health challenges. Rising oxidative stress associated with free radicals contributes to chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions, while antibiotic resistance remains a critical public health crisis. In Indonesia, cancer prevalence increased from 1.4% in 2013 to 1.49% in 2018, with breast, prostate, and lung cancers dominating new cases (Prihantono et al. 2023). Moreover, Antimicrobial Resistance (AMR) caused an estimated 1.3 million deaths globally in 2019, surpassing HIV and malaria, with *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* among the most lethal pathogens (Brüssow 2024). The emergence of biofilm

formation, quorum sensing, and immune evasion mechanisms further exacerbates their clinical impact (Qin et al. 2022; Ziogou et al. 2023; Alhadlaq et al. 2024; Touaitia et al. 2025). In this context, plants with dual antioxidant and antibacterial activities, such as *C. parasiticus*, represent a promising natural source for novel therapeutic agents. While some Gumitir species, such as the orchid *Liparis resupinata* and fern *Asplenium nidus*, have demonstrated such properties (Setyati et al. 2023; Ulum et al. 2023), *C. parasiticus* from this region remains largely unexplored.

Secondary metabolites are central to the pharmacological activity of ferns, driving their antioxidant, antibacterial, and anticancer effects (Bandyopadhyay and Dey 2022). The ecological abundance of *C. parasiticus* further supports its potential as a bioprospecting target (Setyati et al. 2020). However, most studies have relied on basic phytochemical screening methods such as UV-Vis spectrophotometry, which provide limited insights into chemical complexity. Advanced analytical platforms such as Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) provide more comprehensive metabolite profiling. GC-MS is particularly effective for detecting volatile and semi-volatile compounds, while LC-MS enables the characterisation of larger, non-volatile metabolites, offering a more complete picture of chemical diversity (Farhan et al. 2025). Despite their power, these methods have not yet been applied to *C. parasiticus* from Gumitir, representing a significant gap in our understanding of its phytochemical composition.

Given this background, the present study was designed to bridge this knowledge gap by providing the first integrated assessment of biological activity and metabolite identification of *C. parasiticus* from Gumitir Mountain, East Java. Specifically, we aimed to (i) evaluate the antibacterial activity of its methanolic extract against clinically relevant pathogens and assess its antioxidant capacity using the DPPH assay, (ii) comprehensively profile its chemical constituents using complementary GC-MS and LC-MS platforms, and (iii) identify key metabolites and discuss their potential biological significance. By integrating metabolomic profiling with bioactivity screening, this study contributes novel insights into the therapeutic potential of *C. parasiticus*. It reinforces the value of ferns as a source of bioactive natural products for future drug development.

MATERIALS AND METHODS

Plant material

Leaf samples of *Cyclosorus parasiticus* were collected from a pine plantation situated in the montane zone of Gumitir, Jember District, East Java, Indonesia. Species identification was initially performed in the field based on distinct morphological characteristics using established regional pteridophyte identification keys. To ensure taxonomic accuracy, the identification was subsequently confirmed by a certified pteridologist at the Purwodadi Botanical Garden, National Research and Innovation Agency (BRIN), under reference number 1206/IPH.06/HM/VII/2017, and further validated through DNA barcoding analysis (Rizqoni et al. 2024). A voucher specimen was deposited in the Herbarium of the Biology Department at the University of Jember with the accession number JR 0000001801. All collection activities adhered to legal and ethical standards, as authorized by the East Java Forestry Office (Permit No. 35X/803/PI/2017). Upon collection, leaf material was gently rinsed with distilled water to remove dust and surface contaminants without damaging tissue integrity. The cleaned specimens were then air-dried at ambient room temperature (25-27°C) for 24 hours under shaded, dust-free conditions to preserve phytochemical stability. The fully dried leaves were stored in sterile, labeled paper envelopes and kept in a controlled, moisture-free environment until further processing.

Procedures

Extraction procedure

Approximately 30 g of *C. parasiticus* leaf material was macerated in 96% methanol (pro analysis grade, Merck) at a sample-to-solvent ratio of 1:7 (w/v). The extraction process was carried out over 72 hours (3 × 24 hours) at room temperature, with manual stirring performed every 24 hours to enhance solvent penetration and compound diffusion. After the extraction period, the mixture was filtered through a Whatman No. 1 filter paper to remove particulate residues and obtain a clear extract. The resulting filtrate was then concentrated under reduced pressure using a BUCHI Rotavapor R-114 rotary evaporator at a controlled temperature, for approximately 15 minutes, to yield a viscous crude methanolic extract. The concentrated extract was subsequently stored in an airtight amber glass container at 4°C until further analysis (Amalini et al. 2025).

Antimicrobial assay

The antibacterial activity of *C. parasiticus* crude extract was assessed against four bacterial strains: *Staphylococcus aureus*, *Kocuria rosea*, *Pseudomonas aeruginosa*, and *Escherichia coli*. All test bacteria were laboratory isolates and previously used by Nugraha et al. (2020). Bacterial inocula were prepared from rejuvenated isolates in Nutrient Broth (NB), and 100 µL of each inoculum was evenly spread onto sterile petri dishes containing 15 mL of Nutrient Agar (NA). One Petri dish was divided into five sectors to accommodate four concentrations of the methanolic crude extract, i.e., 69.8 mg/mL (P1), 52.3 mg/mL (P2), 34.9 mg/mL (P3), and 17.4 mg/mL (P4) with methanol serving as a negative control (K−). A second plate was used for the positive control (K+), comprising 10 mg/mL chloramphenicol (Jadoun et al. 2016). Wells were made with a sterile cork borer, and 40 µL of extract was dispensed into each well. Plates were incubated at 37°C for 24 h. Antibacterial activity was determined by measuring the Diameter of the Inhibition Zone (DIZ) (Ulum et al. 2023), following the equation:

$$\text{DIZ (mm)} = \frac{\text{D1} + \text{D2} + \text{D3} + \text{D4}}{4} - \text{Ds}$$

Where:

D1-D4 : Inhibition-zone diameters, measured in four replicates

Ds : Well diameter

Antibacterial potency was classified according to Davis and Stout (1971) as follows: weak (<5 mm), moderate (5-10 mm), strong (10-20 mm), or very strong (>20 mm) (Davis and Stout 1971).

Antioxidant activity assay

The antioxidant activity of *C. parasiticus* crude extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The samples tested included a positive control (vitamin C) and the crude extract of *C. parasiticus.* Four serial concentrations of the positive control and the extract were prepared to assess dose-dependent antioxidant activity. Briefly, 0.3 mL of extract was mixed with 1.3 mL of 0.1 mM DPPH in methanol and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Amalini et al. 2025). Radical-scavenging activity was expressed as percentage inhibition using the equation as follows:

$$\text{Inhibition (\%)} = \frac{\text{Abs\_control} - \text{Abs\_sample}}{\text{Abs\_control}} \times 100$$

The IC_{50} value was estimated from the inhibition-concentration relationship by linear regression ($y = bx + a$), where $IC_{50} = (50 - a)/b$. Antioxidant strength was classified as very strong (<50 ppm), strong (50-100 ppm), moderate (101-250 ppm), weak (251-500 ppm), or inactive (>500 ppm) following Manurung et al. (2022).

Determination of phytochemical composition via GC-MS and LC-MS analysis

The phytochemical composition of *C. parasiticus* was investigated using both Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) to capture volatile and non-volatile metabolites. To ensure methodological consistency and enable cross-species comparison within our ongoing Gumitir medicinal plant metabolomics programme, the GC-MS and LC-MS analytical conditions were maintained identically to those previously optimised and published by our group and collaborators (Ulum et al. 2023, 2025; Oktavianawati et al. 2024; Farhan et al. 2025).

GC-MS analysis was performed on a Shimadzu GC-MS-QP 2010 Plus system. The crude extract was filtered through a sterile 0.22 µm syringe filter to remove particulate matter. 1 µL of the filtrate was injected into the GC system using the split injection method (split ratio 1:10), which ensured accurate dosing and prevented column overloading (Choudhury et al. 2021). Chromatographic separation was carried out on an Rtx-50 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) under the following temperature program: an initial temperature of 80°C held for 10 min, increased at 5°C/min and maintained at 230°C for 10 min. The injector and detector were set at 290°C and 280°C, respectively. High-purity helium was used as the carrier gas at a constant flow rate of 3.0 mL/min. For metabolite profiling, chromatograms were evaluated based on retention time, peak area, abundance, and mass spectral features (molecular weight and predicted formula). Compound identification was performed by matching mass spectra against the Wiley 9 library, and only hits with a similarity score ≥90% were accepted as putative annotations (Farhan et al. 2025).

