Trop J Nat Prod Res, July 2025; 9(7): 3100 - 3110 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antioxidant Potentials and Phytochemical Profiling of *Rafflesia zollingeriana* and *Tetrastigma* spp. in Papring Forest, Banyuwangi

Nunuk Nurchayati[1,2*], Jati Batoro[3], Luchman Hakim[3], Rodiyati Azrianingsih[3] and Agus Susatya[4]

[1]*Doctoral Program, Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang 65145, East Java, Indonesia*
[2]*Departement of Biology, Faculty of Mathematics and Natural Sciences, PGRI Banyuwangi University, Banyuwangi 68416, East Java, Indonesia*
[3]*Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang 65145, East Java, Indonesia*
[4]*Department of Forestry, Bengkulu University, Bengkulu 38371, Indonesia*

ARTICLE INFO

Article history:
Received 01 May 2025
Revised 13 June 2025
Accepted 14 June 2025
Published online 01 August 2025

ABSTRACT

Rafflesia zollingeriana, a parasitic plant known for producing the world's largest flower and depends on host plants from the *Tetrastigma* genus for survival. This plant was discovered in the Papring Forest of Banyuwangi, Indonesia, where it has historically been used in traditional herbal medicine. However, scientific understanding of its pharmacological potential remains limited. This study aimed to investigate the total flavonoid content, antioxidant activity, and bioactive compounds in ethanol extract from the perigone of *R. zollingeriana* and the leaves of its host (*Tetrastigma rafflesia*, *Tetrastigma leucostaphylum*, *Tetrastigma dichotomum*) plants and non-host (*Tetrastigma papillosum*) plants. Total flavonoid content was assessed spectrophotometrically, and antioxidant activity was determined using the DPPH assay. The bioactive compounds were identified using the UPLC-MS/MS. The results revealed that *R. zollingeriana* perigone extract exhibited strong antioxidant activity despite having low flavonoid content, suggesting that other secondary metabolites such as glycosides, polyketides, alkaloids, and lipids contribute to it. Among the host plants, *T. dichotomum* had the highest flavonoid content, but had moderate antioxidant activity. In contrast, the non-host *T. papillosum* showed relatively high flavonoid levels, but weak antioxidant activity, possibly due to its distinct metabolite profile in the absence of parasitic interaction. Overall, antioxidant activity in *R. zollingeriana* perigone and *Tetrastigma* species was not directly correlated with flavonoid content but was influenced by diversity and nature of other secondary metabolites. The distinct metabolite profiles between host and non-host species suggest that chemical factors influence parasitic compatibility.

Keywords: Antioxidant, Flavonoid, Phenolic, Parasitic plant, *Rafflesia zollingeriana*, *Tetrastigma* spp.

Introduction

Rafflesia zollingeriana, a species within the *Rafflesia* genus, is renowned for producing the largest flowers in the world.[1] As with other members of this genus, *R. zollingeriana* is also known for its unique and pungent odor, which serves to attract pollinating flies.[2] This species is a holoparasitic plant that depends entirely on the host plant from the *Tetrastigma* species (Vitaceae) for its survival.[3] In Indonesia, this species is found in the Papring Forest of Banyuwangi by parasitizing several *Tetrastigma* species, including *Tetrastigma rafflesiae* (Miq.) Planch., *Tetrastigma dichotomum* (Blume) Planch, and *Tetrastigma leucostaphyllum* (Dennst.) Alston ex Mabb. This forest is one of the primary habitats for both *R. zollingeriana* and its hosts.[4] Traditionally, *R. zollingeriana* has been used in local communities as an herbal medicine.[4,5] Similarly, the *Tetrastigma* species has been used for animal feed and medicinal practices.[6] Despite these traditional uses, the scientific evidence of the medicinal potential of both *R. zollingeriana* and its hosts remains limited.

*Corresponding author. Email: nunuk_nurchayati@yahoo.com
Tel: +62 812-4922-3200

Citation: Nurchayati N, Batoro J, Hakim L, Azrianingsih R, Susatya A.Antioxidant Potentials and Phytochemical Profiling of *Rafflesia zollingeriana* and *Tetrastigma* spp. in Papring Forest, Banyuwangi. Trop J Nat Prod Res. 2025; 9(7): 3100 – 3110 https://doi.org/10.26538/tjnpr/v9i7.33

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Research on the bioactive compounds of *R. zollingeriana* is still limited, primarily due to its rarity and conservation status, but its traditional use in local herbal medicine suggests the presence of pharmacologically active constituents. While specific studies on *R. zollingeriana* are scarce, its host plants from the *Tetrastigma* genus have been more extensively studied and are known to contain a diverse range of bioactive compounds with notable pharmacological activities. Phytochemical analyses of *Tetrastigma* species have identified various flavonoids (kaempferol and quercetin), phenolic compounds (caffeoylquinic acid, orientin, and vitexin), which are known for their strong antioxidant, anti-inflammatory, and antiproliferative activities.[7,8] Additionally, terpenoids and steroids with hepatoprotective and anti-inflammatory effects have also been reported.[9] Alkaloids such as tetrastigmindole A and B isolated from *T. hemsleyanum* further demonstrate anti-inflammatory and potential anticancer activities.[10] These findings underscore the pharmacological relevance of both *R. zollingeriana* and its host plants, warranting further investigation into their phytochemical profiles and potential for novel therapeutic discoveries. Therefore, this study aimed to investigate the phytochemical composition and antioxidant activity of *R. zollingeriana* and its host plants. By evaluating their bioactive compound profiles and antioxidant activities, this research not only contributes to evidence-based validation of traditional uses but also promotes conservation awareness of these ecologically and culturally valuable species.

Materials and Methods

Sample collection and identification

Rafflesia zollingeriana buds in the perigone phase were collected from the Papring Forest (8°09'00.3"S 114°20'45.5"E), Kalipuro Subdistrict, Banyuwangi, during the peak growing season (January to December

2023) to ensure optimal phytochemical content. The leaves of both hosts (*T. rafflesia*, *T. leucostaphylum*, *T. dichotomum*) and non-hosts (*T. papillosum* (Blume) Plach.) of *R. zollingeriana* were also collected. Plant identification was carried out by a botanist (Prof. Dr. Jati Batoro, M.Si) at the Laboratory of Plant Taxonomy, Structure and Development with voucher number 0285/UN10.F09.42/10/2023.

Preparation of plant extracts

The extraction process for *R. zollingeriana* perigone and all *Tetrastigma* species leaves were conducted at the Materia Medica Batu, Malang, using the maceration method. All plant materials (*R. zollingeriana* perigonium and all *Tetrastigma* species leaves) were dried at 40–60°C and finely ground into powder. The powdered samples were then extracted in 70% ethanol (1:10 w/v) at room temperature for 5 x 24 h, with periodic stirring. The mixture was filtered and then concentrated using a rotary evaporator at 40°C. The concentrated extracts were stored in airtight containers at 4°C until further analysis.

Determination of total flavonoid content

The total flavonoid content (TFC) was determined using UV-Vis spectrophotometry based on the Ministry of Health, Republic of Indonesia method.[11] A standard quercetin curve was prepared at concentrations ranging from 3–125 µg/mL. Each sample was diluted with methanol and then mixed with 1 mL of 2% aluminum chloride ($AlCl_3$) solution and 1 mL of 100 g/L potassium acetate (CH_3COOK) solution. The mixture was incubated at room temperature for 30 min. The absorbance of each sample was measured at 415 nm using an Ultrospec 2100 pro UV/Visible Spectrophotometer (Biochrom Ltd., England) against a methanol blank. TFC was expressed as mg quercetin equivalent per gram of extract (mg QE/g extract). All measurements were performed in triplicate.

Determination of DPPH radical scavenging activities

The antioxidant activity of the extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay following the standard protocol described by Egharevba et al.[12] and Iheanacho et al.[13] A total of 0.1 mM DPPH solution in ethanol was prepared and incubated in the dark for 30 min. Sample extracts (10–100 µg/mL) were mixed with equal volumes of DPPH solution (1:1), vortexed, and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using a Ultrospec 2100 pro UV/Visible Spectrophotometer (Biochrom Ltd., England). The percentage of DPPH inhibition was calculated using the formula (equation 1):

$$\%\ inhibition = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100 \quad \text{(Equation 1)}$$

Where:

$A_{control}$ = absorbance of the DPPH solution without the sample

A_{sample} = absorbance of the DPPH solution with the sample extract

The IC_{50} value was calculated from the dose-response curve. All measurements were performed in triplicate.

