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Wild Gingers of Malaysia (Zingiberaceae Martinov): A Source of Bioactive Phytocompounds with Promising Antioxidant and Antidiabetic Potentials

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ABSTRACT

Oxidative stress contributes to chronic diseases including diabetes, cardiovascular disorders and neurodegeneration. Although Zingiberaceae species are valued in ethnomedicine, wild Malaysian gingers remain underexplored for their therapeutic properties, particularly from leaves and stems. This study investigated the phytochemical composition, antioxidant and α-glucosidase inhibitory activities of leaves, stems and rhizomes from seven wild Zingiberaceae species that were collected from Panti Forest Reserve and Endau-Rompin National Park, Johor, Malaysia. Total phenolic content (TPC), total flavonoid content (TFC),

and total terpenoid content (TTC) were quantified using colourimetric assays. Antioxidant activities were assessed via DPPH, ABTS, FRAP and nitric oxide (NO) scavenging assays. The α-glucosidase inhibition was evaluated using enzyme-based colourimetric assays. Leaves consistently exhibited the highest TPC (up to 57.88 mg GAE/g DW), TFC (72.83 mg RE/g DW) and TTC (0.08 mg LE/g DW), followed by rhizomes and stems. Antioxidant assays showed leaves had superior radical scavenging in ABTS and FRAP assays, rhizomes showed stronger DPPH activity, while stems showed the least activity. Correlation analysis indicated that phenolic compounds were the primary contributors to antioxidant activity. α-Glucosidase inhibition was only detected in rhizomes of *Boesenbergia plicata* (IC_{50} = 16.77 µg/mL) and *Sundamomum hastilabium* (IC_{50} = 20.26 µg/mL), comparable to cultivated Zingiberaceae species. Wild Malaysian ginger leaves were rich sources of antioxidant phytochemicals, while rhizomes of selected species showed promising antidiabetic activity. These findings highlight the therapeutic potential of underexplored Zingiberaceae species and support sustainable utilisation beyond rhizomes.

Keywords: α-glucosidase inhibition, antioxidant activity, phytochemical profiling, wild ginger Malaysia, wild Zingiberaceae

INTRODUCTION

Oxidative stress is a major factor in the development of chronic diseases such as diabetes mellitus, cardiovascular disorders, and neurodegenerative conditions (Reddy, 2023). Excessive generation of Reactive Oxygen Species (ROS) can disrupt cellular redox homeostasis, resulting in oxidative damage to proteins, lipids, and nucleic acids, which ultimately contributes to inflammation and metabolic dysfunction (Liguori et al., 2018). Therefore, natural antioxidants derived from plants have gained considerable attention as safer and more biocompatible alternatives to synthetic agents for preventing and managing oxidative stress-related diseases. Phenolic compounds and flavonoids present in Zingiberaceae species have attracted considerable interest due to their potent antioxidant and antidiabetic properties. For example, *Kaempferia galanga* has demonstrated strong DPPH and ABTS radical scavenging activities that are related to its rich phytochemical composition (Nonglang et al., 2022). Similarly, essential oils from *K. galanga* exhibited both in vitro and in vivo antioxidant activities (Wang et al., 2023b). In addition, bioactive constituents isolated from *Alpinia galanga* have shown alpha-glucosidase and protein tyrosine phosphatase 1B (PTP1B) inhibitory activities, which are potentially related to diabetes management (Liu et al., 2023). These previous findings highlight Zingiberaceae species as promising natural alternatives for mitigating oxidative stress and metabolic disorders.

With approximately 1,449 accepted species across 56 genera, the Zingiberaceae family is highly diverse in Southeast Asia and is particularly valued in ethnomedicine for its antioxidant, anti-inflammatory, and antidiabetic properties (Sedek et al., 2024).

Cultivated species such as *Zingiber officinale* (ginger), *Curcuma longa* (turmeric) and *Kaempferia galanga* (kencur) specifically have been extensively studied and demonstrate significant antioxidant activity and glucose-lowering effects due to their high content of phenolic, flavonoid, and terpenoid constituents. For instance, gingerol and shogaol from *Z. officinale* and curcuminoids from *C. longa* are well-known active compounds with antioxidant and antidiabetic activities (Gu et al., 2024; Mashhadi et al., 2013). However, the majority of these studies focus on the rhizome and frequently ignore other plant parts that may have medicinal significance. Recent studies have shown that aerial parts of cultivated Zingiberaceae species are valuable sources of phytochemicals. For instance, leaves of *C. longa* were reported to contain abundant terpenoids and exhibit considerable antioxidant activities (Braga et al., 2023). Similarly, postharvest residues and shoots of *Z. officinale* retained substantial antioxidant capacity (Jorge-Montalvo et al., 2023). Other than that, strong antioxidant properties were reported from essential oils of *K. galanga* in both in vitro and in vivo studies, indicating that bioactive constituents are not limited to rhizomes alone (Wang et al., 2023b). This suggests that relying solely on rhizomes might ignore other plant parts with equal or greater therapeutic potential, as well as contribute to unsustainable harvesting practices.

Despite the extensive utilisation of cultivated gingers, the phytochemical diversity and biological activities of wild Malaysian Zingiberaceae remain poorly explored. Species such as *Boesenbergia plicata* (Ridl.) Holttum, *Boesenbergia prainiana* (King ex Baker) Schltr., *Globba patens* Miq., *Hornstedia leonurus* (J. Koenig) Retz., *Scaphochlamys klossii* (Ridl.) Holttum, *Sundamomum hastilabium* (Ridl.) A.D. Poulsen & M.F. Newman, and *Amomum* sp., have received limited scientific attention. Recent metabolomic studies have shown that wild relatives of cultivated gingers may possess unique chemical profiles shaped by ecological adaptation and environmental factors. According to Singh et al. (2025), there were substantial differences in the composition of secondary metabolites between wild and cultivated Zingiberaceae, including phenolic derivatives, terpenoids, and other specialised metabolites. This study strongly suggests that wild relatives of cultivated gingers are chemically unique resources with untapped medicinal potential. Similar studies conducted in other Southeast Asian countries such as Thailand and Indonesia have demonstrated considerable variations in phytochemical composition and antioxidant activity not just among different species but also among plant organs. This suggests that leaves and stems may contain bioactive compounds similar to those found in rhizomes (Hariyanto et al., 2024; Uthumporn et al., 2016). Nonetheless, comprehensive comparative investigations involving various plant organs across wild Malaysian Zingiberaceae species remain scarce. Therefore, a systematic evaluation of phytochemical contents and biological activities among different plant parts is necessary to identify promising species and support the sustainable utilisation of Malaysia's native ginger resources.

Therefore, this study aims to address this knowledge gap by evaluating the phytochemical content and bioactivities within leaves, stems and rhizomes of seven wild Zingiberaceae species from Panti Forest Reserve and Endau-Rompin National Park, Johor, Malaysia. In each sample, the total phenolic content (TPC), total flavonoid content (TFC), and total terpenoid content (TTC) are measured. These profiles are then correlated with robust antioxidant (DPPH, ABTS, FRAP, and NO) and antidiabetic assays. Ultimately, this study aims to identify promising species and plant parts that may serve as a new source of natural antioxidants and antidiabetic agents while also contributing to sustainable resource utilisation in Malaysian forests.

MATERIALS AND METHODS

Sample Collection and Preparation

The study was conducted in Panti Forest Reserve and Endau-Rompin National Park, Johor, Malaysia, which are characterised as humid lowland rainforest with shaded areas and rocky stream areas near waterfalls. Wild ginger species were sampled from suitable habitats including stream banks, swampy areas, and lowland forests at 200-500 m above sea level (a.s.l.) (Sedek et al., 2024). Six wild ginger species were collected from Panti Forest Reserve (Kota Tinggi, Johor, Malaysia) and one species from Endau-Rompin National Park (Johor, Malaysia). All specimens were taxonomically identified and deposited into the herbarium in Universiti Tun Hussien Onn Malaysia (UTHM) with assigned voucher numbers (*S. klossii* - SM0079; *G. patens* - SM0088*; B. prainiana* - SM0090*; S. hastilabium* - SM0091*; B. plicata* - SM0102*; Amomum* sp. - SM0103; and *H. leonurus* - SM0104) (Figure 1, Figure 2). Plant collection was conducted with permission from the relevant authorities and in accordance with applicable local regulations governing access to forest reserves and protected areas in Johor, Malaysia. For extraction preparation and phytochemical analysis, healthy and fully expanded mature leaves, together with mature stems and rhizomes, were collected from each species. To minimise variation associated with plant developmental stage, all plant materials were sampled from mature individuals at the flowering stage or post-vegetative stage. The rhizomes, stems and leaves of the seven wild ginger species were cleaned and thoroughly rinsed under running tap water to remove any debris and soil. Next, the cleaned samples were sliced into small pieces and dried at 60 °C using the oven-drying method for 72 hours (Uthumporn et al., 2016). After drying, the samples were ground into powder form, sealed properly, and stored in a freezer at -20 °C for further use.