LC-MS analysis was conducted to characterize non-volatile secondary metabolites. The assay was performed at the Research Excellence Laboratory, Institut Pertanian Bogor (Bogor, Indonesia), using a UHPLC Vanquish system coupled with a Q Exactive Plus Orbitrap HRMS (Thermo Scientific). Sample preparation was performed by dissolving 5 mg of crude extract in 2 mL methanol (MeOH), then filtering through a 0.2 µm nylon membrane. A 2 µL aliquot of the filtrate was injected into the LC-MS system. Separation was achieved using an Accucore C18 column (100 × 2.1 mm, 1.5 µm; Thermo Scientific) maintained at 30°C. The mobile phases consisted of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid), delivered at a flow rate of 0.2 mL/min. A gradient program was applied as follows: 0-1 min, 5% B; 1-25 min, 5-95% B; 25-28 min, 95% B; and 28-33 min, 5% B. Detection was performed in positive Electrospray Ionization (ESI^+) mode across a mass range of 100-1500 m/z. The MS/MS acquisition was set with a capillary temperature of 320°C, spray voltage of 3.8 kV, sheath gas and auxiliary gas at 15 and 3 arbitrary units, respectively, and resolving power of 70,000 FWHM. Metabolite features were evaluated based on mass-to-charge (m/z) values and fragmentation patterns. Putative identification was facilitated through library-based matching and annotation workflows in Compound Discoverer 3.2 (Thermo Scientific), including spectral selection, retention-time alignment, feature detection and grouping, composition prediction, mass-list searching, gap filling, area normalisation, and background compound marking (Oktavianawati et al. 2024; Septaningsih et al. 2024).

Data analysis

The data integrated the antioxidant and antibacterial assay outputs with secondary metabolite profiling of *C. parasiticus*. Putatively annotated metabolites were curated through a targeted literature survey to (i) assign each compound to a major phytochemical class (e.g., flavonoids, phenolics, terpenoids, alkaloids) and (ii) summarise reported biological activities relevant to this study, particularly antioxidant and antibacterial effects, and where available, anticancer potential. The resulting dataset was compiled into tables linking metabolite identity, detection platform (GC-MS or LC-MS), and literature-supported bioactivity to facilitate interpretation of the observed bioassay responses (Farhan et al. 2025).

RESULTS AND DISCUSSION

Antimicrobial activity

The qualitative antimicrobial assay demonstrated that *C. parasiticus* exhibits inhibitory activity against *S. aureus*, *K. rosea*, *P. aeruginosa*, and *E. coli*, as evidenced by inhibitory zones around the agar wells (Figure 1). A concentration-dependent antibacterial response was observed, with higher extract concentrations yielding greater activity, as indicated by inhibition zones categories (Table 1). The highest concentration (69.8 mg/mL) possessed moderate inhibition against all four bacterial strains. Concentration of 52.3 mg/mL results in moderate activity against *K. rosea*, and weak activity against *S. aureus*, *P. aeruginosa*, and *E. coli*. Lower concentrations (34.9 and 17.4 mg/mL) showed uniformly weak or no activity, with no detectable inhibition of *E. coli* at the lowest concentration.

The result showed that the *C. parasiticus* extract was more sensitive against Gram-positive bacteria, particularly *S. aureus* and *K. rosea*, as indicated by wider inhibition zones than those observed with Gram-negative strains. It showed that Gram-positive bacteria are more susceptible to plant-derived antimicrobial agents because they lack an outer lipopolysaccharide layer, which restricts the penetration of antimicrobial agents. Moderate inhibition was observed only at comparatively high extract concentrations. The Minimum Inhibitory Concentrations (MICs) of the extracts were not determined, so the antimicrobial potential of *C. parasiticus* can not be quantified. Nevertheless, the detection of reproducible bioactivity indicates that the crude extract likely contains antibacterial constituents that merit further investigation through activity-guided fractionation and compound isolation; subsequent work can then evaluate potency more rigorously and explore optimisation or combination-based applications (e.g., antibiotic potentiation) using standard MIC- and synergy-based frameworks (Binzet et al. 2025).

The present findings align with previous reports on the antimicrobial activity of other orchid and fern species. *L. resupinata* inhibits *S. aureus*, *S. typhi*, and *E. coli*, while *A. nidus* inhibits the growth of *S. aureus* and *E. coli* (Setyati et al. 2023; Ulum et al. 2023). These parallels underscore the antimicrobial promise of historically underexplored plant lineages, orchids included, which are increasingly recognised as reservoirs of structurally diverse secondary metabolites with antibacterial and antioxidant activities. With multidrug-resistant pathogens becoming increasingly prevalent worldwide (Qin et al. 2022; Ziogou et al. 2023; Alhadlaq et al. 2024; Touaitia et al. 2025), the moderate but broad-spectrum activity of *C. parasiticus* suggests that it may provide useful antimicrobial scaffolds. It warrants deeper phytochemical profiling and bioassay-guided fractionation, followed by mechanistic studies to isolate and identify the metabolites underpinning the observed effects.

Antioxidant activity

The crude extract of *C. parasiticus* had an IC_{50} value of 176.66±14.64 ppm (Table 2), indicating that the extract was capable of scavenging 50% of DPPH free radicals at 176.66 ppm. In antioxidant assays, lower IC_{50} values indicate stronger radical-scavenging efficacy. The IC_{50} of *C. parasiticus* was classified as moderate antioxidant activity (Manurung et al. 2022). The antioxidant activity of *C. parasiticus* is substantially higher than that of other fern species, such as *Equisetum debile* from Bali (IC_{50} = 1,604 ppm) and *Plagiogyria glauca* from Kalimantan (IC_{50} = 995 ppm), which are categorized as inactive (Suryaningrum et al. 2017; Nurhasnawati et al. 2019). However, the antioxidant capacity of *C. parasiticus* remains significantly lower than that of vitamin C, which demonstrated a remarkably strong activity with an IC_{50} value of 2.58±1.24 ppm (Table 2). This difference is expected, given that vitamin C is a pure reference compound with well-established efficacy. In contrast, the antioxidant activity of *C. parasiticus* may be attributed to the presence of phenolic acids and flavonoids identified in the extract, which are known for their radical-scavenging properties, albeit typically with lower activity than synthetic standards (Gęgotek and Skrzydlewska 2022).

Several fern species exhibit strong antioxidant-related bioactivity. For example, *Osmunda japonica* root extract suppressed interleukin-1β (IL-1β) expression (IC_{50} = 17.8 µg/mL), a key pro-inflammatory cytokine whose upregulation is commonly triggered by oxidative stress; thus, reduced IL-1β signalling is often interpreted as an indirect marker of antioxidant-mediated attenuation of oxidative-inflammatory responses. By comparison, *Matteuccia orientalis* fronds showed moderate activity at 50.0 µg/mL.

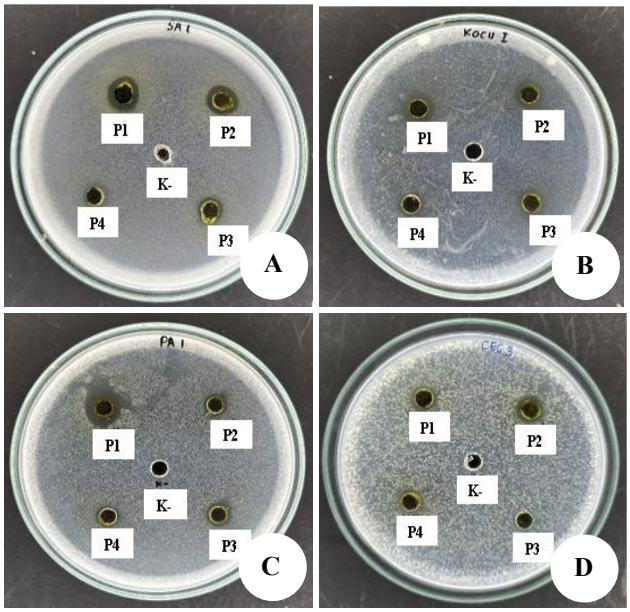

Figure 1. Antibacterial activity of *Cyclosorus parasiticus* extract by agar well diffusion. A. *S. aureus*, B. *K. rosea*, C. *P. aeruginosa*, D. *E. coli*. P1: 69.8 mg/mL, P2: 52.3 mg/mL, P3: 34.9 mg/mL, P4: 17.4 mg/mL, K: Methanol