Identification of Bioactive Compound using UPLC-MS/MS

Bioactive compounds in the *R. zollingeriana* perigone and *Tetrastigma* spp. leaves extract was determined using the Waters® ACQUITY UPLC H-Class System (Waters Corporation, USA. The protocol for UPLC-MS/MS was performed by Mutiah et al.[14] A total of 10 mg of plant sample was dissolved in 10 mL of methanol and loaded into a 5 µL micro syringe. Then, it was subjected to a gradient elution system using a mobile phase composed of a water/formic acid mixture (99.9/0.1 [v/v]) and an acetonitrile/formic acid mixture (99.9/0.1 [v/v]). The separation results were analyzed using UPLC-MS/MS, and the chromatographic data were processed with the MassLynx 4.1 software to generate peak area information and m/z spectra for each detected peak. Compounds were identified by matching m/z values with entries in ChemSpider and PubChem databases.

Results and Discussion

Total flavonoid content and antioxidant properties

The results showed that *T. dichotomum* has the highest total flavonoid content (46.86 mg QE/g), followed by *T. rafflesiae* (34.95 mg QE/g), *T. papillosum* (33.15 mg QE/g), *T. leucostaphyllum* (22.64 mg QE/g), and *R. zollingeriana* (10.15 mg QE/g) (Table 1). Despite its low flavonoid content, *R. zollingeriana* demonstrated the strongest antioxidant activity with an IC_{50} value of 4.35 µg/mL, categorized as very strong. In contrast, despite its relatively high flavonoid content, *T. papillosum* showed the weakest activity (IC_{50}: 293.55 µg/mL). Christina et al.[16] reported that flavonoids are a major contributor of DPPH scavenging activity. However, this study found that the inverse relationship between flavonoid content and antioxidant activity in several species, suggesting that flavonoids are not the sole contributors to antioxidant potential. For instance, *T. papillosum* contains a relatively high flavonoid level but weak antioxidant activity, while *T. leucostaphyllum*, with moderate flavonoid content, exhibits strong antioxidant activity. Similarly, *T. rafflesiae* and *T. dichotomum* had higher flavonoid levels but only moderate antioxidant activity. Notably, *R. zollingeriana* exhibited the lowest total flavonoid content and showed very strong antioxidant activity. These findings highlight the potential role of other bioactive compounds, such as phenolics, alkaloids, and terpenoids, in antioxidant defence.[17] Phenolics are known for their potent antioxidant properties.[18] Additionally, alkaloids known for their antioxidant and anti-inflammatory properties,[19] may enhance the antioxidant activity of *R. zollingeriana* despite its low flavonoid content. Terpenoids also exhibit significant free radical scavenging activity.[20] Therefore, evaluating antioxidant potential in plants should be assessed through a broader phytochemical perspective, not limited to flavonoids alone.[21]

Table 1: Total flavonoid contents and antioxidant activity of *R. zollingeriana*, *T. rafflesiae*, *T. leucostaphyllum*, *T. dichotomum,* and *T. papillosum* ethanol extract

Species	Total Flavonoid Content (mg QE/g)	Antioxidant Activity (IC_{50} µg/mL)	Category*
R. zollingeriana	10.15	4.35	Very Strong
T. rafflesiae	34.95	159.27	Medium
T. leucostaphyllum	22.64	69.10	Strong
T. dichotomum	46.86	151.25	Medium
T. papillosum	33.15	293.55	Weak

*Antioxidant category based on IC_{50} value: very strong: <50 µg/mL, strong: 50-100 µg/mL, medium: 100-150 µg/mL, weak: 150-200 µg/mL, and very weak: >200 µg/mL.[15]

Identification of bioactive compounds in R. zollingeriana perigone extract

UPLC-MS/MS analysis revealed that the ethanol extract of *R. zollingeriana* perigone contains 17 metabolites, belonging to various classes of bioactive compounds, including alkaloids, flavonoids, terpenoids, glycosides, polyketides, polyphenols, stilbenoids, and lipids (Table 2). Glycosides, polyketides, alkaloids, and lipids were the most abundant in the ethanol extract of *R. zollingeriana* perigone (Table 2). In the obtained chromatogram (Figure 1A), two glycosides were identified, including Letaustralin (Peak 1) and Mogroside V (Peak 4), with Letaustralin showing the highest area (22.4%). Several alkaloids were also identified in the perigone extract of *R. zollingeriana*, including Triethanolaminetriacetate (Peak 2), alpha-C-Mannosytryptophan (Peak 5), and Pentadecylpyridine (Peak 14). Among these, pentadecylpyridine is the most abundant compound (11.19%). This alkaloid has been reported to have anti-mycobacterial activity.[22] Several flavonoids were also identified (Figure 1B), including Epicatechin gallate (Peak 7), Theaflavin digallate (Peak 8), and Pinocembrin (Peak 10). These flavonoids are known for their strong antioxidant activity[23], which aligns with the strong antioxidant activity reported for *R. zollingeriana* in this study.

Table 2: Phytochemical identification of ethanol extract from *R. zollingeriana* perigon using UPLC-MS/MS

Peak	Retention Time (RT)	% Area	Measured mass	Calculate Mass	Formula	Compound	Group
1	2.068	**22.41**	262.1298	262.1291	$C_{11}H_{20}NO_6$	Lotaustralin	Glycoside
2	2.399	3.22	276.1454	276.1447	$C_{12}H_{22}NO_6$	Triethanolamine Triacetate	Alkaloid
3	3.039	0.13	467.0822	467.0826	$C_{20}H_{19}O_{13}$	Gibberellin	Terpenoid
4	3.784	0.54	298.1506	298.1502	$C_{11}H_{24}O_8$	Mogroside V	Glycoside
5	4.199	2.68	367.1507	367.1505	$C_{17}H_{23}N_2O_7$	alpha-C-Mannosytryptophan	Alkaloid
6	4.445	**9.55**	619.0945	619.0935	$C_{27}H_{23}O_{17}$	Epigallocatechin gallate	Polyketide
7	5.366	2.08	443.0981	443.0981	$C_{22}H_{19}O_{10}$	Epicatechin gallate	Flavonoid
8	6.027	0.38	883.1714	883.1722	$C_{44}H_{35}O_{20}$	Theaflavin digallate	Flavonoid
9	6.400	0.42	425.0870	425.0873	$C_{22}H_{17}O_9$	Bergenin Acetate	Polyphenol
10	7.848	1.38	257.0822	257.0814	$C_{15}H_{13}O_4$	Pinocembrin	Flavonoid
11	9.078	1.21	357.1698	357.1702	$C_{21}H_{13}O_4$	Gingerenone A	Polyphenol
12	10.72	0.24	310.1415	310.1443	$C_{19}H_{20}NO_3$	Pentanoic acid	Stilbenoid
13	12.108	0.73	329.2701	329.2692	$C_{19}H_{37}O_4$	Octanoylpropyl octanoate	Lipid
14	13.058	**11.19**	304.3008	304.3004	$C_{21}H_{38}N$	Pentadecylpyridine	Alkaloid
15	14.661	**9.10**	332.331	332.3317	$C_{23}H_{42}N$	Benzylalamine	Lipid
16	16.284	2.84	282.2809	282.2797	$C_{18}H_{36}NO$	(Z)-9-Octadeceneamid	Lipid
17	18.281	0.21	338.3419	338.3423	$C_{22}H_{44}NO$	(Z)-13-Docosenamide	Lipid

The terpenoid gibberellin (Peak 3), classically associated with growth regulation, has been shown to modulate stress responses and may contribute to the resilience of the flower.[23] Polyphenols, such as Epigallocatechin gallate (Peak 6) and Bergenin acetate (Peak 9), were also present. These compounds have been shown to have antioxidant, anti-inflammatory, and anticancer effects.[25] Stilbenoids, such as Pentanoic acid (Peak 12), are polyphenolic compounds that have been studied for their potential antioxidant, anti-inflammatory, and anticancer activities.[26] Lipids group, including Octanoylpropyl octanoate (Peak 13), Benzylalamine (Peak 15), and other fatty acid derivatives, were identified. These lipids play a role in maintaining cell membrane integrity, anti-inflammatory and antimicrobial properties.[27] The presence of flavonoids, alkaloids, terpenoids, and other bioactive compounds suggests that *R. zollingeriana* may offer a promising natural source for the development of novel therapeutic agents with multi-target effects.