Figure 1. Wild ginger species (Zingiberaceae) collected from Southern Malaysian Forest Reserva: Panti Forest Reserve and Endau-Rompin National Park, Johor, Malaysia: (A) *Boesenbergia plicata*; (B) *Boesenbergia prainiana*; (C) *Globba patens*; (D) *Scaphochlamys klossii*; (E) *Sundamomum hastilabium*; (F) *Hornstedtia leonurus*. The figure highlights the inflorescence, which was crucial for species identification

Figure 2. Overall vegetative morphology of selected wild ginger species (Zingiberaceae) collected from Southern Malaysian Forest Reserves (Panti Forest Reserve and Endau-Rompin National Park, Johor, Malaysia). Distinct variations in leaf lamina architecture, surface texture, and vegetative habits are observed among the species: (A) *Boesenbergia plicata* (Ridl.) Holttum, demonstrating characteristic deeply plicate, corrugated leaf blades; (B) *Boesenbergia prainiana* (King ex Baker) Schltr., showing a typically monophyllous, broad-ovate leaf habit; (C) *Globba patens* Miq., exhibiting slender, distichous pseudostems with thin, lanceolate leaf blades and undulate margins; (D) *Scaphochlamys klossii* (Ridl.) Holttum, displaying a low-growing terrestrial habit with prominent parallel-pinnate venation; (E) *Sundamomum hastilabium* (Ridl.) A.D. Poulsen & M.F. Newman, indicating an oblong-lanceolate lamina with a long-acuminate apex; (F) *Amomum* sp., illustrating typical large, frond-like forest-canopy pseudostems: and (G) *Hornstedtia leonurus* (J.Koenig) Retz., showcasing robust, coriaceous leaves with elongated, rigid lligules
Source: Taxonomic descriptions and authorities adapted in part from Holttum, 1950; Larsen & Mood, 1998; Sedek et al., 2024, Turner, 1995)

Ethanolic Extraction of Plant Sample

The ethanolic extraction process for each plant part (rhizomes, stems, and leaves), began by weighing 2 g of each dried powder sample and placing them into separate conical flasks. The samples were extracted using 40 mL of absolute ethanol and left for 5 hours at room temperature while being stirred with a magnetic stirrer. Then, the extract was filtered using Whatman filter paper and concentrated using a solvent concentrator for 3 hours at 45°C to obtain a crude extract. The crude extract was then redissolved in absolute ethanol to 8000ppm (8 mg/mL) concentration and stored at 4°C for further use.

Phytocompound Characterisation

Determination of Total Phenolic Content (TPC)

The TPC in leaves, stems, and rhizomes of the wild ginger species was measured separately using the Folin-Ciocalteu method, as described by Sharifi-Rad et al. (2017). The ethanolic extract of each sample (20 µL) was pipette-transferred into a 96-well microplate, and 100 µL of the 10% Folin-Ciocalteu (v/v) reagent was added, and the mixture was incubated for 5 minutes. After 5 minutes, 80 µL of the 7.5% (w/v) sodium carbonate solution (Na_2CO_3) was added, and the mixture was further incubated for 30 minutes. Finally, the absorbance of the mixture was measured at 760 nm using a microplate reader (VersaMax ELISA, United States). The TPC was reported as milligrams of gallic acid equivalents per gram of dry weight sample (mg GAE/g DW). Gallic acid was obtained from Sigma-Aldrich (Hong Kong, China).

Determination of Total Flavonoid Content (TFC)

The TFC in leaves, stem and rhizomes of the wild ginger was measured separately by using the aluminium chloride calorimetric method as described by (Ali et al., 2018) with some modifications. The ethanolic extract of each sample (50 µL) was pipetted and transferred into a 96-well microplate, and 100 µL of 95% ethanol and 20 µL of 10% aluminium chloride [$Al(NO_3)_3$] were added, and the mixture was incubated for 3 minutes in the dark at room temperature. After incubation, 20 µL of the 1.0 M sodium acetate ($C_2H_3NaO_2$) solution was added, and the mixture was further incubated for 40 minutes. Finally, the absorbance of the mixture was measured at 415 nm using a microplate reader (VersaMax ELISA, United States). The TFC was reported as milligrams of rutin equivalents per gram of dry weight sample (mg RE/g DW). Rutin was obtained from Sigma-Aldrich (Hong Kong, China).

Determination of Total Terpenoid content (TTC)

The TTC in leaves, stems, and rhizomes of the wild ginger was measured separately by following the protocol established by Biswas et al. (2021) with slight modifications. The ethanolic extract (200 µL) of each extract sample was mixed with 1 mL of perchloric acid ($HClO_4$) and 300 µL of 5% vanillin solution, followed by 5 mL of glacial acetic acid (CH_3COOH) to the mixture. The mixture was incubated in the dark at room temperature for 45 minutes, and the absorbance of the extract was measured at 548 nm using UV-Vis spectrophotometer (Agilent, United States). TTC was expressed as milligrams of linalool equivalent per gram of dry weight sample (mg LE/g DW). Linalool was obtained from Sigma-Aldrich (Hong Kong, China).

Antioxidant Activities

2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

DPPH radical scavenging assay was determined to assess the antioxidant capacity in accordance with the method described by Idris et al. (2019) with some modification. The ethanolic extract of each sample 200 µL was pipetted and transferred into a 96-well microplate, and 100 µL of ethanol was mixed with the sample using the serial dilution method. Then, 100 µL of ethanolic solution of 0.1 mM DPPH radical solution was added and incubated for 30 minutes in the dark at room temperature. The absorbance of the mixture was measured at 515 nm using a microplate reader (VersaMax ELISA, United States). Inhibition (%) of the extracted sample was calculated using the following Equation 1:

$$DPPH\ Radical\ Scavenging\ Assay\ (\%) = \left[\frac{A_{Control} - A_{sample}}{A_{control}}\right] \times 100\% \qquad [1]$$

where $A_{control}$ is the absorbance of the DPPH solution without antioxidant and A_{sample} is the absorbance of the sample containing antioxidant. The antioxidant activity of each extract sample was expressed as the half maximal inhibitory concentration (IC_{50}) value (mg/mL).

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Radical Scavenging Assay

This assay was conducted following the protocol outlined by Jimoh et al. (2019) with some modifications. The ABTS radical was prepared by mixing equal volumes of 2.45 mM potassium persulfate ($K_2S_2O_8$) and 7 mM ABTS solution prepared in ethanol. The mixture was kept in the dark for 12-18 hours at room temperature and then diluted with ethanol at a 1:50 ratio until an absorbance of 0.700±0.003 was achieved at 734 nm. Then, equal volumes of plant extracts and standard drugs at various concentrations were

mixed with ABTS (1:1 v/v), left in the dark for 6 minutes, and the absorbance was measured at 734 nm. The percentage of ABTS scavenging activity of the crude extracts and standards (rutin) was calculated using the following Equation 2:

$$ABTS\ Radical\ Scavenging\ Assay\ (\%) = \left[\frac{A_{Control} - A_{sample}}{A_{control}}\right] \times 100\% \quad [2]$$

where $A_{control}$ is the absorbance of the ABTS solution without antioxidant and A_{sample} is the absorbance of the sample containing antioxidant. The antioxidant activity of each sample of extract was expressed as the half maximal inhibitory concentration (IC_{50}) value (mg/mL).

Ferric Reducing Antioxidant Power (FRAP)

The iron-reducing efficacy of sample extracts was evaluated using a modified version of the method developed by Hariyanto et al. (2024). This assay measures the reduction of the colourless Fe^{3+}-tripyridyltriazine complex to a blue Fe^{2+}-tripyridyltriazine complex. The FRAP reagent was prepared by mixing 300 mM acetate buffer, 40 mM of hydrogen chloride (HCl), 10 mM (2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) and 20 mM iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$). Standard curves were generated using varying concentrations of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), with all solutions freshly prepared. A reaction mixture consisting of 20 µL of sample solution at different concentrations, 80 µL of distilled water and the FRAP reagent was incubated at 37 °C for 30 minutes. Absorbance was then measured at 593 nm. The difference between the absorbance of the sample and the blank was used to calculate the FRAP value and reduction capacity of the extracts. The FRAP value was expressed in µmol Fe/g.

Nitric Oxide (NO) Radical Scavenging Assay

The inhibitory activity of NO was assessed using the Griess reaction, following the method described by Mfotie Njoya et al. (2017). This approach evaluated the NO scavenging potential of wild ginger extracts and Trolox (used as a standard). Briefly, serial concentrations of the extracts were mixed with sodium nitroprusside ($Na_2[Fe(CN)_5NO]$ and incubated for 20 minutes. After incubation, Griess reagent was added to the mixture, resulting in a pink chromophore, which was measured at 540 nm using a spectrophotometer. The scavenging activity of NO (%) was calculated by using Equation 3:

$$NO\ Radical\ Scavenging\ Assay\ (\%) = \left[\frac{A_{Control} - A_{sample}}{A_{control}}\right] \times 100\% \quad [3]$$

where $A_{control}$ is the absorbance of the NO solution without antioxidant, and A_{sample} is the absorbance of the sample containing antioxidant.

Anti-Diabetic Activity

α-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory activity was carried out following the protocol by Ramadhan et al. (2023). Briefly, 20 µL of the sample was mixed with 30 µL phosphate buffer (pH 6.9), followed by the addition of 50 µL α-glucosidase. After pre-incubation for 10 minutes at 37°C, 45 µL of *p* - nitrophenylglucoside was added to the reaction mixture. Next, 90 µL of sodium carbonate (Na_2CO_3) was added, and the mixture was incubated for 30 minutes at 37°C. Absorbance was then measured at 405 nm. Acarbose and quercetin were used as standard references, and the results were expressed as half-maximal inhibitory concentration (IC_{50}) values.