Table 1. The antibacterial activity of *Cyclosorus parasiticus* was assessed using the agar well diffusion method

Concentration	Gram-positive		Gram-negative	
	Staphylococcus aureus	***Kocuria rosea***	***Pseudomonas auregunosa***	***Escherichia coli***
69.8 mg/mL	6.53^{++}	6.65^{++}	6.13^{++}	5.09^{++}
52.3 mg/mL	4.92^{+}	5.36^{++}	4.78^{+}	4.18+
34.9 mg/mL	4.51^{+}	4.93^{+}	4.05^{+}	0.38
17.4 mg/mL	2.84^{+}	0.41^{+}	2.89^{+}	0.00
Metanol	0.00	0.00	0.00	0.00
Cholamphenicol	14.11^{++}	14.65^{++}	22.16^{+++}	21.16^{+++}

Note: Inhibition zones were categorized as follows: +: Weak, ++: Moderate, +++: Strong, ++++: Very strong (Davis and Stout 1971)

Table 2. Antioxidant activity of *Cyclosorus parasiticus* extract by the DPPH radical scavenging assay

Sampel	IC_{50} (ppm)			Mean±SD	Category
	1	2	3		
Cyclosorus parasiticus	190.89	179.05	161.9	176.66±14.64	Moderate
Vitamin C	1.40	3.89	2.47	2.58±1.24	Very strong

Note: Antioxidant activity classification based on IC_{50} values (Manurung et al. 2022): (Weak, 251-500 ppm); (Moderate, 101-250 ppm); (Strong, 50-100 ppm); (Very strong, <50 ppm)

In a direct radical-scavenging assay, *Stenochlaena palustris* displayed high antioxidant capacity alongside a total polyphenol content of 51.69 mg/g, and *Pteridium aquilinum* achieved a DPPH IC_{50} of <30 µg/mL in DPPH assays and ORAC values >0.5 g Trolox equivalents/g dry weight, supporting its potential nutraceutical relevance (Moussa et al. 2024). The moderate antioxidant activity of *C. parasiticus* suggests the presence of bioactive constituents capable of neutralizing free radicals such as DPPH. These compounds are likely secondary metabolites, specifically terpenoids, phenolics, and alkaloids, which have been widely reported to exhibit antioxidant properties (Hilal et al. 2024; Khan et al. 2025). These chemical groups are known to act primarily via the Hydrogen-Atom Transfer (HAT) mechanism, donating a hydrogen atom to stabilize DPPH radicals (Gülçin and Alwasel 2023). The findings support the pharmacological relevance of *C. parasiticus* as a potential source of natural antioxidants and provide a biochemical basis for its traditional medicinal use.

Secondary metabolite profile and pharmacological potential

The methanolic extract of *C. parasiticus* leaves was subjected to metabolite profiling using both Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS). GC-MS analysis was used to identify volatile constituents, while LC-MS detected both volatile and non-volatile metabolites based on their chromatographic interactions with the stationary phase and elution gradients (Table 3). The GC-MS chromatogram revealed 39 distinct peaks, corresponding to 39 identified compounds (Figure 2), while LC-MS analysis provided complementary information on additional secondary metabolites that are less volatile or thermally stable.

A total of 37 bioactive compounds were confidently identified from the combined GC-MS and LC-MS datasets, representing a broad spectrum of structural classes including furan derivatives, aromatics, terpenoids, alkaloids, steroids, phenylpropanoids, and miscellaneous metabolites. This high level of chemical diversity reflects the species' sophisticated secondary metabolic capacity and highlights its ecological adaptation to diverse environmental conditions. Among the identified compounds, phenol was the most abundant, constituting 15.66% of the total metabolite profile. Phenol and its derivatives serve as precursors for a wide range of polyphenolic compounds, including flavonoids, phenolic acids, lignins, and tannins, which are widely recognised for their roles in plant defence against oxidative stress, pathogen attack, and environmental challenges (Chen et al. 2020).

The chemical composition of *C. parasiticus* underscores its potential as a rich source of pharmacologically active molecules. The identified metabolites exhibited multifunctional biological activities, notably Antioxidant (AO), Antibacterial (AB), and Anticancer (AK) properties. Furan derivatives and aromatic compounds are primarily associated with free-radical scavenging and redox regulation, while terpenoids and alkaloids contribute significantly to antimicrobial and cytotoxic activities (Alizadeh et al. 2020). Steroidal constituents and phenylpropanoids further enhance the pharmacological complexity, potentially offering synergistic therapeutic effects (Sun and Shahrajabian 2025).

The second major compound was pyrocatechol (7.89%), typically derived from catechin degradation. Pyrocatechol functions as a biosynthetic precursor for other natural products such as chalcones, which have been reported in *C. parasiticus* (Wei et al. 2013). In addition, furan derivatives, including furfural, furfuryl alcohol, furanone, butyrolactone, methyl furoate, isosorbide, and hydroxymethylfurfural, were collectively abundant, accounting for 21.66% of the total metabolite content. Notably, coumarin derivatives containing a furan ring have also been identified in related ferns such as *Cyclosorus interruptus* (Quadri-Spinelli et al. 2000). Diterpenes were also prominent in the extract, with neophytadiene representing 9.12% of the profile. This compound is known for diverse biological activities, including anti-inflammatory, antioxidant, and neuroprotective effects. Previous studies have likewise reported neophytadiene as a significant phytochemical in the acetone extracts of *Nephrolepis cordifolia* and *Nephrolepis exaltata* (Rukmini and Suvarnalatha Devi 2014; Sharma et al. 2021). In

addition, alkaloids accounted for 11.6% of the extract, including 2,4-dimethylimidazole, ethyl cyanoacetate, N-acetylpyrrolidone, and piperidine propanoic acid.

LC-MS analysis of the methanolic extract of *C. parasiticus* revealed six significant peaks, of which three major metabolites were identified as the macrocyclic diterpene euphornin and the alkaloids canthiumine and haplophytine, while the remaining peaks corresponded to minor or unidentified compounds (Figure 3). Notably, euphornin was the most abundant feature and is reported here for the first time in a fern, extending the taxonomic distribution of this diterpene beyond its previously recognised association with *Euphorbia* species (Chen et al. 2012; Li et al. 2018; Salehi et al. 2019). Euphornin has been shown to have antitumour potential (Chen et al. 2012; Li et al. 2018). In addition, canthiumine from *Canthium* spp., and haplophytine, previously identified in *Haplophyllum*/*Haplophyton*, are known for their insecticidal activity (Mroue et al. 1996; Ali et al. 2001; Patro et al. 2014; Mohammadhosseini et al. 2021). These compounds are reported for the first time in a fern in this study. The findings of this study broadened current knowledge of fern secondary metabolism and suggest that *C. parasiticus* may harbour previously overlooked diterpene-alkaloid diversity. Further activity-guided fractionation, structural confirmation, and in vivo validation are required to establish the biological significance and therapeutic relevance of these metabolites.

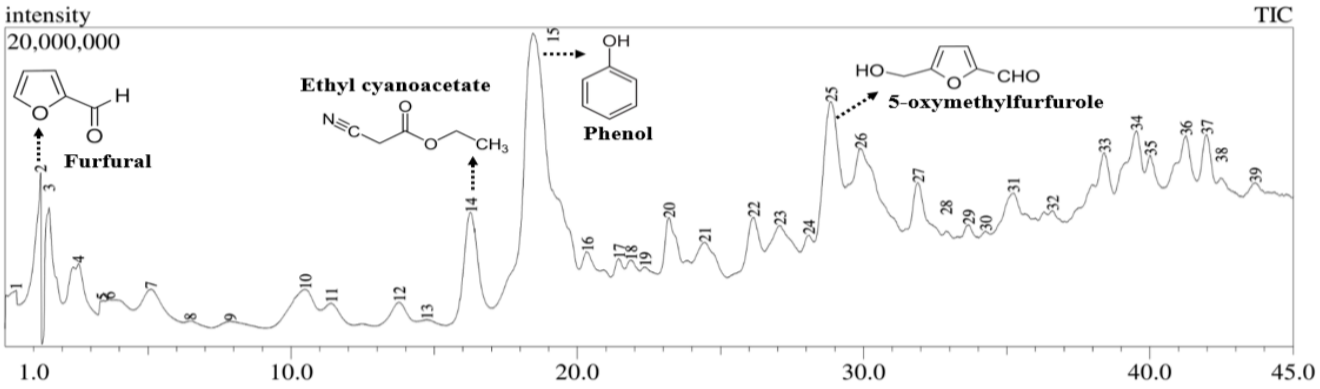

Figure 2. GC-MS chromatogram of the methanolic leaf extract of *Cyclosorus parasiticus*