Identification of Bioactive Compounds in R. zollingeriana's Host Tetrastigma rafflesiae leaves

UPLC-MS/MS analysis of ethanol extract from *T. rafflesiae* leaves revealed various bioactive compounds, including alkaloids, flavonoids, phenolics, naphthalenone, anthraquinone, cyclopentanoids, phenolic acid esters, aromatic esters, and lipids (Table 3, Figure 2 A-B). Luteolin (4.82%) was the predominant flavonoid, while mesalamine (18.37%) and butylated hydroxytoluene (1.72%) were the main phenolic compounds, with mesalamine being the most abundant (Table 3). Flavonoids and phenolics play a crucial role in antioxidant defence mechanisms, contributing to protection against oxidative stress and offering potential health benefits.[6] Their presence suggests that *T. rafflesiae* may have developed biochemical defence mechanisms to ensure its survival in the forest environment.[28] Several alkaloids such as cipamfylline, benzylamine, dasatinib, and ampalex were also identified. Alkaloids are pharmacologically important due to their antimicrobial, cytotoxic, and allelopathic properties,[6] potentially aiding *T. rafflesiae* in defense against herbivores, pathogens, and competing flora.[29,30] Furthermore, several lipid-related compounds were identified, including linolenic acid, glyceryl linolenate, and oleamide. Linolenic acid is a precursor to jasmonic acid, a well-known signaling molecule involved in plant defence responses.[31] The complex metabolite composition provides insights into the ecological roles of *T. rafflesiae*, particularly as a host plant for *R. zollingeriana*. Certain metabolites may influence seed germination or modulate host-microbe interactions.[32] Comparing these findings with previous studies on *Tetrastigma* species, it is suggested that the metabolite composition may vary based on environmental conditions, geographical distribution, and host-parasite interactions. A study on *T. loheri* revealed that benzylisoquinoline alkaloids were more abundant in non-infected shoots, suggesting their role in defence against *Rafflesia* infection.[30] Furthermore, aromatic and phenolic acid esters indicate that potential volatile compounds contribute to attracting pollinators or deterring herbivores.[33,34]

Tetrastigma leucostaphyllum

UPLC-MS/MS analysis of *T. leucostaphyllum* leaves extract successfully identified 21 secondary metabolites, including alkaloids, phenolics, terpenoids, anthraquinones, aromatic esters, amino acids, and lipids (Figure 3, Table 4). The chromatographic analysis (Figure 3A) and corresponding compound structures (Figure 3B) illustrate the diversity of metabolites present in the ethanol extract of *T. leucostaphyllum* leaves. Alkaloids represented the most abundant group, comprising four peaks (compounds 2, 3, 4, and 12) with significant area percentages, especially 5-methoxytryptophol (peak 2, 19.21%) and 3-indoleacrylic acid (peak 3, 6.46%). The amino acid homocycloleucine (Peak 1; 10.60%) was also present in considerable quantity. Phenolic compounds such as sinapinic acid (compound 5), nonylparaben (compounds 6 and 9), and 4-formyl-2,6-di-tert-butylphenol (compound 11) were also detected, contributing to the antioxidant potential of the extract. Additionally, a minor amount of piperlongumine (compound 7) was identified. The detection of multiple indole-based alkaloids, such as 5-methoxytryptophol and 3-indoleacrylic acid, suggests potential neuroactive, anti-inflammatory, and cytotoxic properties.[35,36]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

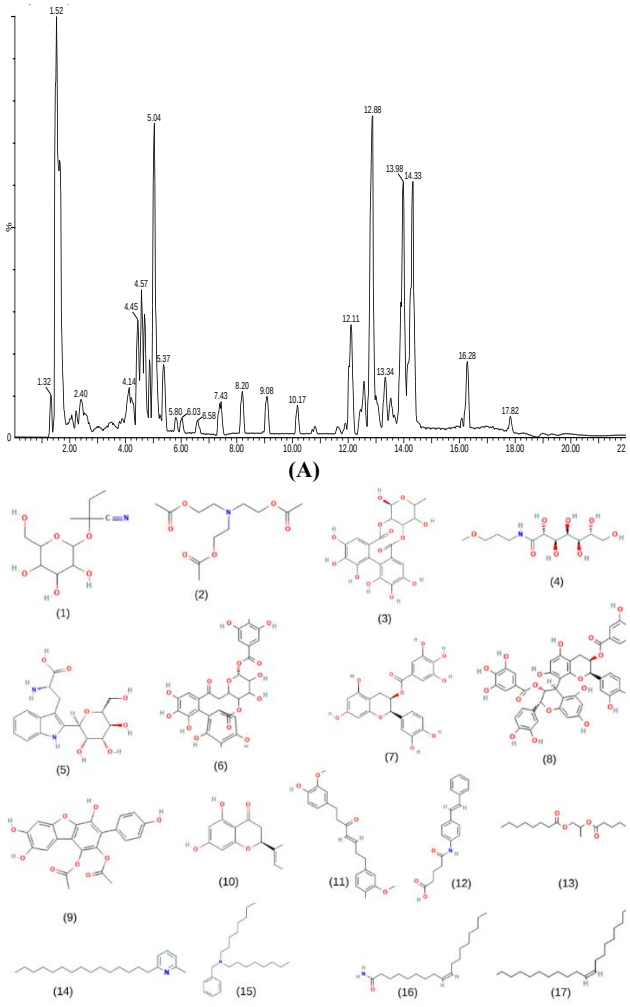
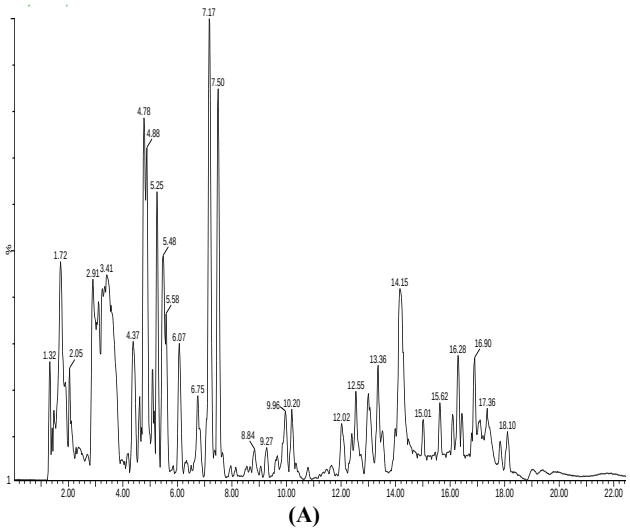


Figure 1: Identification of bioactive compounds in ethanol extract of *R. zollingeriana* perigon using UPLC-MS/MS analysis. (A) Chromatogram of UPLC-MS/MS results. (B) The structure of the identified compound includes glycosides (1,4); alkaloids (2,5,14); flavonoids (7,8,10); polyphenols (9,11); terpenoids (3); stilbenoids (12); polyketides (6); lipid (13,15,16,17).



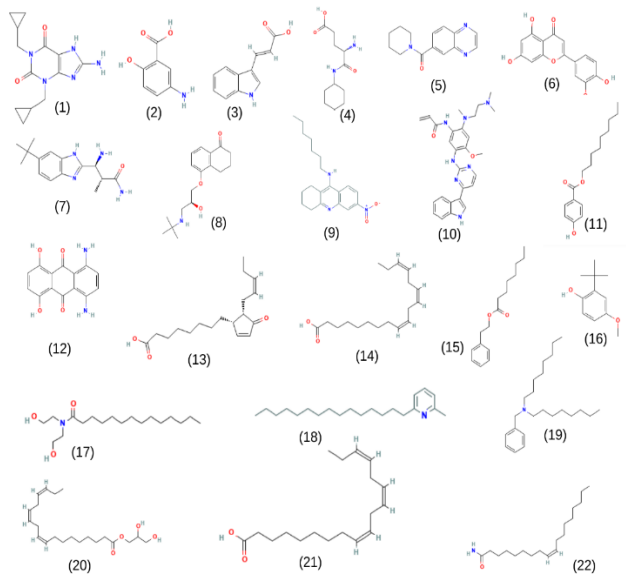


Figure 2: Identification of bioactive compounds in ethanol extract of *T. rafflesiae* leaves using a UPLC-MS/MS instrument. (A) Chromatogram of UPLC-MS/MS results. (B) Structure of identified compounds in ethanol extract of *T. rafflesiae* leaves extract, including alkaloid (1,3,5,7,9,10,18,19); flavonoid (6); phenolic (2,16); naphthalenone (8); anthraquinone (12); cyclopentanoids (13); phenolic acid ester (11); aromatic ester (15); Lipid (14,17,20,21,22).