Data Collection and Statistical Analysis

The experiment was done with three replicates for analysis, and the data generated throughout the experiment were analysed by one-way analysis of variance (ANOVA), whereas the significant differences in means between treatments were separated by using Duncan's Multiple Range test ($p \leq 0.05$) and Pearson's correlation analysis was conducted using IBM SPSS Statistics ver. 30.0.0.0.

RESULTS

Phytocompound Characterisation

In this study, TPC was quantified using the Folin-Ciocalteu colourimetric assay, with gallic acid as the standard. The calibration curve ($y = 2.6769x + 0.4404$, $R^2 = 0.9806$; Supplementary Figure S1) demonstrated strong linearity, and results were expressed as mg gallic acid equivalents (GAE) per gram sample (Table 1). The phenolic content of ethanolic extracts of wild ginger species varied significantly, ranging from 6.53 to 115.77 mg GAE/g DW. Notably, *B. prainiana* and *H. leonurus* displayed the highest TPC in their leaves (115.48 ± 0.36 and 115.77 ± 17.89 mg GAE/g DW, respectively), whereas the lowest phenolic content was observed in the stem of *B. plicata* (3.24 ± 1.47 mg GAE/g DW).

Table 1
Total phenolic content (TPC) for different parts of selected wild ginger species

Species	Total Phenolic Content (mg GAE/g DW)		
	Leaves	Stem	Rhizome
G. patens	$107.23 \pm 3.83_{c}$	$47.71 \pm 17.94_{b}$	$58.21 \pm 9.81_{ab}$
S. klossii	$6.53 \pm 1.03_{a}$	$3.24 \pm 1.47_{a}$	$6.75 \pm 1.31_{a}$
B. plicata	$7.18 \pm 2.90_{a}$	$1.67 \pm 0.49_{a}$	$30.86 \pm 3.01_{ab}$
S. hastilabium	$53.77 \pm 4.04_{b}$	2.38 ± 2.59a	$51.76 \pm 5.42_{ab}$
Amomum sp.	$15.67 \pm 4.13_{a}$	N. D	$45.50 \pm 16.16_{bc}$
H. leonurus	$115.77 \pm 17.89_{c}$	N. D	N. D.
B. prainiana	$115.48 \pm 0.72_{c}$	$26.01 \pm 18.52_{ab}$	$68.43 \pm 20.04_{c}$

Note. *Values are presented as mean ± Standard deviation (*n=3*). For columns of each species, values followed by the same letter (a-c) are not significantly different at $p \leq 0.05$, *as* measured by one-way ANOVA with Duncan's test. N. D indicates Not Detected; calculated values below the detection limit of the assay (negative values obtained after calibration curve). DW indicates Dry Weight

Table 2
Total flavonoid content (TFC) for different parts of selected wild ginger species

Species	Total Flavonoid Content (mg RE/g DW)		
	Leaves	Stem	Rhizome
G. patens	$9.26 \pm 0.01_{b}$	$1.19 \pm 0.05_{a}$	$2.23 \pm 0.05_{a}$
S. klossii	$10.29 \pm 0.05_{b}$	$3.91 \pm 0.04_{a}$	$4.83 \pm 0.03_{a}$
B. plicata	$11.69 \pm 0.05_{bc}$	$0.81 \pm 0.03_{a}$	$4.33 \pm 0.03_{a}$
S. hastilabium	$14.31 \pm 0.02_{cd}$	$2.24 \pm 0.03_{a}$	$3.27 \pm 0.05_{a}$
Amomum sp.	$14.85 \pm 0.04_{d}$	$0.67 \pm 0.05_{a}$	$3.24 \pm 0.04_{a}$
H. leonurus	$3.07 \pm 2.21_{a}$	$0.89 \pm 0.04_{a}$	$1.16 \pm 0.01_{a}$
B. prainiana	$145.66 \pm 1.13_{e}$	$45.89 \pm 13.23_{b}$	$43.53 \pm 15.88_{b}$

Note. *Values are presented as mean ± Standard deviation (*n=3*). For columns of each species, values followed by the same letter (a-e) are not significantly different at $p \leq 0.05$, *as* measured by one-way ANOVA with Duncan's test. DW indicates Dry Weight

TFC was measured using the aluminium chloride colourimetric method with rutin as standard. The assay showed linearity ($y = 0.0021x + 0.0708$, $R^2 = 0.9902$; Supplementary Figure S2) with results expressed as mg rutin equivalents (RE) per gram dry weight sample (Table 2). The analysis revealed flavonoid content among wild ginger species varied significantly, ranging from 0.67 to 145.66 mg RE/g DW. Notably, *B. prainiana* leaves exhibited the highest flavonoid accumulation (145.66 ± 1.13 mg RE/g DW), which represents more than a 200-fold increase compared to the lowest values recorded from *Amomum* sp. stems (0.67 ± 0.05 mg RE/g DW).

Finally, the terpenoid contents in wild ginger species were determined via a vanillin-perchloric acid colourimetric assay standardised against linalool, which produced a

linear calibration (y = 1.526x + 0.4346; R^2 = 0.9982; Supplementary Figure S3). Quantification of TTC revealed that the samples varied significantly between species and plant parts (Table 3). The highest concentration was found in ethanolic extracts of *B. prainiana* leaves containing 0.08 ± 0.002 mg LE/g DW, which was approximately 40-fold greater than the lowest concentration found in *G. patens* and *B. prainiana* stems, which was 0.002 ± 0.005 mg LE/g DW. These significant variations highlighted that terpenoid biosynthesis is species-specific, with *B. prainiana* exhibiting a significantly higher capacity for terpenoid accumulation compared to others. It also reflects the well-documented phenomenon that the distribution of secondary metabolites within plants was often organ-specific, with leaves frequently serving as an important part for the synthesis and storage of bioactive compounds due to their role in defence and environmental interaction (Liu et al., 2022).

Table 3
Total terpenoid content (TTC) for different parts of selected wild ginger species

Species	Total Terpenoid Content (mg LE/g DW)		
	Leaves	Stem	Rhizome
G.patens	$0.07\pm0.02_b$	$0.002\pm0.0005_a$	$0.05\pm0.65_c$
S.klossii	$0.04\pm0.001_a$	$0.02\pm0.001_d$	$0.03\pm0.001_b$
B. plicata	$0.04\pm0.01_a$	$0.02\pm0.001_c$	$0.03\pm0.004_b$
S. hastilabium	$0.04\pm0.003_a$	N. D	$0.03\pm0.04_b$
Amomum sp.	$0.07\pm0.01_b$	N. D	N. D
H. leonurus	$0.03\pm0.001_a$	N. D	N. D
B. prainiana	$0.08\pm0.002_b$	$0.002\pm0.005_a$	$0.07\pm0.01_d$

Note. *Values are presented as mean ± Standard deviation (*n=3*). For columns of each species, values followed by the same letter (a-d) are not significantly different at $p \leq 0.05$, *as* measured by one-way ANOVA with Duncan's test. N. D indicates Not Detected; calculated values below the detection limit of the assay (negative values obtained after calibration curve). DW indicates Dry Weight

Antioxidant Activities

The antioxidant capacity of the ethanolic extracts of wild ginger species varied significantly among species and plant parts (Table 4, Table 5, Table 6, and Table 7). In this study, *S. hastilabium* showed the strongest scavenging activity against both ABTS and NO radicals, with its rhizome being most potent in the ABTS assay (0.03 ± 0.003 mg/mL), while its stem was most potent in the NO assay (2.45 ± 0.881 mg/mL). In *B. prainiana*, the rhizome had the highest DPPH radical scavenging activity (0.02 ± 0.01 mg/mL). Meanwhile, the leaves of *H. leonurus* demonstrated the highest ferric reducing antioxidant power (FRAP) (2.813 ± 0.025 µmol Fe/g). These results indicate that although leaves and rhizome act as major contributors to overall antioxidant capacity, the dominant organ is highly dependent on the specific species and assay type.