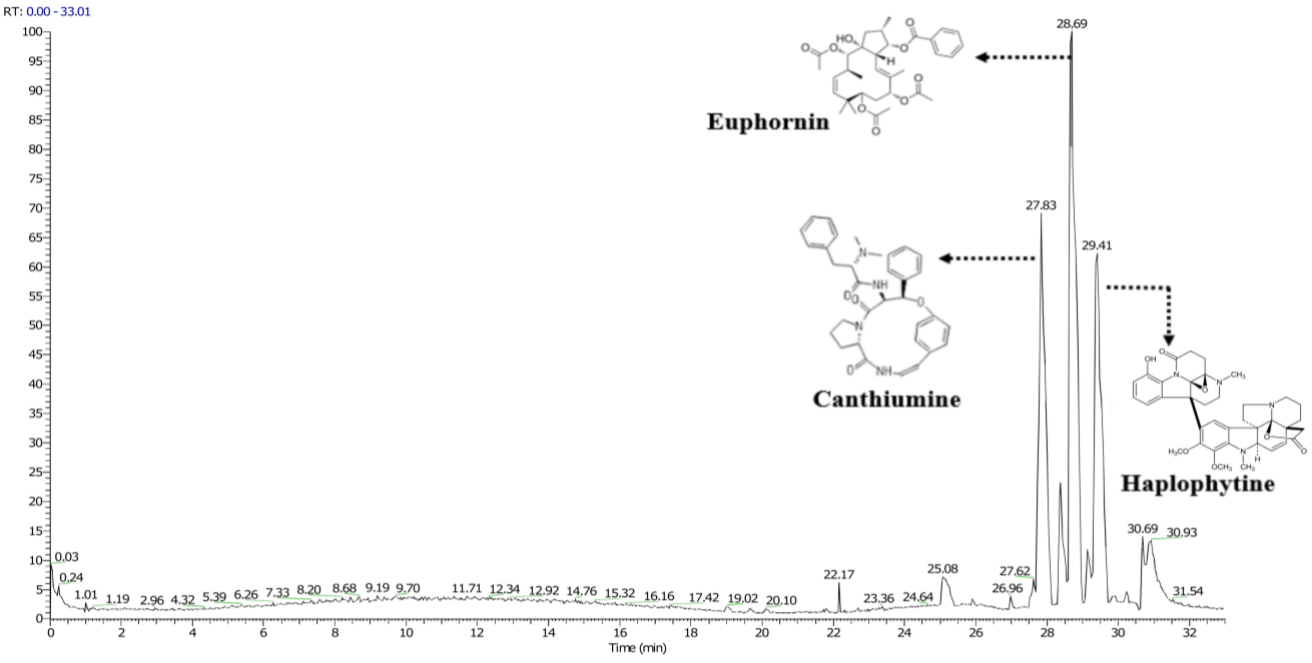

Figure 3. LC-MS chromatogram of the methanolic leaf extract of *Cyclosorus parasiticus*

Table 3. Pharmacological potential of secondary metabolites of *Cyclosorus parasiticus* identified by GC-MS and LC-MS

Compound name	Detection	Class	Pharmacological potential			Reference
			AO	AB	AK	
Furanmethanol ($C_5H_6O_2$)	GC/MS	Furan	√			Kang et al. (2020)
1 Octen 3 Ol ($C_8H_{16}O$)	GC/MS	Misc		√		Xiong et al. (2017)
Cyclohexanedione ($C_6H_8O_2$)	GC/MS	Misc		√		Wagh et al. (2023)
9-Oxabicyclo ($C_8H_{12}O_2$)	GC/MS	Misc		√		Grabarczyk et al. (2015)
Furancarboxylic ($C_6H_6O_3$)	GC/MS	Furan	√	√		Fagbemi et al. (2022)
Benzeneethanol ($C_8H_{10}O$)	GC/MS	Aromatics	√			Rumjuankiat et al. (2018)
Naphthalene ($C_{13}H_{16}$)	GC/MS	Aromatics	√			Özen et al. (2018)
Acetylpropanoate ($C_{10}H_{18}O_3$)	GC/MS	Misc			√	Liu et al. (2011)
Neophytadiene ($C_{20}H_{38}$)	GC/MS	Terpen			√	Selmy et al. (2023)
2-Furancarboxaldehyde ($C_5H_4O_2$)	GC/MS	Furan	√	√		Fagbemi et al. (2022)
Hexanoic Acid ($C_8H_{16}O_2$)	GC/MS	Misc	√			Fu et al. (2011)
alpha-springene ($C_{20}H_{32}$)	GC/MS	Terpen	√			Safavi et al. (2021)
Curcumene ($C_{15}H_{22}$)	LC/MS	Terpen		√		da Silva et al. (2015)
Octadecanediol ($C_{18}H_{38}O_2$)	LC/MS	Misc			√	Tan et al. (2010)
Fucosterolepoxide ($C_{29}H_{48}O_2$)	LC/MS	Steroid	√	√		Meinita et al. (2021)
Bis2-ethylhexyl phtalate ($C_{24}H_{38}O_4$)	LC/MS	Misc		√		Herwin et al. (2020)
Echinenone ($C_{40}H_{54}O$)	LC/MS	Terpen	√			Matsuura et al. (2012)
Euphornin ($C_{33}H_{44}O_9$)	LC/MS	Terpen	√		√	Li et al. (2018); Mustafa et al. 2021)
Antheraxanthin ($C_{40}H_{56}O_3$)	LC/MS	Terpen	√			Shimode et al. (2018)
Imidazole-2,4-Dimethyl ($C_5H_8N_2$)	GC/MS	Alkaloid		√		Abbood et al. (2021)
Benzimidazole Carboxaldehyde ($C_8H_6N_{2O}$)	GC/MS	Alkaloid		√		Abbood et al. (2021)
Acetylpyrrolidine ($C_6H_9NO_2$)	GC/MS	Alkaloid	√			Hussain et al. (2019)
Choline ($C_5H_{13}NO$)	LC/MS	Alkaloid	√	√		Ivanović et al. (2022)
Fluoro-α-pyrrolidinobutiophenone ($C_{14}H_{18}FNO$)	LC/MS	Alkaloid	√			Hussain et al. (2019)
Haplophytine ($C_{37}H_{40}N_4O_7$)	LC/MS	Alkaloid	√		√	Galasso (2010; Won et al. (2010)
Galagine ($C_6H_{13}N_3$)	LC/MS	Alkaloid		√	√	Coqueiro et al. (2014); Arjmand et al. (2022)
Pyrrolidin-1-yl-5-(trifluoromethyl)phenyl]-2,3-dihydro-1-benzofuran-5-carboxamide ($C_{20}H_{19}F^3N_2O_2$)	LC/MS	Alkaloid	√			Hussain et al. (2019)
Pheophorbide A ($C_{35}H_{36}N_4O_5$)	LC/MS	Alkaloid	√			Kandagatla et al. (2019)
Canthiumine ($C_{33}H_{36}N_4O_4$)	LC/MS	Alkaloid	√	√		Amalraj et al. (2021)
Furancarboxaldehyde ($C_5H_4O_2$)	GC/MS	Furan		√		Rodrigues et al. (2020)
Furanone, Dihydro (C_4 H_6O^2)	GC/MS	Furan	√	√		Youssef et al. (2023)
2,5 Furanone ($C_4H_4O^2$)	GC/MS	Furan	√	√		Youssef et al. (2023)
Phenol (CAS) Izal $$ Monophenol (C_6H_6O)	GC/MS	Aromatics	√	√	√	Godlewska-Żyłkiewicz et al. (2020); Menshchikova et al. (2023)
2-Tert-Butyl-4-Methyl-5-Oxo ($C_9H_{14}O_5$)	GC/MS	Misc	√			Chen et al. (2020)
2-Benzenediol (CAS) Pyrocatechol ($C_6H_6O_2$)	GC/MS	Aromatics	√	√	√	Kosanić et al. (2012)
Methyl-3-3,5-Ditertbutyl-4-Hydroxyphenyl-Propionate ($C_{18}H_{28}O_3$)	GC/MS	Phenyl propanoid	√			Mayer et al. (2023)
3beta-hydroxy-4beta-methyl-5alpha-cholest-7-ene-4alpha-carboxylic acid ($C_{29}H_{48}O^3$)	LC/MS	Steroid	√			Godlewska-Żyłkiewicz et al. (2020)
Acetyl hexamethyl tetralin ($C_{18}H_{26}O$)	LC/MS	Aromatics	√			Wu et al. (2022)
4-[(2E)-3,7-dimethyl-2,6-octadien-1-yl]-2-formyl-3-hydroxy-5- methoxybenzylstearate ($C_{37}H_{60}O_5$)	LC/MS	Misc	√			Chen et al. (2020)