Roscovitine, a purine alkaloid identified at lower concentration, is noteworthy due to its role as a cyclin-dependent kinase inhibitor with well-documented anticancer effects.[37] Phenolic compounds like sinapinic acid are widely recognized for their antioxidant capacity and may contribute to the plant's ability to mitigate oxidative stress and inflammation.[38,39] The identification of piperlongumine is of particular interest due to its established anticancer and pro-oxidative properties, which have been studied in various tumor models.[40] Cocamidopropyl betaine, a surfactant compound found in personal care products, was detected at a low level.[41]

Tetrastigma dichotomum

UPLC-MS/MS analysis of *T. dichotomum* leaves extract identified diverse bioactive compounds, including alkaloids, phenolics, terpenoids, aromatic esters, amino acids, and lipids (Table 5, Figure 4). Alkaloids were predominant, with 2-Methyl-5-vinylpyridine being detected as a major component. Flavonoids represented a significant portion of the extract, with compounds such as apioside (Peak 2; 7.34%), liquiritigenin (Peak 11; 7.12%), and neohesperidin dihydrochalcone (Peak 7; 4.04%) detected in notable amounts. Phenolic compounds included yatein (Peak 4; 0.33%) and butylated hydroxyanisole (BHA) (Peak 10; 2.64%), while terpenoid and phenylpropanoid groups were represented by beta-ionone epoxide and asarone, respectively. Additionally, steroids like nandrolone and 3β-androstanediol and essential fatty acids such as stearidonic acid and linolenic acid were identified. The dominance of alkaloids and flavonoids in *T. dichotomum* leaves indicates a strong pharmacological potential, consistent with findings in related *Tetrastigma* species. The identification of 2-methyl-5-vinylpyridine, a pyridine-based alkaloid, suggests potential neuroprotective or anti-inflammatory effects,[42,] aligning with the bioactivities of related nitrogenous compounds in medicinal plants. Flavonoids such as apioside, liquiritigenin, and kuersetin are well-documented for their antioxidant, anti-inflammatory, and estrogenic activities.[43,44] The phenolic compound sinapinic acid, previously reported in *T. leucostaphyllum*, was not detected here, but other phenolics such as BHA were detected. BHA is widely used as an antioxidant preservative and may also exhibit protective effects against oxidative damage in biological systems.[45]

Table 3: Phytochemical identification of ethanol extract from *T. rafflesiae* leaves using UPLC-MS/MS

Peak	Retention Time (RT)	% Area	Measured mass	Calculate Massa	Formula	Compound	Group
1	2.512	**8.19**	276.1453	276.1460	$C_{13}H_{18}N_5O_2$	Cipamfylline	Alkaloid
2	2.905	**18.37**	154.0510	154.0504	$C_7H_8NO_3$	Mesalamine	Phenolic
3	4.374	2.31	188.0718	188.1712	$C_{11}H_{10}NO_2$	Indole-3-acrylate	Alkaloid
4	4.621	**8.86**	242.1308	242.1308	$C_{13}H_{25}N_2O_3$	Neboglamine	Amino acids
5	4.776	3.91	367.1507	367.1505	$C_{14}H_{16}N_3O$	Ampalex	Alkaloid
6	5.098	4.82	287.0563	287.0556	$C_{15}H_{11}O_6$	Luteolin	Flavonoid
7	6.329	1.85	292.1917	292.1913	$C_{15}H_{23}N_4O$	Navitoclax	Alkaloid
8	6.504	1.05	292.1917	292.1913	$C_{17}H_{26}NO_3$	Levobunolol	Naphthalenone
9	6.751	1.05	342.2182	342.2182	$C_{20}H_{28}N_3O_2$	Nabumetone	Alkaloid
10	7.166	**6.74**	486.2613	486.1617	$C_{27}H_{32}N_7O_2$	Dasatinib	Alkaloid
11	8.488	0.07	265.1806	265.1804	$C_{16}H_{25}O_3$	Nonylparaben	Phenolic acid esters
12	8.664	0.06	271.0722	271.0719	$C_{14}H_{11}N_2O_4$	Doxorubicin	Anthraquinone
13	8.839	0.70	293.2127	293.2117	$C_{18}H_{29}O_3$	Piperlongumine	Cyclopentanoid
14	9.057	0.07	279.2334	279.2334	$C_{18}H_{31}O_2$	linolenic acid	Lipid
15	9.275	0.38	249.1866	249.1855	$C_{16}H_{25}O_2$	Phenethyl octanoate	Ester Aromatik
16	9.957	1.72	181.1234	181.1229	$C_{11}H_{17}O_2$	Butylated Hydroxytoluene	Phenolic
17	12.024	1.06	316.2849	316.2852	$C_{18}H_{38}NO_3$	Myristic diethanolamide	Lipid
18	13.008	1.90	304.3017	304.3004	$C_{21}H_{38}N$	**Scolopamine**	Alkaloid
19	14.154	**7.41**	332.3321	332.3317	$C_{23}H_{42}N$	Benzylamine	Alkaloid
20	15.102	1.20	353.2694	353.2692	$C_{21}H_{37}O_4$	Glyceryl linoleate	Lipid
21	15.623	2.00	279.2330	279.2324	$C_{18}H_{31}O_2$	Linolenic acid	Lipid
22	16.284	4.29	282.2803	282.2797	$C_{18}H_{36}NO$	Oleamide	Lipid

The terpenoid beta-ionone epoxide, though present in small amounts, has known cytotoxic and antimicrobial properties. Similarly, asaronea (phenylpropanoid class) found in two peaks (8 and 12) is linked to cognitive-enhancing and anti-cancer properties, though its safety profile requires cautious interpretation due to possible carcinogenicity in high doses.[46]

Identification of Bioactive Compounds in R. zollingeriana's Non-Host Tetrastigma papillosum

UPLC-MS/MS analysis of ethanol extract from *T. papillosum* leaves revealed a diverse range of secondary metabolites, including flavonoids, phenolics, terpenoids, anthraquinones, steroids, and lipids (Table 6). A total of 16 compounds were identified, with flavonoids being the predominant class, constituting 8 of the detected compounds (50%). The chromatographic profile (Figure 5A) showed distinct peaks corresponding to different compounds, with retention times (RT) ranging from 2.336 to 17.628 minutes. Genistin exhibited the highest relative abundance at 35.58% area (RT = 5.479 min), indicating its dominance in the extract.

Genistin is a glycosylated isoflavone that has been reported for its antioxidant, anti-inflammatory, and estrogenic activities[47], suggesting a significant contribution to the potential pharmacological effects of *T. papillosum*. Other notable flavonoids included diosmetin (13.01%), cyanidanol (9.79%), and quercetin (2.12%), all known for their potent free-radical scavenging and cardioprotective properties.[48] Phenolic compounds such as Helichrysoside, Gingerenone, and Butylated HydroxyToluene (BHT) were detected in smaller amounts (0.31–0.81%). Despite their low abundance, these compounds are recognized for their antioxidant activity.[49]

The phytochemical profiles of *Tetrastigma* species demonstrate significant differences between host and non-host plants of *R. zollingeriana*. These differences indicate specific biochemical adaptations related to parasitic compatibility and ecological defence strategies. *T. papillosum*, a non-host, showed high levels of flavonoids, including genistin, diosmetin, and cyanidanol, which are recognized for potent antioxidant, anti-inflammatory, and cytoprotective properties.[6] The abundance of these compounds may indicate a strong defence mechanism, potentially inhibiting *Rafflesia* seed germination or attachment through chemical barriers or allelopathic effects. Furthermore, *T. papillosum* also contains anthraquinones and terpenoids, known for antimicrobial and antiparasitic activities.[50] These bioactive compounds may contribute to parasite resistance either by directly deterring invasion or modulating the plant's internal biochemistry and associated microbiota to create an inhospitable environment for parasitism.