Table 4
DPPH antioxidant assay for different parts of wild ginger species

Species	IC50 (mg/mL)		
	Leaves	Stem	Rhizome
G. patens	N.D.	$6.10 \pm 0.36_{b}$	$5.34 \pm 5.14_{b}$
S. klossii	$0.40 \pm 0.17_{a}$	$4.28 \pm 0.75_{ab}$	$3.280 \pm 0.17_{ab}$
B. plicata	$0.46 \pm 0.17_{a}$	$4.94 \pm 1.54_{b}$	$0.05 \pm 0.04_{a}$
S. hastilabium	$0.25 \pm 0.01_{a}$	$0.6 \pm 0.21_{a}$	$0.15 \pm 0.01_{a}$
Amomum sp.	$1.08 \pm 0.07_{b}$	$3.69 \pm 0.68b_{ab}$	$5.29 \pm 1.14_{b}$
H. leonurus	$0.11 \pm 0.07_{a}$	$4.28 \pm 0.75_{ab}$	$0.39 \pm 0.12_{a}$
B. prainiana	$0.02 \pm 0.01_{a}$	$0.37 \pm 0.47_{a}$	$0.21 \pm 0.12_{a}$
Ascorbic acid		0.006 ± 0.01	

Note. *Values are presented as mean ± Standard Deviation (*n=3*). For each column of each species, values followed by the same letter (a-d) are not significantly different at $p \leq 0.05$ measured by one-way ANOVA with Duncan's test. N. D indicates Not Detected; calculated values below the detection limit of the assay (negative values obtained after calibration curve). Ascorbic acid was used as standard

Table 5
ABTS antioxidant assay for different parts of wild ginger species

Species	IC50 (mg/mL)		
	Leaves	Stem	Rhizome
G. patens	$0.09 \pm 0.01_{a}$	$0.45 \pm 0.06_{a}$	$0.38 \pm 0.09_{b}$
S. klossii	$1.58 \pm 0.18_{c}$	$4.46 \pm 0.46_{e}$	$1.54 \pm 0.02_{c}$
B. plicata	$0.73 \pm 0.24_{b}$	$3.50 \pm 0.06_{d}$	$0.39 \pm 0.13_{b}$
S. hastilabium	$0.04 \pm 0.01_{a}$	$0.72 \pm 0.1_{ab}$	$0.03 \pm 0.003_{a}$
Amomum sp.	$2.76 \pm 0.03_{d}$	$2.77 \pm 0.6_{c}$	$4.05 \pm 0.3_{d}$
H. leonurus	$0.04 \pm 0.01_{a}$	$0.81 \pm 0.05_{ab}$	N.D.
B. prainiana	$0.17 \pm 0.03_{a}$	$1.39 \pm 0.7_{b}$	N. D.
Ascorbic acid		0.04 ± 0.00	

Note. *Values are presented as mean ± Standard Deviation (*n=3*). For columns of each species, values followed by the same letter (a-e) are not significantly different at $p \leq 0.05$, *as* measured by one-way ANOVA with Duncan's test. N. D indicates Not Detected; calculated values below the detection limit of the assay (negative values obtained after calibration curve). Ascorbic acid was used as standard

Table 6
FRAP antioxidant assay for different parts of wild ginger species

Species	FRAP (µmol Fe/g)		
	Leaves	Stem	Rhizome
G.patens	$1.547 \pm 0.03_{e}$	$1.186 \pm 0.02_{f}$	$0.984 \pm 0.02_{c}$
S.klossii	$1.063 \pm 0.03_{d}$	$0.613 \pm 0.004_{c}$	$0.405 \pm 0.03_{a}$
B. plicata	$0.891 \pm 0.02_{c}$	$0.545 \pm 0.02_{b}$	$1.178 \pm 0.02_{d}$

Table 6 (*continued*)

Species	FRAP (µmol Fe/g)		
	Leaves	Stem	Rhizome
S. hastilabium	$0.905 \pm 0.02_{c}$	$0.805 \pm 0.03_{d}$	$1.770 \pm 0.07_{f}$
Amomum sp.	$0.736 \pm 0.03_{b}$	$0.405 \pm 0.03_{a}$	$0.564 \pm 0.04_{b}$
H. leonurus	$2.813 \pm 0.03_{f}$	$1.173 \pm 0.02_{f}$	$1.252 \pm 0.02_{e}$
B. prainiana	$0.474 \pm 0.01_{a}$	$1.134 \pm 0.01_{e}$	$1.008 \pm 0.03_{c}$
Iron (II) sulfate ($FeSO_4$)		1.513 ± 0.01	

Note. *Values are presented as mean ± Standard Deviation (*n=3*). For columns of each species, values followed by the same letter (a-f) are not significantly different at $p \leq 0.05$, *as* measured by one-way ANOVA with Duncan's test. $FeSO_4$ was used as standard

Table 7
Nitric Oxide (NO) antioxidant assay for different parts of wild ginger species

Species	IC50 (µg/mL)		
	Leaves	Stem	Rhizome
G.patens	$7.37 \pm 0.82_{b}$	$14.46 \pm 1.13_{a}$	$36.53 \pm 1.27_{b}$
S.klossii	$15.34 \pm 0.60_{d}$	$24.27 \pm 0.62_{b}$	$14.34 \pm 1.76_{d}$
B. plicata	$22.40 \pm 0.59_{g}$	$33.07 \pm 0.53_{d}$	$38.27 \pm 0.53_{f}$
S. hastilabium	$6.86 \pm 1.29_{f}$	$2.45 \pm 0.88_{a}$	$50.27 \pm 0.33_{a}$
Amomum sp.	$17.38 \pm 0.72_{e}$	$53.15 \pm 0.23_{c}$	$72.43 \pm 0.48_{g}$
H. leonurus	$37.64 \pm 0.69_{c}$	$30.33 \pm 1.75_{e}$	$34.15 \pm 0.78_{e}$
B. prainiana	$8.72 \pm 1.68_{a}$	$21.16 \pm 2.91_{a}$	$19.40 \pm 0.17_{c}$
Trolox		183.14 ± 26.05	

Note. *Values are presented as mean ± Standard Deviation (*n=3*). For columns of each species, values followed by the same letter (a-g) are not significantly different at $p \leq 0.05$, as measured by Duncan's test. Trolox was used as standard

Correlation between Phytochemical Contents and Antioxidant Activities

Pearson correlation analysis (Table 8) revealed significant associations between phenolic content and antioxidant activities. TPC showed a moderate negative correlation with ABTS IC50 values ($r = -0.481$, $p < 0.05$) and a moderate positive correlation with FRAP values ($r = 0.510$, $p < 0.05$), indicating that phenolics significantly contributed to ABTS radical scavenging and ferric-reducing capacities. There were no significant correlations between TPC and either DPPH or NO assays. On the other hand, TFC and TTC did not show significant correlations with any of the antioxidant assays.

Table 8
Pearson's correlation coefficient values (r) between phytochemical and antioxidant activities of wild ginger species

Variable	DPPH	ABTS	FRAP	NO
TPC	r = -0.196	r = -0.481*	r = 0.510*	r =-0.096
TFC	r = 0.266	r = -0.215	r = -0.184	r =-0.329
TTC	r = -0.109	r = 0.273	r =0.018	r =-0.403

Note. *Pearson correlation coefficient values are significant at the $p < 0.05$ level (2-tailed). r refers to the Pearson coefficient

α-Glucosidase Inhibitory Activity

In this study, ethanolic extracts of leaves, stems, and rhizomes of seven wild ginger species from Panti Forest Reserve and Endau-Rompin National Park, Johor, Malaysia were evaluated for α-glucosidase inhibitory activity. The majority of the extracts showed negligible or even negative inhibition, indicating no measurable enzyme suppression at the evaluated concentrations. Only the rhizomes of *B. plicata* and *S. hastilabium* showed detectable inhibition, with IC50 values of 16.77 ± 0.138 µg/mL and 20.26 ± 0.145 µg/mL, respectively (Table 9).

Table 9
α-glucosidase inhibitory assay for different parts of wild ginger species

Species	IC50 (µg/mL)		
	Leaves	Stem	Rhizome
G.patens	NI	NI	NI
S.klossii	NI	NI	NI
B. plicata	NI	NI	16.77 ± 0.138
S. hastilabium	NI	NI	20.26 ± 0.145
Amomum sp.	NI	NI	NI
H. leonurus	NI	NI	NI
B. prainiana	NI	NI	NI
Acarbose		39.85 ± 0.09	
Quercetin		8.36 ± 1.47	

Note. *Values are presented as mean ± Standard Deviation ($n=3$). NI indicates No Inhibition; inhibitory effects were less than 30% at 40 µg/ml. Acarbose and quercetin were used as standards

DISCUSSION

Phytocompound Characterisation

Phenolic compounds are a major class of phytochemicals, exhibiting significant antioxidant activity due to their redox properties, primarily mediated by hydroxyl groups

that facilitate free radical scavenging (Aryal et al., 2019). The reported TPC in leaves may reflect their role in reducing oxidative stress and pathogen defence, which is often caused by phenolic accumulation (Salunke & Koche, 2023). The TPC values in this study were generally lower than those reported for other ginger species. For instance, Fahmi et al. (2024) documented significantly higher TPC in *Etlingera elatior* leaves (483.79 ± 2.57 mg GAE/g) compared to its flowers and stems. On the other hand, a study reported TPC values of 5.95-19.68 mg GAE/g in ethanolic extracts of hybrid and local ginger varieties, while another study observed a range of 9.42-10.09 mg GAE/g in ethanolic ginger extracts (Akullo et al., 2023; Ezez & Tefera, 2021). Large-scale screenings of 24 Zingiberaceae species revealed TPC ranges in 23.80-321.23 mg GAE/g, which indicates Zingiberaceae variability (Generalić Mekinić et al., 2019). These variations may arise from species-specific differences, growth conditions or extraction protocols (Sytar et al., 2018). Despite these variations, our findings suggest that all parts of *B. prainiana* are promising phenolic-rich species among the evaluated wild gingers, warranting further investigation into its bioactive potential.