Note: AO: Antioxidant, AB: Antibacterial, AC: Anticancer

The phytochemical screening of *C. parasiticus* revealed a diverse array of secondary metabolites with pharmacological relevance, highlighting its potential as a medicinal fern. Among the identified compounds, several furan derivatives such as furanmethanol, furancarboxylic acid, 2-furancarboxaldehyde, furanone, and 2,5-furanone were among the dominant constituents of the C. parasiticus extract (Kang et al. 2020; Rodrigues et al. 2020; Fagbemi et al. 2022; Youssef et al. 2023). These compounds are well known for their potent radical-scavenging activity, which plays a central role in mitigating oxidative stress, a major pathogenic factor linked to cancer, cardiovascular disorders, neurodegeneration, and other chronic diseases (Godlewska-Żyłkiewicz et al. 2020). In particular, furancarboxylic acid and 2-furancarboxaldehyde displayed dual antioxidant and antibacterial properties, demonstrating their capacity to neutralise free radicals while simultaneously disrupting microbial growth. The detection of furanone derivatives, reported to interfere with microbial quorum sensing and prevent oxidative damage, further

indicates that *C. parasiticus* metabolites may act through multiple complementary mechanisms. In addition to furans, the antioxidant profile of the extract was enriched by aromatic compounds such as benzeneethanol and naphthalene, which have also been reported to possess strong radical-scavenging capacity (Özen et al. 2018; Rumjuankiat et al. 2018).

Aromatic compounds formed another major group of bioactives, including benzeneethanol, naphthalene, phenol, pyrocatechol, and acetyl hexamethyl tetralin (Kosanić et al. 2012; Özen et al. 2018; Rumjuankiat et al. 2018; Godlewska-Żyłkiewicz et al. 2020; Wu et al. 2022). These molecules are characterised by their strong electron-donating ability, which enables them to stabilise reactive oxygen species and prevent oxidative damage at the cellular level. Particularly, phenol and pyrocatechol exhibited broad pharmacological potential, contributing to antioxidant, antibacterial, and anticancer activities (Menshchikova et al. 2023). Their roles in regulating oxidative signalling pathways and inducing apoptosis in cancer cells highlight their potential as lead molecules for therapeutic development. Additionally, lipophilic aromatics such as methyl-3,3,5-ditertbutyl-4-hydroxyphenyl-propionate enhance the bioavailability of antioxidant molecules, suggesting a synergistic effect within the extract's metabolite matrix (Mayer et al. 2023).

Terpenoids were also abundantly represented and contributed significantly to the extract's pharmacological profile. Identified compounds such as neophytadiene, α-springene, curcumene, echinenone, antheraxanthin, and euphornin are known for their wide-ranging biological activities, including anti-inflammatory, antioxidant, and anticancer properties (Matsuura et al. 2012; da Silva et al. 2015; Li et al. 2018; Shimode et al. 2018; Safavi et al. 2021; Selmy et al. 2023). Euphornin, reported here for the first time in *C. parasiticus*, is particularly notable for its dual antioxidant and cytotoxic potential, positioning it as a promising candidate for anticancer drug development (Mustafa et al. 2021). Carotenoid-derived terpenes such as echinenone and antheraxanthin contribute to the neutralisation of Reactive Oxygen Species (ROS), while neophytadiene and curcumene have demonstrated antiproliferative activity against tumour cells, suggesting multiple mechanisms of action.

Alkaloids were among the most pharmacologically versatile groups among the identified compounds. This class included imidazole derivatives, benzimidazole carboxaldehyde, haplophytine, canthiumine, choline, galagine, and several pyrrolidine-based molecules (Galasso 2010; Won et al. 2010; Coqueiro et al. 2014; Abbood et al. 2021; Ivanović et al. 2022). Many of these alkaloids displayed dual or triple bioactivities, functioning as antioxidants, antimicrobials, and anticancer agents. Haplophytine and canthiumine, in particular, are cytotoxic alkaloids with documented anticancer potential and serve as scaffolds for antitumour drug development (Amalraj et al. 2021). Choline contributes to membrane integrity and antioxidant defence, indicating possible neuroprotective roles (Ivanović et al. 2022). The antibacterial activities of galagine and pyrrolidine derivatives, which target bacterial DNA replication and cell wall biosynthesis, further underscore the therapeutic relevance of this chemical class (Arjmand et al. 2022).

In addition to these major groups, steroids and other miscellaneous compounds added further bioactivity diversity. Steroidal metabolites such as fucosterol epoxide and 3β-hydroxy-4β-methyl-5α-cholest-7-ene-4α-carboxylic acid showed antioxidant and antimicrobial properties and are known to enhance membrane permeability, facilitating the bioactivity of other compounds (Godlewska-Żyłkiewicz et al. 2020; Meinita et al. 2021). Miscellaneous metabolites, such as octadecanediol and acetylpropanoate, exhibited anticancer potential, possibly through the induction of apoptosis and modulation of cell membrane dynamics (Tan et al. 2010; Liu et al. 2011).

The antibacterial potential of *C. parasiticus* was equally pronounced. Compounds such as 1-octen-3-ol, cyclohexanedione, and 9-oxabicyclo structures demonstrated significant inhibitory effects on bacterial growth. These findings align with previous reports that octenol derivatives exhibit antimicrobial effects against both Gram-positive and Gram-negative bacteria (Xiong et al. 2017). Moreover, furan derivatives, including furancarboxylic acid and 2-furancarboxaldehyde, exhibit dual antioxidant and antibacterial properties (Fagbemi et al. 2022), indicating their multifunctional therapeutic potential. Such dual-action metabolites are particularly valuable in the management of infections where oxidative stress amplifies tissue damage, positioning *C. parasiticus* as a promising candidate for integrated antimicrobial therapies.

The anticancer potential of *C. parasiticus* was supported by the detection of acetylpropanoate and neophytadiene, both known for their chemopreventive and cytotoxic activities. Neophytadiene, a diterpene hydrocarbon, has been widely reported to exhibit anti-inflammatory, antioxidant, and anticancer effects (Selmy et al. 2023). Its identification in *C. parasiticus* underscores the fern's potential as a natural chemotherapeutic reservoir. Similarly, acetylpropanoate has been associated with apoptosis induction and inhibition of cancer cell proliferation (Liu et al. 2011). The co-occurrence of these metabolites with potent antioxidant compounds suggests a synergistic mechanism by which the fern may prevent oxidative DNA damage while inhibiting tumor progression.

This study presents the first integrative pharmacological and metabolomic characterisation of *C. parasiticus* from Gumitir Mountain, East Java, revealing its unique bioactive potential and chemical diversity. The crude extract exhibited concentration-dependent antibacterial activity, with notably greater sensitivity against Gram-positive bacteria (*S. aureus* and *K. rosea*) than against Gram-negative bacteria, consistent with differences in cell envelope structure and permeability barriers between the groups. Although the overall antibacterial and antioxidant activities were moderate, the results indicate that *C. parasiticus* contains bioactive compounds that warrant further investigation. Importantly, because crude extracts represent complex metabolite mixtures, the observed activities may reflect additive or synergistic interactions and may mask the contribution of individual active compounds. Therefore, activity-guided fractionation and isolation of the most active constituents

are necessary for further study to confirm bioactivity at the compound level and to identify specific metabolite markers responsible for the observed effects. Likewise, any future consideration of structural optimisation or combination-based applications should be supported by purified-compound testing and synergism assays (e.g., MIC-based combination analysis), rather than being inferred from crude-extract potency alone (Nugraha et al. 2020). Its moderate antioxidant activity (IC_{50} = 176.66±14.64 ppm), surpassing that of several other ferns, further indicates the presence of terpenoids, phenolics, and alkaloids with potent radical-scavenging properties.