In contrast, host plants like *T. rafflesiae*, *T. dichotomum*, and *T. leucostaphylum* contain a greater variety of alkaloids, including 2-methyl-5-vinylpyridine and cipamfylline, which are associated with stress response, signaling, and nutrient transport.[33] These characteristics may provide a conducive environment for *Rafflesia* seed germination and development. Lipids and steroids such as linolenic acid and nandrolone were also found in higher levels in the host species.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

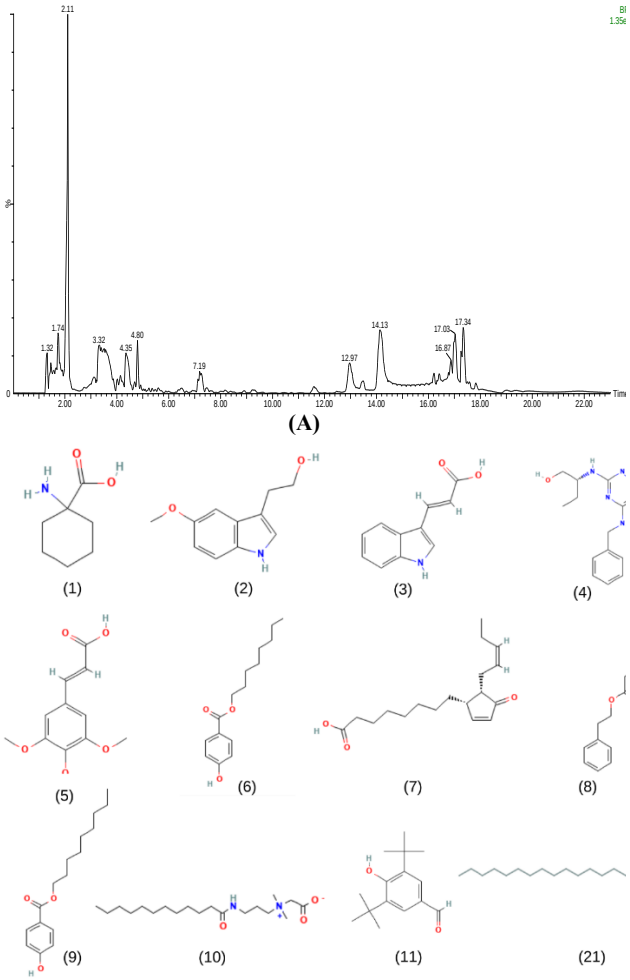
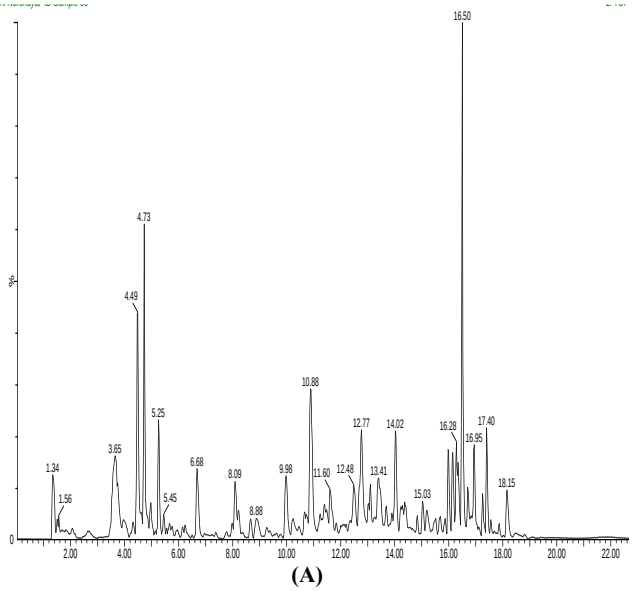


Figure 3: Identification of bioactive compounds in the ethanol extract of *T. leucostaphyllum* leaves using a UPLC-MS/MS instrument. (A) Chromatogram of UPLC-MS/MS results. (B) Structure of identified compounds in ethanol extract of *T. leucostaphyllum* leaves, including alkaloid (2,3,4,12); phenolic (5,6,9,11); amino acid (1); aromatic ester (8); lipid (10).



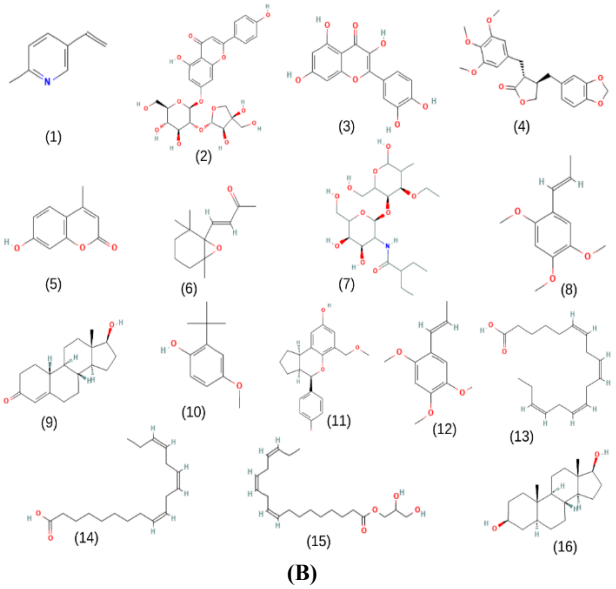


Figure 4: Identification of bioactive compounds in the ethanol extract of *T. dichotomum* leaves using a UPLC-MS/MS instrument. (A) Chromatogram of UPLC-MS/MS results. (B) Structure of identified compounds in ethanol extract of *T. dichotomum* leaves, including alkaloid (1); flavonoid (2,3,5,7,11); phenolic (4,10); terpenoid (6), phenylpropanoid (8,12); steroid (9,16); lipid (13,14,15).

These compounds can affect membrane dynamics, hormonal pathways, and systemic plant responses, which may favor parasitic establishment.[27] The different metabolite profiles between host and non-host species suggest that *Rafflesia* compatibility may depend on more than a single compound and complex interplay between secondary metabolites, hormones, and environmental signals.[51] Specific alkaloids and lipids in host species may act as chemical cues or physiological facilitators for *Rafflesia* endophyte development.[52,53] Moreover, the non-host *T. papillosum* may produce inhibitory compounds that disrupt the parasitic life cycle.[54] The absence of *Rafflesia* parasitism on this species supports the hypothesis that chemical composition is a key determinant in host selection and compatibility.

R. zollingeriana displayed a distinctive phytochemical profile characterized by strong antioxidant activity despite low total flavonoid content. UPLC-MS/MS analysis of its perigone extract revealed a wide array of non-flavonoid secondary metabolites, including glycosides, alkaloids, polyketides, lipids, terpenoids, and polyphenols. This antioxidant potential likely results from synergistic interactions among these compounds, a pattern seen in parasitic or stress-adapted plants that require enhanced protection of reproductive tissues during short-lived flowering stages.[54] While *T. papillosum* (non-host) was rich in flavonoids but showed weak antioxidant activity, *R. zollingeriana* exhibited strong antioxidant activity despite low flavonoid content. This underscores the importance of considering the complete phytochemical spectrum when assessing antioxidant potential. In contrast to its host species, *R. zollingeriana* appears to rely more on alkaloids, polyphenols, and lipid-associated antioxidants, which may offer more targeted or efficient ROS-scavenging during flowering. *T. papillosum* may maintain a high-flavonoid defence strategy as a barrier to parasitism, while *R. zollingeriana*, dependent on the biochemical environment of its host, utilizes a broader metabolite strategy for oxidative protection.

Table 4: Phytochemical identification of ethanol extract from *T. leucostaphyllum* leaves using UPLC-MS/MS

Peak	Retention Time (RT)	% Area	Measured Mass (m/z)	Calculate Massa (m/z)	Formula	Compound	Group
1	1.809	**10.60**	144.1028	144.1025	$C_7H_{14}NO_2$	Homocycloleucine	Amino acid
2	3.123	**19.21**	192.1030	192.1025	$C_{11}H_{14}NO_2$	5-Methoxytryptophol	Alkaloid
3	4.353	**6.46**	188.0721	188.0712	$C_{11}H_{10}NO_2$	3-Indoleacrylic acid	Alkaloid
4	5.366	0.91	355.2228	355.2246	$C_{19}H_{27}N_6O$	roscovitine	Alkaloid
5	7.124	**3.07**	225.0770	225.0763	$C_{11}H_{13}O_5$	Sinapinic acid	Phenolic
6	8.438	0.49	265.1801	165.1804	$C_{16}H_{25}O_3$	Nonylparaben	Phenolic
7	8.789	0.19	293.2120	293.2117	$C_{18}H_{29}O_3$	Piperlongumine	Terpenoid
8	9.233	0.44	249.1859	249.1855	$C_{16}H_{25}O_2$	Phenethyl octanoate	Aromatic ester
9	9.563	0.08	265.1808	265.1804	$C_{16}H_{25}O_3$	Nonylparaben	Phenolic
10	10.723	0.03	343.2957	343.2961	$C_{19}H_{39}N_2O_3$	Cocamidopropyl betaine	Lipid
11	10.970	0.07	235.1701	235.1698	$C_{15}H_{23}O_2$	4-Formyl-2,6-di-tert-butylphenol	Phenolic
12	12.966	**4.01**	304.3011	304.3004	$C_{21}H_{38}N$	pentadecylpyridine	Alkaloid