Flavonoids are another subclass of polyphenolic compounds that are characterised by their benzopyrone backbone and hydroxylated aromatic rings. They have important ecological functions in plants and provide significant health benefits for humans (Nicolescu et al., 2025). As secondary metabolites, they contribute to plant defence against UV radiation, pathogens and oxidative stress. These compounds are becoming increasingly valued for use in pharmaceutical and nutraceutical applications due to their strong antioxidant, anti-inflammatory and anticancer properties. Previous studies revealed that ginger leaves accumulate 2-3 times higher flavonoid content than rhizomes, while another study documented solvent-dependent variations (1.84-2.54 mg QE/g) in local ginger cultivars (Akullo et al., 2023; Ghasemzadeh et al., 2010b). This tissue-specific variation aligns with previous reports where leaves often accumulate higher flavonoid levels than stems, suggesting their functions in protecting photosynthetic tissues and mediating ecological interactions such as UV protection and herbivore deterrence (Davies et al., 2020). *B. prainiana* has a high potential as a source of natural antioxidants due to its exceptional flavonoid concentrations. These findings suggest further investigation into the specific flavonoid subclasses present, optimal extraction protocols and potential synergistic effects with other phytochemicals in this understudied species.

Terpenoids constitute one of the largest and most structurally diverse classes of plant secondary metabolites that mediate essential ecological interactions in plants by serving as chemical defences against herbivores, diseases, and competing vegetation (Das et al., 2022). However, quantitative analyses remain scarce, with most research emphasising rhizomes despite previous studies having often focused on qualitative investigations of the presence or lack of terpenoids in cultivated ginger leaves (Kim et al., 2024; Koo & Gang, 2012).

In this study, *B. prainiana* accumulates terpenoids at concentrations similar to those reported in well-characterised medicinal species such as *K. galanga*, *Z. officinale*, and *C. longa,* which are known for their terpenoid-rich essential oils and related antimicrobial and antioxidant properties (Wang et al., 2023a; Kim et al., 2024; Gu et al., 2024). These results provide the first comprehensive quantification of both foliar and rhizomic terpenoids across wild Zingiberaceae species. This finding also indicates *B. prainiana* as a particularly promising candidate for detailed phytochemical characterisation of specific terpenoid profiles, investigation of biosynthetic pathway regulation and development of standardised extraction protocols for potential antimicrobial applications. The demonstrated variability across wild ginger species highlights the untapped chemical diversity within this taxon and emphasises the need for expanded phytochemical surveys of understudied Zingiberaceae.

Several limitations should be considered when evaluating the phytochemical results. The first limitation is that absolute ethanol was used as the only solvent for extraction, which may underestimate the abundance of lipophilic constituents, particularly non-polar terpenoids and preferentially extracts polar and moderately polar molecules (Azmir et al., 2013; Khaw et al., 2017). The second limitation is that TPC was measured using the vanillin-perchloric acid colourimetric assay, which provides only an estimation of total terpenoid abundance and lacks specificity to any particular terpenoid classes. Previous studies have reported that this assay may also react with certain phenolic compounds, which could influence the accuracy of the measured values (Das et al., 2022). As a result, the total terpenoid contents reported in this study should be interpreted as approximate values rather than absolute concentrations. Future studies using sequential extraction combined with chromatographic techniques such as GC-MS and LC-MS/MS are recommended to achieve comprehensive characterisation of terpenoid contents.

Overall, the leaf part consistently contained higher phenolic, flavonoid and terpenoid levels compared to stems and rhizomes across all the evaluated wild ginger species (leaves > stems and rhizomes). This is because leaves are continuously exposed to environmental stresses such as oxidative stress, UV radiation and herbivores, which require greater production of protective secondary metabolites. Rhizomes served as storage and regenerative organs that exhibited intermediate levels, whereas stems generally had the lowest content due to their structural role. These results highlight the importance of evaluating multiple plant parts rather than focusing solely on rhizomes as commonly done in many Zingiberaceae studies.

Antioxidant Activities

The antioxidant activity reported in this study reflects the general distribution of phytochemicals among different plant parts. Leaves with higher total phenolic and flavonoid contents consistently demonstrated potent radical scavenging activities in DPPH,

ABTS, and nitric oxide assays, supporting the established roles of phenolic compounds as significant contributors to antioxidant defence. Phenolics and flavonoids possess hydroxyl groups that are capable of donating electrons or hydrogen atoms to neutralise reactive oxygen species and inhibit oxidation chain reactions (Aryal et al., 2019; Ulewicz-Magulska & Wesolowski, 2023). On the other hand, rhizomes showed considerably higher ferric-reducing capacities in several species, despite having moderate phenolic levels, suggesting the presence of compounds with stronger reducing potential. Stems generally had weaker antioxidant activities due to their predominantly structural role and lower accumulation of secondary metabolites. Similar organ-specific variations have been reported in cultivated Zingiberaceae species and other medicinal plants, where the genetic background, tissue specialisation, and environmental adaptation affect the antioxidant production and accumulation (Avci et al., 2019; Sinkovič et al., 2024; Yang et al., 2022).

This organ-specific antioxidant variation is strongly correlated with the distribution of phytochemicals present in the same plant parts. The high TPC and TFC accumulation in leaves consistently showed stronger radical scavenging activity in DPPH, ABTS and NO, supporting the well-established role of phenolics and flavonoids as main antioxidant compounds. Meanwhile, rhizomes with intermediate phenolic levels showed significant reducing power (FRAP) likely due to accumulated storage metabolites and defence. On the other hand, stems primarily serve as structural organs and contain the lowest phytochemical concentrations and correspondingly weaker antioxidant potential (Mahmudati et al., 2023). These variations are commonly attributed to species-specific genetic factors that affect the biosynthesis and accumulation of secondary metabolites, as reported in recent phytochemical studies (Avci et al., 2019; Sinkovič et al., 2024; Yang et al., 2022).

The variations observed among antioxidant assays highlight the importance of using multiple analytical approaches to fully assess antioxidant potential. DPPH and ABTS assays mainly measure radical scavenging through hydrogen atom transfer and single-electron transfer mechanisms, while FRAP evaluates ferric ion reducing capacity (Ulewicz-Magulska & Wesolowski, 2023). Therefore, the same extract may show different antioxidant capabilities depending on the underlying reaction mechanism and the chemical nature of the bioactive constituents present. For instance, the superior ABTS radical scavenging activity exhibited by *S. hastilabium* rhizomes and the strong ferric reducing capacity observed in *H. leonurus* leaves suggest that various metabolite classes contribute to antioxidant activity through different mechanisms. Similar assay-dependent variations have been reported in *Z. officinale* and *C. longa*, in which radical scavenging and reducing capacities differed among plant organs and extraction systems (Ghasemzadeh et al., 2010a; Ulewicz-Magulska & Wesolowski, 2023). These findings highlight that no single antioxidant assay is sufficient to fully characterise the antioxidant potential of medicinal plants.

Overall, leaves emerged as the principal reservoirs of antioxidant phytochemicals in most of the investigated species, followed by rhizomes, whereas stems generally exhibited the lowest antioxidant activities. This pattern reflects the physiological roles of the different organs. Leaves are metabolically active tissues that are continuously exposed to ultraviolet radiation, herbivores, and oxidative stress, thereby requiring higher concentrations of protective secondary metabolites. Rhizomes serve as storage and regenerative organs and thus maintain moderate concentrations of defence compounds, whereas stems mainly provide structural support and consequently invest less in secondary metabolite biosynthesis (Mahmudati et al., 2023). These findings highlight the importance of evaluating multiple plant organs rather than focusing exclusively on rhizomes, as commonly practised in many Zingiberaceae studies. Furthermore, the strong antioxidant activities observed, particularly in leaf extracts, suggest that wild Malaysian gingers represent promising and sustainable sources of natural antioxidants for nutraceutical and pharmaceutical applications.

Correlation Between Phytochemical Contents and Antioxidant Activities

Phenolic compounds appeared to be the main contributors to antioxidant activity in the investigated wild ginger species. Significant correlations between TPC and both ABTS and FRAP assays indicate that phenolic constituents are largely responsible for radical scavenging and reducing capacities. Similar relationships have been reported in *Zingiber officinale* and *Curcuma longa*, where phenolic compounds were identified as major determinants of antioxidant performance (Ghasemzadeh et al., 2010a; Ulewicz-Magulska & Wesolowski, 2023). Differences in radical chemistry and the selectivity of specific oxidising species may be the cause for the absence of significant correlations between TPC and DPPH or nitric oxide assays (Nicolescu et al., 2025).

Similarly, the lack of significant correlations between TFC and TTC suggests that antioxidant potential is not always determined by total flavonoid and terpenoid contents alone. Previous studies have shown that the chemical structures of individual compounds have a significant influence on antioxidant capabilities rather than their total concentration. The ability of flavonoids and terpenoids to scavenge radicals is significantly influenced by structural features such as the number and position of hydroxyl groups, conjugated double bonds, and glycosylation patterns (Lu et al., 2024; Wang et al., 2023a). Therefore, total phytochemical contents should be interpreted cautiously because they may not accurately reflect the biological activities of specific metabolites. Future investigations using chromatographic and metabolomic approaches such as LC-MS/MS are required to identify the compounds responsible for the observed antioxidant activities.

Overall, the present findings demonstrate that phenolic compounds play a central role in the antioxidant properties of wild Malaysian gingers, particularly in leaf tissues, and further support their potential as valuable natural sources of antioxidant compounds.