In conclusion, the integrated bioassay-metabolomics approach revealed a chemically rich profile in *C. parasiticus* from Gumitir Mountain and documented the first fern record of the diterpene euphornin and the alkaloids canthiumine and haplophytine, underscoring the novelty of this study. Collectively, these results establish *C. parasiticus* as a promising natural source of antioxidant, antibacterial, and potentially anticancer agents. Future research should focus on the isolation and structural elucidation of individual bioactive compounds, mechanistic studies to define their pharmacological modes of action, and conservation strategies to safeguard this species as a valuable reservoir for natural product-based drug discovery.

ACKNOWLEDGEMENTS

This research was funded by the DPPM *Penelitian Riset Fundamental* (PFR) Grant Year 2025 (Contract Number: 4551/UN25.3.1/LT/2025) to Dra. Dwi Setyati, M.Si, Universitas Jember, Indonesia.

REFERENCES

Abbood AF, Abdula AM, Baqi Y. 2021. New benzimidazoles bearing 2-pyrazoline moiety: Synthesis and antimicrobial activity. J Wasit Sci Med 14 (3): 1-12. https://doi.org/10.31185/jwsm.401.

Alhadlaq MA, Aljurayyad OI, Almansour A, Al-Akeel SI, Alzahrani KO, Alsalman SA, Yahya R, Al-Hindi RR, Hakami MA, Alshahrani SD, Alhumeed NA, Al Moneea AM, Al-Seghayer MS, AlHarbi AL, Al-Reshoodi FM, Alajel S. 2024. Overview of pathogenic *Escherichia coli*, with a focus on Shiga toxin-producing serotypes, global outbreaks (1982-2024) and food safety criteria. Gut Pathog 16: 57. https://doi.org/10.1186/s13099-024-00641-9.

Ali MS, Pervez MK, Saleem M, Tareen RB. 2001. Haplophytin-A and B: The alkaloidal constituents of *Haplophyllum acutifolium*. Phytochemistry 57 (8): 1277-1280. https://doi.org/10.1016/S0031-9422(01)00188-1.

Alizadeh M, Jalal M, Hamed K, Saber A, Kheirouri S, Pourteymour Fard Tabrizi F, Kamari N. 2020. Recent updates on anti-inflammatory and antimicrobial effects of furan natural derivatives. J Inflamm Res 13: 451-463. https://doi.org/10.2147/jir.s262132.

Amalini YR, Muzakhar K, Su'udi M, Setyati D, Dwinanti EF, Ulum FB. 2025. Morphological, molecular, and phytochemical characteristics of wild edible fern (*Diplazium esculentum*) from Jember, East Java, Indonesia. Biodiversitas 26 (6): 2794-2805. https://doi.org/10.13057/biodiv/d260624.

Amalraj S, Krupa J, Sriramavaratharajan V, Mariyammal V, Murugan R, Ayyanar M. 2021. Chemical characterization, antioxidant, antibacterial and enzyme-inhibitory properties of *Canthium coromandelicum*, a valuable source for bioactive compounds. J Pharm Biomed Anal 192: 113620. https://doi.org/10.1016/j.jpba.2020.113620.

Arjmand MH, Sabri H, Maghrouni A, Zarei E, Hotelchi M, Afshari AR. 2022. Potential cytotoxic activity of galegine on human melanoma cells. Res J Pharmacogn 9 (3): 15-23. https://doi.org/10.22127/rjp.2022.324923.1829.

Bandyopadhyay A, Dey A. 2022. Medicinal pteridophytes: Ethnopharmacological, phytochemical, and clinical attributes. Beni-Suef Univ J Basic Appl Sci 11: 113. https://doi.org/10.1186/s43088-022-00283-3.

Binzet G, Yıldırım M, Binzet R, Yabalak E. 2025. Phytochemical profiling and evaluation of antioxidant, antimicrobial, and cytotoxic activities of endemic *Onosma angustissima*. Intl J Environ Health Res 18: 1-15. https://doi.org/10.1080/09603123.2025.2576592.

Brüssow H. 2024. The antibiotic resistance crisis and the development of new antibiotics. Microb Biotechnol 17 (7): e14510. https://doi.org/10.1111/1751-7915.14510.

Chen H, Wang Z, Yang L. 2012. Analysis of euphornin in *Euphorbia helioscopia* L. and its cytotoxicity to mouse Lung Adenocarcinoma cells (LA795). Nat Prod Res 26 (22): 2112-2116. https://doi.org/10.1080/14786419.2011.622275.

Chen J, Yang J, Ma L, Li J, Shahzad N, Kim CK. 2020. Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl and carboxylic acid groups of phenolic acids. Sci Rep 10: 2611. https://doi.org/10.1038/s41598-020-59451-z.

Choudhury FK, Pandey P, Meitei R, Cardona D, Gujar AC, Shulaev V. 2021. GC-MS/MS profiling of plant metabolites. In: Shulaev V (eds). Plant Metabolic Engineering: Methods in Molecular Biology. Humana, New York. https://doi.org/10.1007/978-1-0716-1822-6_9.

Coqueiro A, Regasini LO, Stapleton P, da Silva Bolzani V, Gibbons S. 2014. In vitro antibacterial activity of prenylated guanidine alkaloids from *Pterogyne nitens* and synthetic analogues. J Nat Prod 77 (8): 1972-1975. https://doi.org/10.1021/np500281c.

da Silva GNS, Pozzatti P, Rigatti F, Hörner R, Alves SH, Mallmann CA, Heinzmann BM. 2015. Antimicrobial evaluation of sesquiterpene α-curcumene and its synergism with imipenem. J Microbiol Biotechnol Food Sci 4 (5): 434-436. https://doi.org/10.15414/jmbfs.2015.4.5.434-436.

Davis WW, Stout TR. 1971. Disc plate method of microbiological antibiotic assay. II. Novel procedure offering improved accuracy. Appl Microbiol 22: 666-670. https://doi.org/10.1128/am.22.4.666-670.1971.

Fagbemi KO, Aina DA, Adeoye-Isijola MO, Naidoo KK, Coopoosamy RM, Olajuyigbe OO. 2022. Bioactive compounds, antibacterial and antioxidant activities of methanol extract of *Tamarindus indica* Linn. Sci Rep 12: 9432. https://doi.org/10.1038/s41598-022-13716-x.

Farhan AM, Setyati D, Gilani NA, Su'udi M, Ulum FB. 2025. Phytochemistry profile and antioxidant activity of *Dumortiera hirsuta* (Sw.) Nees from Gumitir, East Java. Hayati J Biosci 32: 1643-1653. https://doi.org/10.4308/hjb.32.6.1643-1653.

Fu X-J, Maimaiti AS, Mou H-M, Yang Q, Liu G-J. 2011. Hexanoic acid 2-(diethylamino)ethyl ester enhances chilling tolerance in strawberry seedlings by impact on photosynthesis and antioxidants. Biol Plant 55: 793. https://doi.org/10.1007/s10535-011-0190-8.

Galasso V. 2010. A DFT investigation of structure and spectroscopic properties of haplophytine and its half-molecules. Chem Phys Lett 487 (1-3): 32-37. https://doi.org/10.1016/j.cplett.2010.01.008.

Gęgotek A, Skrzydlewska E. 2022. Antioxidative and anti-inflammatory activity of ascorbic acid. Antioxidants 11 (10): 1993. https://doi.org/10.3390/antiox11101993.

Godlewska-Żyłkiewicz B, Świsłocka R, Kalinowska M, Golonko A, Świderski G, Arciszewska Ż, Nalewajko-Sieliwoniuk E, Naumowicz M, Lewandowski W. 2020. Biologically active compounds of plants: Structure-related antioxidant, microbiological and cytotoxic activity of selected carboxylic acids. Materials 13 (19): 4454. https://doi.org/10.3390/ma13194454.

Grabarczyk M, Wińska K, Mączka W, Żołnierczyk AK, Żarowska B, Anioł M. 2015. Lactones with methylcyclohexane systems obtained by chemical and microbiological methods and their antimicrobial activity. Molecules 20 (2): 3335-3353. https://doi.org/10.3390/molecules20023335.

Gülçin İ, Alwasel SH. 2023. DPPH radical scavenging assay. Processes 11 (8): 2248. https://doi.org/10.3390/pr11082248.