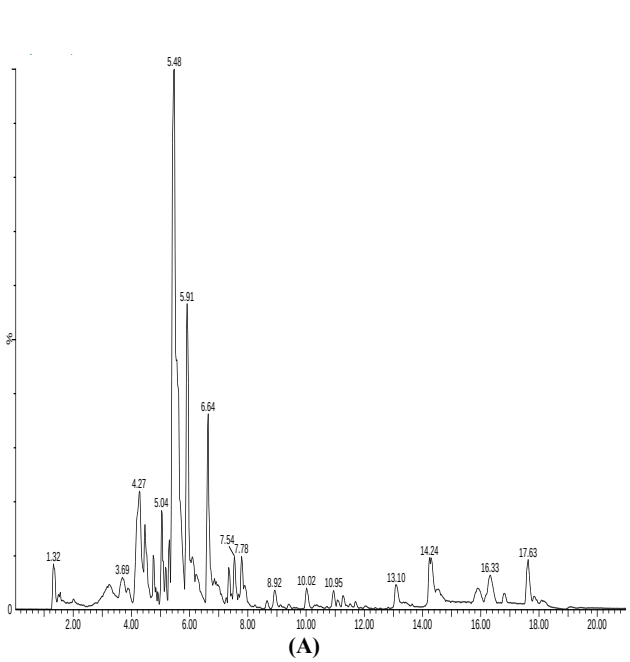
Table 5: Phytochemical identification of ethanol extract from *T. dichotomum* leaves using UPLC-MS/MS

Peak	Retention Time (RT)	% Area	Measured Mass (m/z)	Calculate Massa(m/z)	Formula	Compound	Group
1	3.650	**8.81**	120.0818	120.0813	$C_8H_{10}N$	2-Methyl-5-vinylpyridine	Alkaloid
2	4.973	**7.34**	565.1555	565.1557	$C_{26}H_{29}O_{14}$	Apioside	Flavonoid
3	5.253	2.97	303.0517	303.0505	$C_{15}H_{11}O_7$	Quercetin	Flavonoid
4	5.676	0.33	401.1620	401.1600	$C_{22}H_{25}O_7$	Yatein	Phenolic
5	5.956	0.33	177.0565	177.0552	$C_{10}H_9O_3$	Hymecromone	Flavonoid
6	6.245	0.48	209.1552	209.1542	$C_{13}H_{21}O_2$	beta-Ionone epoxide	Terpenoid
7	8.086	**4.04**	466.2654	466.2652	$C_{21}H_{40}NO_{10}$	Neohesperidin dihydrochalcone	Flavonoid
8	8.312	0.49	209.1192	209.1178	$C_{12}H_{17}O_3$	Asarone	Phenylpropanoid
9	8.881	1.11	275.2018	275.2011	$C_{18}H_{27}O_2$	Nndrolone	Steroid
10	9.957	2.64	181.1237	181.1229	$C_{11}H_{17}O_2$	Butylated Hydroxyanisole	Phenolic
11	10.878	**7.12**	327.1606	327.1596	$C_{20}H_{23}O_4$	**Liquiritigenin**	Flavonoid
12	11.250	3.47	209.1183	209.1178	$C_{12}H_{17}O_3$	Asarone	Phenylpropanoid
13	12.769	**3.98**	277.2178	277.2168	$C_{18}H_{29}O_2$	Stearidonic acid	Lipid
14	13.388	2.05	279.2325	279.2324	$C_{18}H_{31}O_2$	Linolenic acid	Lipid
15	14.836	0.11	353.2688	353.2692	$C_{21}H_{37}O_4$	Glyceryl linolenate	Lipid
16	15.188	1.37	293.2488	293.2481	$C_{19}H_{33}O_2$	3beta-androstanediol	Steroid

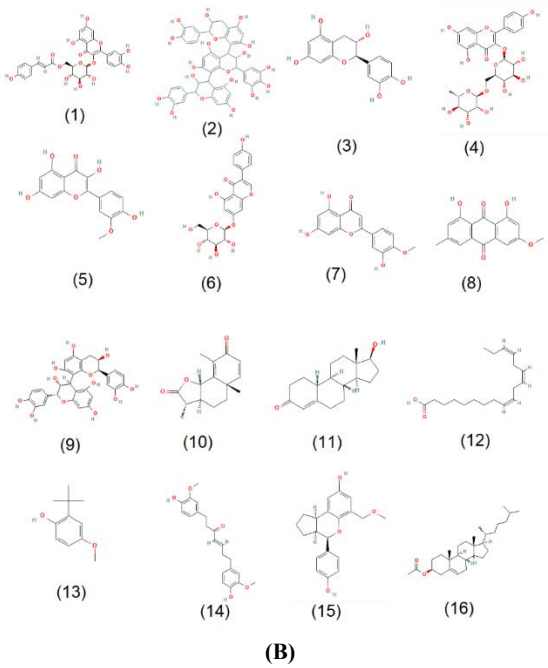
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 6: Phytochemical identification of ethanol extract from *T. papillosum* leaves using UPLC-MS/MS

Peak	Retention Time (RT)	%Area	Measured Mass (m/z)	Calculate Mass (m/z)	Formula	Compound	Group
1	2.336	0.79	611.1385	611.1401	$C_{30}H_{27}O_{14}$	Helichrysoside	Phenolic
2	3.868	1.83	883.2084	883.2086	$C_{45}H_{39}O_{19}$	Cathecin	Flavonoid
3	4.466	**9.79**	291.0882	291.0869	$C_{15}H_{15}O_6$	Cianidanol	Flavonoid
4	4.747	0.44	595.1662	595.1663	$C_{27}H_{31}O_{15}$	Kaempferol	Flavonoid
5	5.303	2.12	317.0667	317.0661	$C_{16}H_{13}O_7$	Quercetin	Flavonoid
6	5.479	**35.58**	433.1136	433.1135	$C_{21}H_{21}O_{10}$	Genistin	Flavonoid
7	6.090	**13.01**	301.0719	301.0712	$C_{16}H_{13}O_6$	Diosmetin	Flavonoid
8	6.638	6.84	285.0772	285.0763	$C_{16}H_{13}O_5$	Physcion	Anthraquinone
9	7.693	2.49	579.1494	579.1503	$C_{30}H_{27}O_{12}$	Procyanidin B2	Flavonoid
10	7.911	2.30	247.1340	247.1334	$C_{15}H_{19}O_3$	Santonin	Terpenoid
11	8.923	0.96	275.2023	275.2011	$C_{18}H_{27}O_2$	Nandrolone	Steroid
12	9.120	0.21	279.2336	279.2324	$C_{18}H_{31}O_2$	Linolenic acid	Lipid
13	10.020	0.81	181.1237	181.1229	$C_{11}H_{17}O_2$	Butylated Hydroxytoluene	Phenolic
14	10.723	0.31	357.1697	357.1702	$C_{21}H_{25}O_5$	Gingerenone	Phenolic
15	10.949	0.83	327.1589	327.1596	$C_{20}H_{23}O_4$	Liquiritigenin	Flavonoid
16	17.628	2.66	429.3751	429.3733	$C_{29}H_{49}O_2$	Cholesteryl acetate	Steroid



(A)



(B)

Figure 5: Identification of bioactive compounds in the ethanol extract of *T. papillosum* leaves using a UPLC-MS/MS instrument. (A) Chromatogram of UPLC-MS/MS results. (B) Structure of identified compounds in ethanol extract of *T. papillosum* leaves, including flavonoid (2,3,4,5,6,7,9,15); phenolic (113,14,10); terpenoid (10), anthraquinone (8); steroid (11); lipid (12).

Conclusion

R. zollingeriana showed very strong antioxidant activity despite low flavonoid levels, likely due to non-flavonoid compounds such as alkaloids, glycosides, and lipids. Among its hosts, *T. dichotomum* had the highest flavonoid content with moderate antioxidant activity. While *T. leucostaphylum* showed strong antioxidant activity with low flavonoid levels. The distinct metabolite profiles between host and non-host species suggest that chemical factors influence parasitic compatibility. These findings highlight the need for broader phytochemical analysis in evaluating antioxidant activity and plant-parasite interactions.