α-Glucosidase Inhibitory Activity

α-Glucosidase is a key enzyme involved in postprandial carbohydrate digestion, and its inhibition is an established therapeutic strategy for controlling hyperglycaemia in type 2 diabetes mellitus (Han et al., 2025). Although acarbose is widely used as a clinical α-glucosidase inhibitor, its use is often associated with gastrointestinal side effects, thereby stimulating interest in plant-derived alternatives with fewer adverse effects (Akmal et al., 2024). The α-glucosidase inhibitory activities observed in this study were comparable to those reported for several cultivated Zingiberaceae species. For instance, methanolic rhizome extracts of *Z. officinale* exhibited an IC50 value of 18.5 µg/mL (Maniam et al., 2024), while *Aframomum melegueta* fruits rich in 6-gingerol and oleanolic acid demonstrated potent inhibitory activity with an IC50 value of 17.35 ± 0.88 µM (Mohammed et al., 2017). A similar significant inhibitory effect was shown by the rhizome extracts of *B. plicata* and *S. hastilabium,* suggesting that these wild species may represent promising sources of natural antidiabetic agents.

Interestingly, leaves and stems showed negligible α-glucosidase inhibitory activity despite possessing higher total phenolic and flavonoid contents. This observation suggests that total phytochemical abundance alone does not necessarily translate into enzyme inhibition. Previous studies have shown that inhibitory activity is mainly dependent on the presence of specific metabolites and their structural characteristics rather than overall phenolic or flavonoid concentrations (Wu et al., 2023). Diarylheptanoids, phenylpropanoids, flavonoids, and terpenoids are examples of bioactive compounds that have been reported to inhibit α-glucosidase through different mechanisms, highlighting the significance of compound composition rather than bulk metabolite content (Han et al., 2025). Therefore, the absence of measurable inhibition in most species and organs may reflect either the absence of these active constituents or their low abundance in the ethanolic extracts.

Although the active compounds responsible for the potent inhibitory activities observed in *B. plicata* and *S. hastilabium* rhizomes remain unknown, the present findings indicate that specific metabolites are likely involved. Consequently, future studies employing bioassay-guided fractionation and metabolomic approaches such as LC-MS/MS are required to identify and characterise the compounds responsible for the observed α-glucosidase inhibitory activity. Such investigations would facilitate a better understanding of the antidiabetic potential of these underexplored wild gingers and may contribute to the discovery of novel natural inhibitors.

Several limitations should be considered when interpreting the antidiabetic findings of this study. The α-glucosidase inhibitory activity was evaluated using a single-endpoint assay without detailed kinetic characterisation. Therefore, the mode of inhibition and enzyme-substrate interactions remain unclear. Enzyme kinetic analyses to differentiate competitive, non-competitive, and mixed modes of inhibition could provide deeper insights

into the mechanisms underlying enzyme suppression (Lam et al., 2024; Lu et al., 2023). Accordingly, future studies should incorporate enzyme kinetic analyses with bioassay-guided fractionation and metabolomic profiling to elucidate the active compounds and their mechanisms of action.

CONCLUSION

In conclusion, this study represents the first comprehensive comparative study that evaluates phytochemical composition, antioxidant activities, and α-glucosidase inhibitory potential between different plant parts (leaves, stems, and rhizomes) of seven wild Zingiberaceae species that were collected from Panti Forest Reserve and Endau-Rompin National Park, Johor, Malaysia. The findings showed significant variations in phytochemical accumulation and biological activities across different plant parts. In general, leaves contained higher total phenolic, flavonoid, and terpenoid contents and exhibited superior antioxidant activities, highlighting their importance as metabolically active tissues rich in protective secondary metabolites. The antioxidant properties of these wild ginger species are mainly attributed to phenolic compounds, as demonstrated by significant correlations between total phenolic content and ABTS and FRAP assays.

B. prainiana demonstrated the highest phytochemical contents among the studied species, whereas *H. leonurus* and *S. hastilabium* showed exceptional antioxidant capacities in selected assays. The rhizomes of *B. plicata* and *S. hastilabium* showed measurable enzyme inhibition with IC50 values lower than that of acarbose, suggesting the presence of potent antidiabetic constituents. These findings indicate that enzyme inhibition is governed by specific metabolites rather than total phytochemical abundance alone.

Overall, the present study highlights the untapped phytochemical diversity and therapeutic potential of wild Malaysian Zingiberaceae, particularly their leaves, which may serve as sustainable alternative sources of natural antioxidants for nutraceutical and pharmaceutical applications. Furthermore, the promising α-glucosidase inhibitory activities observed in *B. plicata* and *S. hastilabium* rhizomes warrant further investigation through bioassay-guided fractionation, enzyme kinetic studies, and metabolomic approaches such as LC-MS/MS and GC-MS to identify the compounds responsible for their biological activities. Expanding phytochemical and pharmacological investigations to other underexplored wild Zingiberaceae species may facilitate the discovery of novel bioactive compounds while supporting the sustainable utilisation and conservation of Malaysia's rich botanical resources.

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SUPPLEMENTARY DATA

Raw Absorbance Values for Total Phenolic Content of Wild Ginger Species

Table S1
Raw absorbance values of gallic acid used for total phenolic content standard curve

Concentration (mg/ml)	Absorbance			Blank Sample
	1	2	3	
1.000	3.206	3.0564	3.1888	3.1455
0.500	1.6486	1.4124	1.7845	0.3108
0.250	1.6771	1.0412	1.247	1.1871
0.125	0.8332	0.8214	0.7125	0.706
0.063	0.6025	0.4462	0.4881	0.4924

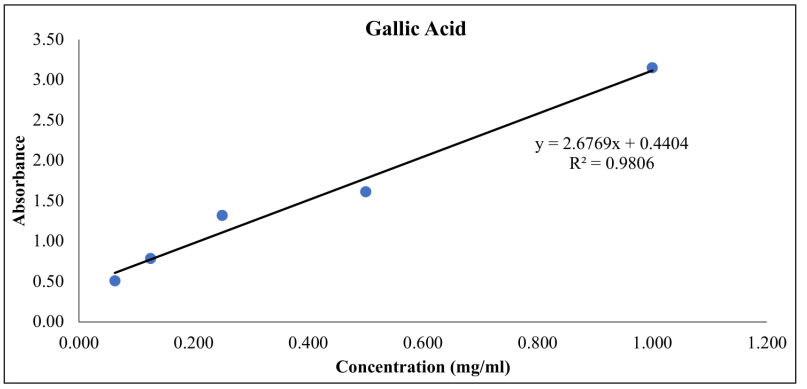

Figure S1. The standard curve of gallic acid

Standard Rutin for Total Flavonoid Content

Table S2
Raw absorbance values of rutin used for total flavonoid content standard curve

Concentration (mg/ml)	Absorbance			Blank Sample
	1	2	3	
0.250	1.2415	1.2452	1.2761	1.1523
0.125	0.7349	0.7277	0.762	0.5028
0.063	0.4427	0.4284	0.4256	0.4176
0.032	0.2511	0.2365	0.2422	0.2314
0.016	0.19	0.2034	0.2102	0.1587

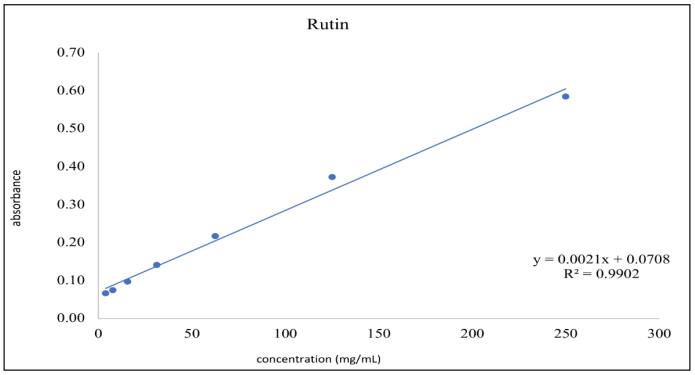

Figure S2. The standard curve of Rutin

Standard Linalool for Total Terpenoid Content

Table S3
Raw absorbance of linalool values used for total terpenoid content standard curve

Concentration (mg/ml)	Absorbance
1.00	1.0705
0.80	0.812
0.50	0.3407
0.40	0.1675
0.30	0.014

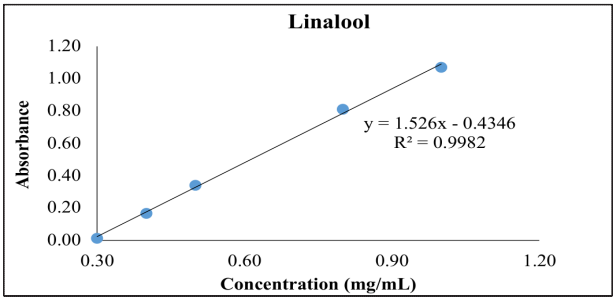

Figure S3. The standard curve of Linalool

Standard Ascorbic acid for 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

Table S4
Raw absorbance of ascorbic acid values used for DPPH standard curve

Concentration (mg/mL)	Absorbance			Blank Sample
	1	2	3	
0.0125	0.1083	0.1303	0.1176	0.0384
0.0063	0.151	0.148	0.1449	0.0388
0.0031	0.1636	0.1765	0.1657	0.0402
0.0016	0.1743	0.18	0.1783	0.0408