Herwin, Nuryanti S, Sjafaraenan, Baits M. 2020. Antibacterial activity of bis (2-ethylhexyl) phthalate leaves fraction of *Colocasia esculenta* L. against enteric Gram-negative bacteria. J Glob Pharm Technol 12 (11): 130-139.

Hilal B, Khan MM, Fariduddin Q. 2024. Recent advancements in deciphering therapeutic properties of plant secondary metabolites: Phenolics, terpenes and alkaloids. Plant Physiol Biochem 211: 108674. https://doi.org/10.1016/j.plaphy.2024.108674.

Hussain F, Khan Z, Jan MS, Ahmad S, Ahmad A, Rashid U, Ullah F, Ayaz M, Sadiq A. 2019. Synthesis and biological evaluation of pyrrolidine-2,5-dione and thiazolidine-2,4-dione derivatives. Bioorg Chem 91: 103128. https://doi.org/10.1016/j.bioorg.2019.103128.

Ivanović M, Grujić D, Cerar J, Razboršek MI, Topalić-Trivunović L, Savić A, Kočar D, Kolar M. 2022. Extraction of bioactive metabolites from *Achillea millefolium* L. with choline-chloride-based natural deep eutectic solvents: A study of the antioxidant and antimicrobial activity. Antioxidants 11 (4): 724. https://doi.org/10.3390/antiox11040724.

Jadoun J, Yazbak A, Rushrush S, Rudy A, Azaizeh H. 2016. Identification of a new antibacterial sulfur compound from *Raphanus sativus* seeds. Evid Based Complement Alternat Med 2016 (1): 9271285. https://doi.org/10.1155/2016/9271285.

Kandagatla SK, Uhl RT, Graf TN, Oberlies NH, Raner GM. 2019. Pheophorbide derivatives isolated from Açaí berries (*Euterpea oleracea*) activate an antioxidant response element in vitro. Nat Prod Commun 14: 33214801. https://doi.org/10.1177/1934578x19852443.

Kang D-E, Lee H-U, Davaatseren M, Chung M-S. 2020. Comparison of acrylamide and furan concentrations, antioxidant activities, and volatile profiles in cold or hot brew coffees. Food Sci Biotechnol 29: 141-148. https://doi.org/10.1007/s10068-019-00644-2.

Khan A, Kanwal F, Ullah S, Fahad M, Tariq L, Altaf MT, Riaz A, Zhang G. 2025. Plant secondary metabolites—Central regulators against abiotic and biotic stresses. Metabolites 15 (4): 276. https://doi.org/10.3390/metabo15040276.

Kosanić M, Ranković B, Stanojković T. 2012. Antioxidant, antimicrobial and anticancer activity of 3 *Umbilicaria* species. J Food Sci 77 (1): T20-T25. https://doi.org/10.1111/j.1750-3841.2011.02459.x.

Li X-Q, Bai Y-L, Zhang D-L, Jiao H-S, He R-X. 2018. Euphornin reduces proliferation of human cervical adenocarcinoma HeLa cells through induction of apoptosis and G2/M cell cycle arrest. OncoTargets Ther 11: 4395-4405. https://doi.org/10.2147/ott.s166018.

Liu W, Zhou J, Zhang H, Qian H, Yin J, Bensdorf K, Gust R. 2011. Synthesis and biological evaluation of licofelone derivatives as anticancer and anti-inflammatory agents. Lett Drug Design Discov 8 (10): 911-917. https://doi.org/10.2174/157018011797655223.

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. https://doi.org/10.13057/biodiv/d231104.

Matsuura H, Watanabe MM, Kaya K. 2012. Echinenone production of a dark red-coloured strain of *Botryococcus braunii*. J Appl Phycol 24: 973-977. https://doi.org/10.1007/s10811-011-9719-7.

Mayer J, Steinbrecher R, Metzsch-Zilligen E, Pfaendner R. 2023. Antioxidant activity of biogenic cinnamic acid derivatives in polypropylene. Polymers 15 (17): 3621. https://doi.org/10.3390/polym15173621.

Meinita MDN, Harwanto D, Tirtawijaya G, Negara BFSP, Sohn J-H, Kim J-S, Choi J-S. 2021. Fucosterol of marine macroalgae: Bioactivity, safety and toxicity on organism. Mar Drugs 19 (10): 545. https://doi.org/10.3390/md19100545.

Menshchikova EB, Khrapova MV, Kozhin PM, Chechushkov AV, Serykh AE, Romakh LP, Kandalintseva NV. 2023. Protective effect of a new monophenolic antioxidant TS-13 in a mouse model of Parkinson's disease. Bull Exp Biol Med 175: 265-269. https://doi.org/10.1007/s10517-023-05847-6.

Mohammadhosseini M, Venditti A, Frezza C, Serafini M, Bianco A, Mahdavi B. 2021. The genus *Haplophyllum* Juss.: Phytochemistry and bioactivities—A review. Molecules 26 (15): 4664. https://doi.org/10.3390/molecules26154664.

Moussa AY, Luo J, Xu B. 2024. Insights into chemical diversity and potential health-promoting effects of ferns. Plants 13 (18): 2668. https://doi.org/10.3390/plants13182668.

Mroue MA, Euler KL, Ghuman MA, Alam M. 1996. Indole alkaloids of *Haplophyton crooksii*. J Nat Prod 59 (9): 890-893. https://doi.org/10.1021/np960070c.

Mustafa I, Faisal MN, Hussain G, Muzaffar H, Imran M, Ijaz MU, Sohail MU, Iftikhar A, Shaukat A, Anwar H. 2021. Efficacy of *Euphorbia helioscopia* in context to a possible connection between antioxidant and antidiabetic activities: A comparative study of different extracts. BMC Complement Med Ther 21: 62. https://doi.org/10.1186/s12906-021-03237-x.

Nugraha AS, Permatasari AEN, Kadarwenny CP, Pratoko DK, Triatmoko B, Rosyidi VA, Norcahyanti I, Dewi IP, Dianasari D, Sary IP, Wangchuk P. 2020. Phytochemical screening and the antimicrobial and antioxidant activities of medicinal plants of Meru Betiri National Park-Indonesia. J Herbs Spices Med Plants 26 (3): 303-314. https://doi.org/10.1080/10496475.2020.1734136.

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. https://doi.org/10.13057/biodiv/d200238.

Oktavianawati I, Santoso M, Fatmawati S. 2024. The chemical profiles and cytotoxicity of gaharu bouya oil from Borneo's *Gonystylus bancanus* wood. Sci Rep 14: 12064. https://doi.org/10.1038/s41598-024-58529-2.

Özen T, Macit M, Toka M. 2018. Screening and evaluation of antioxidant activities of selected naphthalene compounds. Jurnal Sains Kesihatan Malaysia 16 (1): 45-55. https://doi.org/10.17576/jskm-2018-1601-06.

Patro SK, Sasmal D, Mazumndar P, Behera P, Lal UR, Dash SK, Padhy RK. 2014. Review on genus *Canthium*: Special reference to *Canthium coromandelicum*—an unexplored traditional medicinal plant of Indian Subcontinent. Am J Phytomed Clin Ther 2 (6): 796-813.

Prihantono, Rusli R, Christeven R, Faruk M. 2023. Cancer incidence and mortality in a tertiary hospital in Indonesia: An 18-year review. Ethiop J Health Sci 33 (3): 515. https://doi.org/10.4314/ejhs.v33i3.15.

Qin S, Xiao W, Zhou C, Pu Q, Deng X, Lan L, Liang H, Song X, Wu M. 2022. *Pseudomonas aeruginosa*: Pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. Signal Transduct Target Ther 7: 199. https://doi.org/10.1038/s41392-022-01056-1.

Quadri-Spinelli T, Heilmann J, Rali T, Sticher O. 2000. Bioactive coumarin derivatives from the fern *Cyclosorus interruptus*. Planta Med 66 (8): 728-733. https://doi.org/10.1055/s-2000-9908.

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

Rodrigues AC, de Almeida FA, André C, Vanetti MCD, Pinto UM, Hassimotto NMA, Vieira ÉNR, de Andrade NJ. 2020. Phenolic extract of *Eugenia uniflora* L. and furanone reduce biofilm formation by *Serratia liquefaciens* and increase its susceptibility to antimicrobials. Biofouling 36 (9): 1031-1048. https://doi.org/10.1080/08927014.2020.1844881.