Conflicts of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Malabrigo Jr P, Tobias AB, Witono J , Mursidawati S, Susatya A, Siti-Munirah MY, Wicaksono A, Raihandhany R, Edwards S, Thorogood CJ. Most of the World's Largest Flowers (Genus *Rafflesia*) Are Now on the Brink of Extinction. Plants People Planet. 2023; 7(2):331–346. Doi: 10.1002/ppp3.10431
2. Sofiyanti N, Mat-Salleh K, Mahmud K, Mazlan NZ, Hasein MRA, Burslem DFRP. *Rafflesia parvimaculata* (Rafflesiaceae), a new species of *Rafflesia* from Peninsular Malaysia. Phytotaxa. 2016; 253(3):207-213. Doi: 10.11646/phytotaxa.253.3.4
3. Siahaan FA, Iryadi R, Lestari D. Edaphic Characteristics of *Rafflesia* Habitats in Indonesia: Implications for Conservation and Propagation. J Trop Biodiv Biotechnol. 2024; 9(3):1-11. Doi: 10.22146/jtbb.89231
4. Nurchayati N, Batoro J, Hakim L, Azrianingsih R. Ethnobotanical study of *Rafflesia zollingeriana* and its host (*Tetrastigma* spp.) at Papring forest, Kalipuro, Banyuwangi, East Java, Indonesia. BIO Web Conf. 2024; 117(01019):1-10. Doi: 10.1051/bioconf/202411701019
5. Lestari D, Rianto N. Utilization and community attitude toward *Rafflesia zollingeriana* and its conservation in Meru Betiri National Park. Jurnal Penelitian Sosial dan Ekonomi Kehutanan. 2019; 16(3):211-226. Doi: 10.20886/jpsek.2019.16.3.211-226
6. Zhang L, Li B, Wang M, Lin H, Peng Y, Zhou X, Peng C, Zhan J, Wang W. Genus *Tetrastigma*: A review of its folk uses, phytochemistry and pharmacology. Chin Herb Med. 2022; 14(2):210-233. Doi: 10.1016/j.chmed.2022.03.003
7. Zheng W, Wang H, Wang X, Li X, Hu J, Zi X, Zhou Y, Pan D, Fu Y. Kaempferol 3-O-Rutinoside, a Flavone Derived from *Tetrastigma hemsleyanum* Diels et Gilg, Reduces Body Temperature through Accelerating the Elimination of IL-6 and TNF-α in a Mouse Fever Model. Molecules. 2024; 29(7):1-20. Doi: 10.3390/molecules29071641
8. Shu J, Zhao Y, Zhou Y, Lin F, Song J, Li X. Optimization of *Tetrastigma hemsleyanum* extraction process based on GA-BPNN model and analysis of its antioxidant effect. Heliyon. 2023; 9(10):1-21. Doi: 10.1016/j.heliyon.2023.e20200
9. Sun Y, Guo F, Peng X, Cheng K, Xiao L, Zhang H, Li H, Jiang L, Deng Z. Metabolism of phenolics of *Tetrastigma hemsleyanum* roots under in vitro digestion and colonic fermentation as well as their in vivo antioxidant activity in rats. Foods. 2021; 10(9):1-14. Doi: 10.3390/foods10092123.
10. Wang CY, Jang HJ, Han YK, Su XD, Lee SW, Rho MC, Wang HS, Yang SY, Kim YH. Alkaloids from *Tetrastigma hemsleyanum* and their anti-inflammatory effects on LPS-Induced RAW264.7 cells. Molecules. 2018; 23(6):1-11. Doi: 10.3390/molecules23061445
11. Ministry of Health Republic Indonesia. Indonesian Herbal Pharmacopoeia Edition II: Supplement I. Jakarta: Ministry of Health Republic Indonesia; 2022.
12. Egharevba E, Chukwuemeke-Nwani P, Eboh U, Okoye E, Bolanle IO, Oseghale IO, Imieje VO, Erharuyi O, Falodun A. Evaluation of the antioxidant and hypoglycaemic potentials of the leaf extracts of *Stachytarphyta jamaicensis* (Verbenaceae). Trop J Nat Prod Res. 2019; 3(5):170-174. doi: 10.26538/tjnpr/v3i5.4.
13. Iheanacho CM, Akubuiro PC, Oseghale IO, Imieje VO, Erharuyi O, Ogbeide KO, Jideonwo AN, Falodun A. Evaluation of the antioxidant activity of the stem bark extracts of *Anacardium occidentale* (Linn) Anacardiaceae. Trop J Phytochem Pharm Sci. 2023; 2(2):65-69. Doi: 10.26538/tjpps/v2i2.4.
14. Mutiah R, Rachmawati E, Fitrianingsih AA, Zahiro SR. Metabolite profiling of anticancer compounds in *Saussure lappa* based on UPLC-QToFMS/MS. Pharm Educ. 2023; 23(4):37-42. Doi: 10.46542/pe.2023.234.3742
15. Pujiawati Y, Khotijah L, Wiryawan IKG. Screening of antioxidant activities and their bioavailability of tropical plants. IOP Conf Ser Earth Environ Sci. 2023; 1182:1-5. Doi: 10.1088/1755-1315/1182/1/012083
16. Christina YI, Nafisah W, Widodo, Rifa'i M, Djati MS. Evaluation of total phenolic, flavonoid contents, antioxidant and cytotoxicity activities of various parts of *Phaleria macrocarpa* (Scheff.) Boerl fruit. IOP Conf Ser Earth Environ Sci. 2021; 743:1-7. Doi: 10.1088/1755-1315/743/1/012026
17. Maury GL, Rodríguez DM, Hendrix S, Arranz JCE, Boix YF, Pacheco AO, Díaz JG, Morris-Quevedo HJ, Dubois AF, Aleman EI, Beenaerts N, Méndez-Santos IE, Ratón TO, Cos P, Cuypers A. Antioxidants in plants: a valorization potential emphasizing the need for the conservation of plant biodiversity in Cuba. Antioxidants. 2020; 9(11):1-36. Doi: 10.3390/antiox9111048
18. Kruk J, Aboul-Enein BH, Duchnik E, Marchlewicz. Antioxidative properties of phenolic compounds and their effect on oxidative stress induced by severe physical exercise. J Physion Sci. 2022; 19:1-24. Doi: 10.1186/s12576-022-00845-1
19. Sirin S, Dolanbay SN, Aslim B. Role of plant derived alkaloids as antioxidant agents for neurodegenerative diseases. Health Sci Rev. 2023; 6:1-11. Doi: 10.1016/j.hsr.2022.100071
20. Gutiérrez-Del-Río I, López-Ibáñez S, Magadán-Corpas P, Fernández-Calleja L, Pérez-Valero Á, Tuñón-Granda M, Miguélez EM, Villar CJ, Lombó F. Terpenoids and Polyphenols as natural antioxidant agents in food preservation. Antioxidants. 2021; 10(8):1-33. Doi: 10.3390/antiox10081264
21. Hassanpour SH, Doroudi A. Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. Avicenna J Phytomed. 2023; 13(4):354-376. Doi: 10.22038/AJP.2023.21774
22. Mata-Espinosa D, Molina-Salinas GM, Barrios-Payán J, Navarrete-Vázquez G, Marquina B, Ramos-Espinosa O, Bini EI, Baeza I, Hernández-Pando R. Therapeutic efficacy of liposomes containing 4-(5-pentadecyl-1,3,4-oxadiazol-2-yl)pyridine in a murine model of progressive pulmonary tuberculosis. Pulm Pharmacol Ther. 2015; 32:7-14. Doi: 10.1016/j.pupt.2015.03.004
23. Liu K, Chen Q, Luo H, Li R, Chen L, Jiang B, Liang Z, Wang T, Ma Y, Zhao M. An in vitro catalysis of tea polyphenols by polyphenol oxidase. Molecules. 2023; 28(4):1-22. Doi: 10.3390/molecules28041722