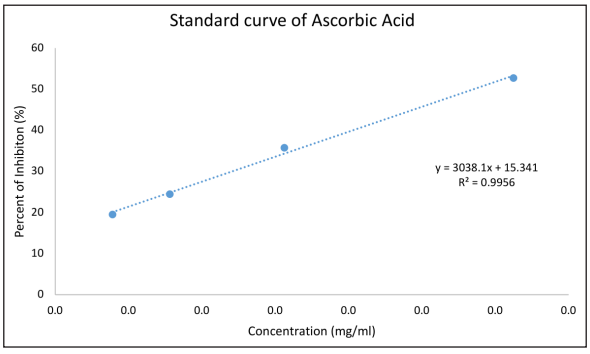

Figure S4. Standard calibration curve of ascorbic acid for DPPH assay

Standard Ascorbic acid for 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Radical Scavenging Assay

Table S5

Raw absorbance of standard ascorbic acid values used for ABTS

Concentration (mg/mL)	Absorbance			Blank Sample
	1	2	3	
0.100	0.0392	0.0405	0.0395	0.0374
0.050	0.3608	0.4229	0.4261	0.0505
0.025	0.631	0.6656	0.6883	0.0379
0.013	0.7768	0.807	0.7902	0.037
0.006	0.8414	0.8697	0.852	0.036
0.003	0.8806	0.8947	0.8864	0.0374

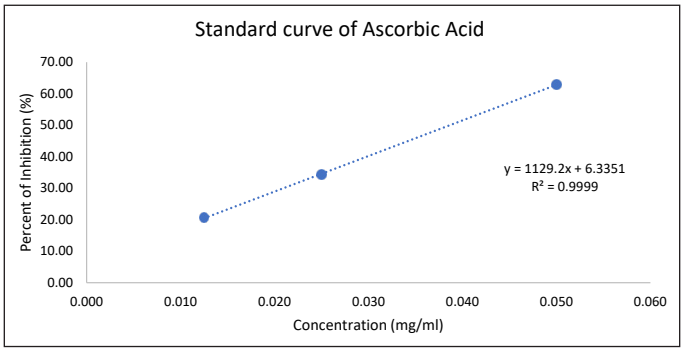

Figure S5. Standard calibration curve of ascorbic acid for ABTS assay

Standard Iron (II) sulphate for Ferric Reducing Antioxidant Power (FRAP)

Table S6
Raw absorbance of standard $FeSO_4$

Concentration (ppm)	Absorbance			Blank Sample
	1	2	3	
500	1.6236	1.6142	1.6021	0.0997
400	1.3123	1.4784	1.3550	0.0978
300	1.1154	1.0251	1.1950	0.0960
200	0.9189	0.8397	0.7115	0.1046
100	0.5101	0.5005	0.4836	0.1024
500	1.6236	1.6142	1.6021	0.0997

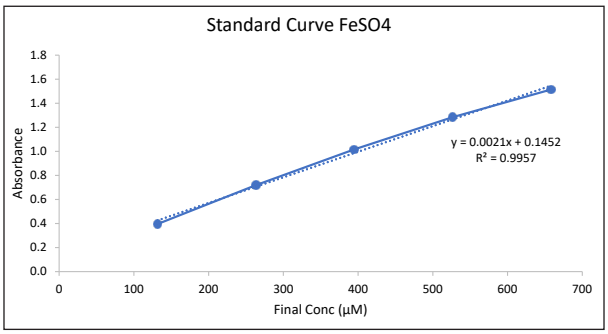

Figure S6. Standard calibration curve of FeSO4 for FRAP assay

Standard Trolox for Nitric Oxide (NO) Radical Scavenging Assay

Table S7
Raw absorbance of standard Trolox

Concentration (ppm)	Absorbance			Blank Sample
	1	2	3	
400	0.5596	0.5504	0.5579	0.0997
200	0.2422	0.2322	0.2331	0.0978
100	0.2814	0.2807	0.2734	0.0960
50	0.3027	0.3081	0.3184	0.1046
25	0.3217	0.3221	0.3229	0.1024
13	0.3376	0.3350	0.3246	0.0997

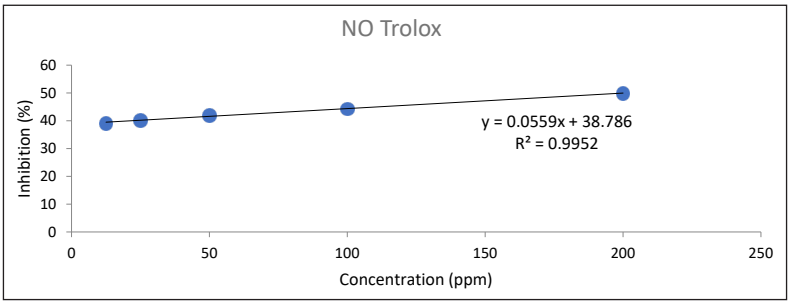

Figure S7. Standard calibration curve of Trolox for NO assay

Statistical Analysis

Total Phenolic Content

Table S8
ANOVA test total phenolic content for stem wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.000	6	0.000	49.289	<0.001
Within Groups	0.000	14	0.000		
Total	0.000	20			

Table S9
Duncan's test total phenolic content for leaves of wild ginger species

	Species	N	Subset for Alpha = 0.05		
			1	2	3
Duncan[a]	*S. klossii*	3	0.000833		
	B. plicata	3	0.000867		
	Amomum sp.	3	0.001167		
	S. hastilabium	3		0.002533	
	G. patens	3			0.004433
	B. prainiana	3			0.004733
	H. leonurus	3			0.004767
	Sig.		.411	1.000	0.411

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S10
ANOVA test total phenolic content for stem wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.000	6	0.000	3.662	0.021
Within Groups	0.000	14	0.000		
Total	0.000	20			

Table S11
Duncan's test total phenolic content for stem wild ginger species

	Species	N	Subset for Alpha = 0.05	
			1	2
Duncan[a]	*Amomum* sp.	3	0.000367	
	H. leonurus	3	0.000533	
	B. plicata	3	0.000667	
	S. klossii	3	0.000700	
	S. hastilabium	3	0.000700	
	B. prainiana	3	0.001533	0.001533
	G. patens	3		0.002333
	Sig.		0.063	0.146

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S12
ANOVA test total phenolic content for rhizome wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.657	5	0.531	40.352	<0.001
Within Groups	0.158	12	0.013		
Total	2.815	17			

Table S13
Duncan's test total phenolic content for rhizome wild ginger species

	Species	N	Subset for Alpha = 0.05		
			1	2	3
Duncan[a]	S. klossii	3	0.030804		
	Amomum sp.	3	0.204481		
	S. hastilabium	3	0.215143		
	B. plicata	3	0.231199		
	G. patens	3		0.607730	
	B. prainiana	3			1.178491
	Sig.		0.070	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Total Flavonoid Content

Table S14
ANOVA test total flavonoid content for leaves of wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	188375.893	6	31395.982	2644.961	<0.001
Within Groups	166.182	14	11.870		
Total	188542.074	20			

Table S15
Duncan's test total flavonoid content for leaves of wild ginger species

	Species	N	Subset for Alpha = 0.05				
			1	2	3	4	5
Duncan[a]	*H. leonurus*	3	5.09333				
	G. patens	3		17.46000			
	S. klossii	3		19.53333	19.53333		
	B. plicata	3			24.14667	24.14667	
	S. hastilabium	3				27.56000	
	Amomum sp.	3				28.64333	
	B. prainiana	3					290.25333
	Sig.		1.000	0.473	0.123	0.151	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S16
ANOVA test total flavonoid content for stem wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	54369178.234	6	9061529.706	67.922	<0.001
Within Groups	1867748.318	14	133410.594		
Total	56236926.552	20			

Table S17
Duncan's test total flavonoid content for stem wild ginger species

	Species	N	Subset for Alpha = 0.05	
			1	2
Duncan[a]	*Amomum* sp.	3	4.5238	
	H. leonurus	3	7.4603	
	B. plicata	3	8.4788	
	G. patens	3	16.6601	
	S.klossii	3	63.5913	
	S. hastilabium	3	64.4444	
	B. prainiana	3		4625.2230
	Sig.		0.858	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S18
ANOVA test total flavonoid content for rhizome wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11849134.732	6	1974855.789	122.540	<0.001
Within Groups	225624.247	14	16116.018		
Total	12074758.979	20			

Table S19
Duncan's test total flavonoid content for rhizome wild ginger species

	Species	N	Subset for Alpha = 0.05	
			1	2
Duncan[a]	*H. Leonurus*	3	3.9667	
	S. hastilabium	3	23.0200	
	Amomum sp.	3	28.4333	
	G. patens	3	35.3100	
	S. klossii	3	35.7333	
	B. plicata	3	55.4967	
	B. prainiana	3		2176.5600
	Sig.		0.660	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Total Terpenoid Content

Table S20
ANOVA test total terpenoid content for leaves of wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.007	6	0.001	32.571	<0.001
Within Groups	0.000	14	0.000		
Total	0.007	20			

Table S21
Duncan's test total terpenoid content for leaves of wild ginger species

	Species	N	Subset for Alpha = 0.05	
			1	2
Duncan[a]	*H.leonurus*	3	0.0300	
	S. klossii	3	0.0400	
	B.plicata	3	0.0400	
	S. hastilabium	3	0.0400	
	G. patens	3		0.0700
	Amomum sp.	3		0.0700
	B. prainiana	3		0.0767
	Sig.		0.069	0.200