Rudrapal M, Khan J, Dukhyil AAB, Alarousy RMII, Attah EI, Sharma T, Khairnar SJ, Bendale AR. 2021. Chalcone scaffolds, bioprecursors of flavonoids: Chemistry, bioactivities and pharmacokinetics. Molecules 26 (23): 7177. https://doi.org/10.3390/molecules26237177.

Rukmini K, Suvarnalatha Devi P. 2014. GC-MS analysis and phytochemical screening of a rare Pteridophyte *Nephrolepis cardifolia* (L.) Presl. From Tirumala Hills. Intl J Pharm Res Rev 3 (12): 13-19.

Rumjuankiat K, Sonhom N, Showpanish K, Somsri A, Pilasombut K. 2018. In vitro antioxidant activities and volatile compounds from Karanda (*Carissa carandas*) fruit wine. Intl J Agric Technol 14 (7): 1843-1860.

Safavi M, Olia MSJ, Abolhasani MH, Amini M, Kianirad M. 2021. Optimization of the culture medium and characterization of antioxidant compounds of a marine isolated microalga as a promising source in aquaculture feed. Biocatal Agric Biotechnol 35: 102098. https://doi.org/10.1016/j.bcab.2021.102098.

Salehi B, Iriti M, Vitalini S, Antolak H, Pawlikowska E, Kręgiel D, Sharifi-Rad J, Oyeleye SI, Ademiluyi AO, Czopek K, Staniak M, Custódio L, Coy-Barrera E, Segura-Carretero A, Cádiz-Gurrea MdlL, Capasso R, Cho WC, Seca AML. 2019. Euphorbia-Derived Natural Products with Potential for Use in Health Maintenance. Biomolecules 9 (8):337. https://doi.org/10.3390/biom9080337.

Selmy AH, Hegazy MM, El-Hela AA, Saleh AM, El-Hamouly MM. 2023. In vitro and in silico studies of Neophytadiene; A diterpene isolated from *Aeschynomene elaphroxylon* (Guill. & Perr.) Taub. as apoptotic inducer. Egypt J Chem 66: 149-161. https://doi.org/10.21608/ejchem.2023.178261.7296.

Septaningsih DA, Suparto IH, Achmadi SS, Heryanto R, Rafi M. 2024. Untargeted metabolomics using UHPLC-Q-Orbitrap HRMS for identifying cytotoxic compounds on MCF-7 breast cancer cells from *Annona muricata* Linn leaf extracts as potential anticancer agents. Phytochem Anal 35 (6): 1418-1427. https://doi.org/10.1002/pca.3373.

Setiawan A. 2022. Keanekaragaman hayati Indonesia: Masalah dan upaya konservasinya. Indones J Conserv 11 (1): 13-21. https://doi.org/10.15294/ijc.v11i1.34532. [Indonesian]

Setyati D, Adawiyah R, 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. https://doi.org/10.19184/bioedu.v21i3.43368.

Setyati D, Sulistiyowati H, Erizcy MP, Ratnasari T. 2020. The flavonoid and alkaloid content of *Cyclosorus parasiticus* (Linn.) Farwell ferns at the plantation areas of Jember Regency. Biolink 7 (1): 23-37. https://doi.org/10.31289/biolink.v7i1.3026.

Sharma DK, Dave RS, Shah KR. 2021. Phytochemical screening and characterization of volatile compounds by Gas Chromatography-Mass Spectrometry from *Nephrolepis exaltata*. Asian J Pharm Clin Res 14 (7): 93-98. https://doi.org/10.22159/ajpcr.2021.v14i7.41869.

Shimode S, Miyata K, Araki M, Shindo K. 2018. Antioxidant activities of the antheraxanthin-related carotenoids, antheraxanthin, 9-cis-antheraxanthin, and mutatoxanthins. J Oleo Sci 67 (8): 977-981. https://doi.org/10.5650/jos.ess18060.

Singh C, Chauhan N, Rani A, Kishore K, Singh K, Singh R. 2021. Ethnobotanical and pharmacological studies of Pteridophytes (ferns) from Mussoorie Region, Dehradun District, Uttarakhand. Intl J Bot Stud 6 (6): 694-702.

Sun J, Liu B, Rustiami H, Xiao H, Shen X, Ma K. 2024. Mapping Asia plants: Plant diversity and a checklist of vascular plants in Indonesia. Plants 13 (16): 2281. https://doi.org/10.3390/plants13162281.

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

Suryaningrum RD, Puspawati NM, Astiti NPA. 2017. Aktivitas antioksidan ekstrak tumbuhan Paku Ekor Kuda (*Equisetum debile* L.) terhadap peroksidasi lipid plasma darah mencit (*Mus musculus*). Metamorfosa J Biol Sci 4 (1): 48-53. https://doi.org/10.24843/metamorfosa.2017.v04.i01.p08. [Indonesian]

Tan KB, Ling LU, Chiu GNC. 2010. In vitro efficacy of a novel liposomal formulation of a protein kinase C inhibitor in the treatment of acute myeloid leukemia. Blood 116 (21): 3282. https://doi.org/10.1182/blood.V116.21.3282.3282.

Tangavelou AC, Viswanathan MB. 2017. Anti-inflammatory activity of epidermal glandular extract of *Cyclosorus parasiticus* in experimental animals. J Biol Active Prod Nat 7 (3): 245-250. https://doi.org/10.1080/22311866.2017.1342560.

Touaitia R, Mairi A, Ibrahim NA, Basher NS, Idres T, Touati A. 2025. *Staphylococcus aureus*: A review of pathogenesis and virulence mechanisms. Antibiotics 14 (5): 470. https://doi.org/10.3390/antibiotics14050470.

Ulum FB, Putri YA, Farhan AM, Setyati D, Rajamani R, Sugiharto B, Su'udi M. 2025. Phytochemical and pharmacological evaluation of *Vanda tricolor* from Gumitir, East Java, Indonesia. Biodiversitas 26 (9): 4783-4792. https://doi.org/10.13057/biodiv/d260947.

Ulum FB, Setyati D, Rizqoni MIA, Su'udi M. 2023. Antimicrobial and phytochemistry study of *Liparis resupinata* Ridl. from Mount Gumitir, East Java, Indonesia. J Pharm Sci Clin Res 8 (3): 373-384. https://doi.org/10.20961/jpscr.v8i3.70856.

Wagh SS, Patil AM, Kasralikar HM. 2023. Ru(II)/PEG-400: A green synthesis of indolyl-oxindoles and indolyl-cyclohexane-dione hybrids as potential antimicrobial agents. Indian J Chem 62 (2): 107-114. https://doi.org/10.56042/ijc.v62i2.71255.

Wei H, Zhang X, Wu G, Yang X, Pan S, Wang Y, Ruan J. 2013. Chalcone derivatives from the fern *Cyclosorus parasiticus* and their anti-proliferative activity. Food Chem Toxicol 60: 147-152. https://doi.org/10.1016/j.fct.2013.07.045.

Won K-J, Chung K-S, Lee YS, Alia MS, Pervez MK, Fatima S, Choi J-H, Lee K-T. 2010. Haplophytin-A induces caspase-8-mediated apoptosis via the formation of death-inducing signaling complex in human promyelocytic leukemia HL-60 cells. Chem Biol Interact 188 (3): 505-511. https://doi.org/10.1016/j.cbi.2010.09.001.

Wu F, Tian F, Jin Z, Liu J, Jin W, Chen X, Wang Z, Jia X, Liu B, Liu L, Shen W. 2022. Antioxidant capacities of heat-treated wheat germ and extruded compounded bran. Cereal Chem 99 (3): 582-592. https://doi.org/10.1002/cche.10520.

Xiong C, Li Q, Li S, Chen C, Chen Z, Huang W. 2017. In vitro antimicrobial activities and mechanism of 1-octen-3-ol against food-related bacteria and pathogenic fungi. J Oleo Sci 66 (9): 1041-1049. https://doi.org/10.5650/jos.ess16196.

Youssef YM, Azab ME, Elsayed GA, El-Sayed AA, Hassaballah AI, El-Safty MM, Soliman RA, El-Helw EAE. 2023. Synthesis and antioxidant, antimicrobial and antiviral activity of some pyrazole-based heterocycles using a 2(3H)-furanone derivative. J Iran Chem Soc 20: 2203-2216. https://doi.org/10.1007/s13738-023-02814-w.

Ziogou A, Giannakodimos I, Giannakodimos A, Baliou S, Ioannou P. 2023. *Kocuria* species infections in humans-A narrative review. Microorganisms 11 (9): 2362. https://doi.org/10.3390/microorganisms11092362.