24. Ritonga FN, Zhou D, Zhang Y, Song R, Li C, Li J, Gao J. The roles of gibberellins in regulating leaf development. Plants. 2023; 12(6):1-19. Doi: 10.3390/plants12061243
25. Capasso L, De Masi L, Sirignano C, Maresca V, Basile A, Nebbioso A, Rigano D, Bontempo P. Epigallocatechin Gallate (EGCG): Pharmacological properties, biological activities and therapeutic potential. Molecules. 2025; 30(3):1-34. Doi: 10.3390/molecules30030654
26. Duta-Bratu CG, Nitulescu GM, Mihai DP, Olaru OT. Resveratrol and other natural oligomeric stilbenoid compounds and their therapeutic applications. Plants. 2023; 12(16):1-16. Doi: 10.3390/plants12162935
27. Shrestha H, Bala R, Arora S. Lipid-based drug delivery systems. J Pharm. 2014; 2014:1-10. Doi: 10.1155/2014/801820
28. Mierziak J, Kostyn K, Kulma A. Flavonoids as important molecules of plant interactions with the environment. Molecules. 2014; 19(10):16240-16265. Doi: 10.3390/molecules191016240
29. Molina J, de Guzman RC, Abzalimov R, Huang W, Guruprasad A, Pedales R, Wicaksono A, Davis D, Callado JR, Bänziger H, Suksathan P, Eaton W, Yin P, Bürger M, Erickson M, Jones S, Adams J, Pell S. Microbes and metabolites of a plant-parasite interaction: Deciphering the ecology of *Tetrastigma* host choice in the world's largest parasitic flower, *Rafflesia*. Curr Plant Biol. 2025; 42:1-16. Doi: 10.1016/j.cpb.2025.100456
30. Molina J, Nikolic D, Jeevarathanam JR, Abzalimov R, Park EJ, Pedales R, Mojica EE, Tandang D, McLaughlin W, Wallick K, Adams J, Novy A, Pell SK, van Breemen RB, Pezzuto JM. Living with a giant, flowering parasite: metabolic differences between *Tetrastigma loheri* Gagnep. (Vitaceae) shoots uninfected and infected with *Rafflesia* (Rafflesiaceae) and potential applications for propagation. Planta. 2021; 255(1):1-12. Doi: 10.1007/s00425-021-03787-x
31. Wang Y, Mostafa S, Zeng W, Jin B. Function and Mechanism of Jasmonic Acid in Plant Responses to Abiotic and Biotic Stresses. Int J Mol Sci. 2021; 22:1-26. Doi: 10.3390/ijms22168568
32. Pang Z, Chen J, Wang T, Gao C, Li Z, Guo L, Xu J, Cheng Y. Linking Plant Secondary Metabolites and Plant Microbiomes: A Review. Front Plant Sci. 2021; 12:1-22. Doi: 10.3389/fpls.2021.621276
33. Divekar PA, Narayana S, Divekar BA, Kumar R, Gadratagi BG, Ray A, Singh AK, Rani V, Singh V, Singh AK, Kumar A, Singh RP, Meena RS, Behera TK. Plant secondary metabolites as defense tools against herbivores for sustainable crop protection. Int J Mol Sci. 2022; 23(5):1-24. Doi: 10.3390/ijms23052690
34. Serdo DF. Insects' perception and behavioral responses to plant semiochemicals. PeerJ. 2024; 12:1-20. Doi: 10.7717/peerj.17735
35. Anastassova N, Stefanova D, Hristova-Avakumova N, Georgieva I, Kondeva-Burdina M, Rangelov M, Todorova N, Tzoneva R, Yancheva D. New indole-3-propionic acid and 5-methoxy-indole carboxylic acid derived hydrazone hybrids as multifunctional neuroprotectors. Antioxidants. 2023; 12(4):1-33. Doi: 10.3390/antiox12040977
36. Wlodarska M, Luo C, Kolde R, d'Hennezel E, Annand JW, Heim CE, Krastel P, Schmitt EK, Omar AS, Creasey EA, Garner AL, Mohammadi S, O'Connell DJ, Abubucker S, Arthur TD, Franzosa EA, Huttenhower C, Murphy LO, Haiser HJ, Vlamakis H, Porter JA, Xavier RJ. Indoleacrylic acid produced by commensal peptostreptococcus species suppresses inflammation. Cell Host Microbe. 2017; 22(1):25-37. Doi: 10.1016/j.chom.2017.06.007
37. Cicenas J, Kalyan K, Sorokinas A, Stankunas E, Levy J, Meskinyte I, Stankevicius V, Kaupinis A, Valius M. Roscovitine in cancer and other diseases. Ann Transl Med. 2015; 3(10):1-12. Doi: 10.3978/j.issn.2305-5839.2015.03.61
38. Sun W, Shahrajabian MH. Therapeutic potential of phenolic compounds in medicinal plants-natural health products for human health. Molecules. 2023; 28(4):1-43. Doi: 10.3390/molecules28041845
39. Bin Jardan YA, Ansari MA, Raish M, Alkharfy KM, Ahad A, Al-Jenoobi FI, Haq N, Khan MR, Ahmad A. Sinapic acid ameliorates oxidative stress, inflammation, and apoptosis in acute doxorubicin-induced cardiotoxicity via the NF-κB-mediated pathway. Biomed Res Int. 2020; 2020:1-10. Doi: 10.1155/2020/3921796
40. Parama D, Rana V, Girisa S, Verma E, Daimary UD, Thakur KK, Kumar A, Kunnumakkara AB. The promising potential of piperlongumine as an emerging therapeutics for cancer. Explor Target Antitumor Ther. 2021; 2(4):323-354. Doi: 10.37349/etat.2021.00049
41. Wang PG, Zhou W. Rapid determination of cocamidopropyl betaine impurities in cosmetic products by core-shell hydrophilic interaction liquid chromatography-tandem mass spectrometry. J Chromatogr. 2016; 1461-78-83. Doi: 10.1016/j.chroma.2016.07.056
42. Lin SX, Curtis MA, Sperry J. Pyridine alkaloids with activity in the central nervous system. Bioorg Med Chem. 2020; 28(24):1-20. Doi: 10.1016/j.bmc.2020.115820
43. Al-Khayri JM, Sahana GR, Nagella P, Joseph BV, Alessa FM, Al-Mssallem MQ. Flavonoids as Potential Anti-Inflammatory Molecules: A Review. Molecules. 2022; 27(9):1-24. Doi: 10.3390/molecules27092901
44. Wu Y, Wang Z, Du Q, Zhu Z, Chen T, Xue Y, Wang Y, Zeng Q, Shen C, Jiang C, Liu L, Zhu H, Liu Q. Pharmacological effects and underlying mechanisms of licorice-derived flavonoids. Evid Based Complement Alternat Med. 2022; 2022:1-25. Doi: 10.1155/2022/9523071
45. Ren J, Li Z, Li X, Yang L, Bu Z, Wu Y, Li Y, Zhang S, Meng X. Exploring the Mechanisms of the antioxidants BHA, BHT, and TBHQ in hepatotoxicity, nephrotoxicity, and neurotoxicity from the perspective of network toxicology. Foods. 2025; 14(7):1-21. Doi: 10.3390/foods14071095
46. Chellian R, Pandy V, Mohamed Z. Pharmacology and toxicology of α- and β-Asarone: A review of preclinical evidence. Phytomedicine. 2017; 32:41-58. Doi: 10.1016/j.phymed.2017.04.003
47. Sharifi-Rad J, Quispe C, Imran M, Rauf A, Nadeem M, Gondal TA, Ahmad B, Atif M, Mubarak MS, Sytar O, Zhilina OM, Garsiya ER, Smeriglio A, Trombetta D, Pons DG, Martorell M, Cardoso SM, Razis AFA, Sunusi U, Kamal RM, Rotariu LS, Butnariu M, Docea AO, Calina D. Genistein: an integrative overview of its mode of action, pharmacological properties, and health benefits. Oxid Med Cell Longev. 2021; 2021:1-36. Doi: 10.1155/2021/3268136
48. Zahra M, Abrahamse H, George BP. Flavonoids: Antioxidant powerhouses and their role in nanomedicine. Antioxidants. 2024; 13(8):1-26. Doi: 10.3390/antiox13080922
49. Yehye WA, Rahman NA, Ariffin A, Abd Hamid SB, Alhadi AA, Kadir FA, Yaeghoobi M. Understanding the chemistry behind the antioxidant activities of butylated hydroxytoluene (BHT): a review. Eur J Med Chem. 2015; 101:295-312. doi: 10.1016/j.ejmech.2015.06.026.
50. Singh B, Sharma RA. Plant terpenes: defense responses, phylogenetic analysis, regulation and clinical applications. 3 Biotech. 2015; 5(2):129-151. Doi: 10.1007/s13205-014-0220-2
51. Wicaksono A, Mursidawati S, Molina J. A plant within a plant: Insights on the development of the *Rafflesia* endophyte within its host. Bot Rev. 2021; 87(2):233–242. Doi: 10.1007/s12229-020-09236-w
52. Susatya A, Lestari D, Mahyuni R, Kusuma YWC, Dalimunthe SH, Nurchayati N, Ardiyansyah F, As'ari H. Morphological variation and distribution of a newly recorded *Rafflesia zollingeriana*. J Trop Forest Sci. 2023; 35(4):465–475. Doi: 10.26525/jtfs2023.35.4.465

53. Lestari D, Susatya A. Morphological variation of *Rafflesia zollingeriana* Koord. and its recent distribution in East Java, Indonesia. Biosaintifika J Biol Biol Educ. 2022; 14(1):117-124 Doi: 10.15294/biosaintifika.v14i1.33089
54. Ali M, Cheng Z, Ahmad H, Hayat S. Reactive oxygen species (ROS) as defenses against a broad range of plant fungal infections and case study on ROS employed by crops against *Verticillium dahliae* wilts. J. Plant Interact. 2018; 13(1):353–363. Doi: 10.1080/17429145.2018.1484188