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S22
ANOVA test total terpenoid content for stem wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.002	6	0.000	71.000	<0.001
Within Groups	0.000	14	0.000		
Total	0.002	20			

Table S23
Duncan's test total terpenoid content for stem wild ginger species

	Species	N	Subset for Alpha = 0.05			
			1	2	3	4
Duncan[a]	*G. patens*	3	0.0000			
	H. leonurus	3	0.0000			
	B. prainiana	3	0.0000			
	S. hastilabium	3		0.0100		
	Amomum sp.	3		0.0100		
	B.plicata	3			0.0200	
	S. klossii	3				0.0267
	Sig.		1.000	1.000	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S24
ANOVA test total terpenoid content for rhizome wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.009	6	0.002	29.030	<0.001
Within Groups	0.001	14	0.000		
Total	0.010	20			

Table S25
Duncan's test total terpenoid content for rhizome wild ginger species

	Species	N	Subset for Alpha = 0.05			
			1	2	3	4
Duncan[a]	*H. leonurus*	3	0.0000			
	Amomum sp.	3	0.0100			
	S. klossii	3		0.0300		
	S. hastilabium	3		0.0300		
	B. plicata	3		0.0333		
	G. patens	3			0.0500	
	B. prainiana	3				0.0667
	Sig.		0.113	0.601	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

DPPH Radical Scavenging Assay

Table S26
ANOVA test DPPH assay for leaves of wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	79.894	6	13.316	28.094	<0.001
Within Groups	6.636	14	0.474		
Total	86.529	20			

Table S27
Duncan's test DPPH for leaves of wild ginger species

	Species	N	Subset for Alpha = 0.05	
			1	2
Duncan[a]	*B. prainiana*	3	0.0233	
	H. leonurus	3	0.1100	
	S. hastilabium	3	0.2500	
	S. klossii	3	0.4033	
	B. plicata	3	0.4600	
	Amomum sp.	3	1.0825	
	G. patens	3		5.8867
	Sig.		0.112	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S28
ANOVA test DPPH assay for stem wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	76.974	6	12.828	2.718	0.58
Within Groups	66.071	14	4.719		
Total	143.041	20			

Table S29
Duncan's test DPPH for stem wild ginger species

	Species	N	Subset for Alpha = 0.05	
			1	2
Duncan[a]	*B. prainiana*	3	0.3733	
	S. hastilabium	3	0.6000	
	S. klossii	3	1.7200	1.7200
	Amomum sp.	3	3.6891	3.6891
	H. leonurus	3	4.2767	4.2767
	B. plicata	3	1.0825	4.9400
	G. patens	3		5.3433
	Sig.		0.065	0.084

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S30
ANOVA test DPPH assay for rhizome wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	109.757	6	18.293	4.636	0.009
Within Groups	55.239	14	3.946		
Total	164.996	20			

Table S31
Duncan's test DPPH for rhizome wild ginger species

	Species	N	Subset for Alpha = 0.05	
			1	2
Duncan[a]	B. plicata	3	.0467	
	S. hastilabium	3	.1467	
	B. prainiana	3	.2100	
	H. leonurus	3	.3933	
	S. klossii	3	3.2767	3.2767
	Amomum sp.	3		5.2883
	G. patens	3		5.3433
	Sig.		.092	.246

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

ABTS Radical Scavenging Assay

Table S32
ANOVA test ABTS assay for leaves of wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19.504	6	3.251	253.113	<0.001
Within Groups	0.180	14	0.013		
Total	19.684	20			

Table S33
Duncan's test ABTS for leaves of wild ginger species

	Species	N	Subset for Alpha = 0.05			
			1	2	3	4
Duncan[a]	S. hastilabium	3	0.0367			
	H. leonurus	3	0.0367			
	G. patens	3	0.0867			
	B. prainiana	3	0.1700			
	B. plicata	3		0.7333		
	S. klossii	3			1.5767	
	Amomum sp.	3				2.7567
	Sig.		0.204	1.000	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S34
ANOVA test ABTS assay for stem wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	44.197	6	7.366	50.590	<0.001
Within Groups	2.038	14	0.146		
Total	46.235	20			

Table S35
Duncan's test ABTS for stem wild ginger species

	Species	N	Subset for Alpha = 0.05				
			1	2	3	4	5
Duncan[a]	*G. patens*	3	0.4500				
	S. hastilabium	3	0.7167	0.7167			
	H. leonurus	3	0.8100	0.8100			
	B. prainiana	3		1.3900			
	Amomum sp.	3			2.7667		
	B. plicata	3				3.4967	
	S. klossii	3					4.4633
	Sig.		0.291	0.058	1.000	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S36
ANOVA test ABTS assay for rhizome wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	39.738	6	6.623	356.992	<0.001
Within Groups	0.260	14	0.019		
Total	39.998	20			

Table S37
Duncan's test ABTS for rhizome wild ginger species

	Species	N	Subset for Alpha = 0.05			
			1	2	3	4
Duncan[a]	*H. leonurus*	3	0.0000			
	B. prainiana	3	0.0000			
	S. hastilabium	3	0.0300			
	G. patens	3		0.3767		
	B. plicata	3		0.3900		
	S. klossii	3			1.5433	
	Amomum sp.	3				4.0500
	Sig.		0.802	0.906	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Ferric Reducing Antioxidant Power (FRAP)

Table S38
ANOVA test (FRAP) for leaves of wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.158	6	1.860	3446.505	<0.001
Within Groups	0.008	14	0.001		
Total	11.165	20			

Table S39
Duncan's test (FRAP) for leaves of wild ginger species

	Species	N	Subset for Alpha = 0.05					
			1	2	3	4	5	6
Duncan[a]	*B. prainiana*	3	0.437767					
	Amomum sp.	3		0.736367				
	plicata	3			0.890800			
	S. hastitalibum	3			0.905400			
	S. klossii	3				1.063500		
	G. patens	3					1.547000	
	H. leonurus	3						2.813033
	Sig.		1.000	1.000	0.454	1.000	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S40
ANOVA test (FRAP) for stem wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.937	6	0.323	919.887	<0.001
Within Groups	0.005	14	0.000		
Total	1.942	20			

Table S41
Duncan's test for stem wild ginger species

	Species	N	Subset for Alpha = 0.05					
			1	2	3	4	5	6
Duncan[a]	*Amomum* sp.	3	0.404567					
	B. plicata	3		0.545067				
	S. klossii	3			0.612567			
	S. hastilabium	3				0.805400		
	B. prainiana	3					1.133667	
	H. leonurus	3						1.172700
	G. patens	3						1.185567
	Sig.		1.000	1.000	1.000	1.000	0.052	

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S42
ANOVA test (FRAP) for rhizome wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.517	6	0.586	534.769	<0.001
Within Groups	0.015	14	0.001		
Total	3.532	20			

Table S43
Duncan's test (FRAP) for stem wild ginger species

	Species	N	Subset for Alpha = 0.05					
			1	2	3	4	5	6
Duncan[a]	*S. klossii*	3	0.452867					
	Amomum sp.	3		0.563967				
	G. patens	3			0.983633			
	B. prainiana	3			1.007933			
	B. plicata	3				1.178100		
	H. leonurus	3					1.252100	
	S. hastilabium	3						1.770300
	Sig.		1.000	1.000	.384	1.000	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Nitric Oxide (NO) Antioxidant Assay

Table S44
ANOVA test (NO) for leaves of wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5498.219	6	916.370	1097.742	<0.001
Within Groups	11.687	14	0.835		
Total	5509.906	20			

Table S45
Duncan's test (NO) for leaves of wild ginger species

	Species	N	Subset for Alpha = 0.05						
			1	2	3	4	5	6	7
Duncan[a]	*B. prainiana*	3	.000000						
	G. patens	3		14.336233					
	H. leonurus	3			19.398267				
	S. klossii	3				34.148167			
	Amomum sp.	3					36.532533		
	S. hastilabium	3						38.270033	
	B. plicata	3							51.921767
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S46
ANOVA test (NO) for stem wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2161.730	6	360.288	313.485	<0.001
Within Groups	16.090	14	1.149		
Total	2177.820	20			

Table S47
Duncan's test (NO) for stem wild ginger species

	Species	N	Subset for Alpha = 0.05				
			1	2	3	4	5
Duncan[a]	*S. hastilabium*	3	6.859722				
	G. patens	3	7.371192				
	B. prainiana	3	8.717563				
	S. klossii	3		15.336593			
	Amomum sp.	3			17.374953		
	B. plicata	3				22.399398	
	H. leonurus	3					37.638210
	Sig.		0.062	1.000	1.000	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S48
ANOVA test (NO) for rhizome wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4680.847	6	780.141	364.192	<.001
Within Groups	29.990	14	2.142		
Total	4710.836	20			

Table S49
Duncan's test (NO) for rhizome wild ginger species

	Species	N	Subset for Alpha = 0.05						
			1	2	3	4	5	6	7
Duncan[a]	*S. hastilabium*	3	2.452050						
	G. patens	3		14.464084					
	B. prainiana	3			21.158330				
	S. klossii	3				24.264761			
	H. leonurus	3					30.327191		
	B. plicata	3						33.065062	
	Amomum sp.	3							53.899962
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000