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Original Article

Chemical fingerprinting and antioxidant properties of *Glochidion philippicum*

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Abstract

Glochidion philippicum has been suggested to exhibit considerable pharmacological potential, yet its chemical composition and bioactivity remain inadequately explored. The aim of this study was to investigate the chemical fingerprint and antioxidant properties of *G. philippicum* leaf extracts using Fourier-transform infrared spectroscopy (FTIR) with chemometric analyses, and in vitro and in vivo evaluations. Four extraction methods (maceration, reflux, ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE)) were optimized with water, 70% ethanol, ethyl acetate, and n-hexane as solvents. FTIR profiles were analyzed with principal component analysis (PCA), hierarchical cluster analysis, and orthogonal partial least squares discriminant analysis. An in vitro study assessing the free radical scavenging capacity was conducted using the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric-reducing antioxidant power (FRAP) methods, while in vivo evaluations were conducted using *Drosophila melanogaster* to measure antioxidant enzyme activity and expression of endogenous antioxidant-related genes. FTIR profiles identified functional groups contributing to antioxidant activity. In vitro assays using ABTS and FRAP methods revealed that extracts obtained with 70% ethanol and water exhibited the highest antioxidant activity, attributed to key functional groups such as C=C (aromatic), O–H (acidic), N=O (nitro), and C–O (ester). In vivo studies showed that ethanol-based MAE extracts (MAEEO) significantly improved the survival of autoinflammatory *PGRP-LB*$^{\Delta}$ mutant larvae exposed to heat-killed *Escherichia coli*. Real-time quantitative PCR analysis indicated this effect was dependent on endogenous antioxidant gene activation. The study highlights that *G. philippicum* leaf extracts as a natural source of bioactive compounds with exogenous antioxidant properties, offering potential for therapeutic applications.

Keywords: Phyllanthaceae, chemometric analysis, antioxidant, autoinflammatory model, *Drosophila melanogaster*

Introduction

Glochidion philippicum (Cav.) C.B. Rob., locally known as *sampare* in Indonesia, is a member of the Phyllanthaceae family widely distributed across tropical Southeast Asia, including Indonesia [1]. This plant commonly thrives along gravel roads, forest margins, and roadsides at low to medium altitudes [2]. Traditionally, *G. philippicum* has been used as an ethnomedicine for

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treating bacterial infections and malaria [3,4]. Phytochemical investigations have identified bioactive compounds such as flavonoids, tannins, and phenolics in its leaves, which are thought to contribute to its pharmacological properties [5]. A previous study has shown that ethanol extracts of *G. philippicum* leaves exhibited antimalarial activity by inhibiting *Plasmodium falciparum* [6]. While the mechanism underlying this activity is attributed to the plant's bioactive compounds, it may also involve their capacity to reduce reactive oxygen species (ROS), which play a critical role in malaria pathogenesis [7]. However, limited research has explored this connection, emphasizing the need for further investigations into the antioxidant effects of *G. philippicum*.

The pharmacological activity of *G. philippicum*, including its antioxidant potential, is closely linked to the solubility of its bioactive compounds, which is affected by the extraction conditions [8]. Variations in solvent type, temperature, pH, and extraction duration affect the extraction efficiency of these compounds, highlighting the importance of choosing and optimizing the proper method to maximize antioxidant activity [9]. Once extracted, bioactive compounds play an important role in determining the pharmacological efficacy of plants. Their profile can be directly linked to their measurable chemical composition, which can be analyzed using Fourier-transform infrared spectroscopy (FTIR) [10]. Techniques such as chemometric analysis and metabolomics are particularly effective in characterizing the profiles of these compounds. These approaches provide detailed and holistic insights into metabolite composition, allowing researchers to identify the functional groups of compounds, even those present in small quantities [10]. Such precision is critical to understanding the relationship between extracted compounds and functional groups, including their antioxidant potential. By integrating these modern analytical tools, researchers can better evaluate the quality and efficacy of *G. philippicum* extracts, paving the way for more consistent and reliable applications in pharmacological contexts.

To address these gaps, the aim of this study was to evaluate the antioxidant activity of *G. philippicum* extracts using both in vitro and in vivo approaches. By integrating in vitro assays, FTIR-based chemometric analyses, and in vivo studies, this research represents a comprehensive and practical strategy for advancing the study of natural products like *G. philippicum*. This approach also establishes an innovative framework for addressing the need for high-quality herbal materials in therapeutic applications.

Methods

Study design and setting

The antioxidant activity of *G. philippicum* extracts was assessed using in vitro and in vivo studies. The in vitro assays assessed the free radical scavenging capacity of the extracts using the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric-reducing antioxidant power (FRAP) methods. Additionally, FTIR-based chemometric analyses were employed to profile the chemical composition of extracts obtained using various extraction methods and solvents. These analyses identified functional groups associated with antioxidant activity and discriminated between extracts based on their chemical and biological properties. The in vivo evaluation used the fruit fly *Drosophila melanogaster* as a model organism to investigate the effects of the extracts on oxidative stress. This evaluation focused on the upregulation of antioxidant enzymes and their genes and the protection of tissues from ROS-induced damage.

Glochidion philippicum extraction

Leaves of *G. philippicum* were collected from the Samofa District, Biak Numfor Regency, Papua Province, Indonesia. The plant specimens were authenticated and deposited at the Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia, under voucher specimen number B-705. Only the leaves were used in this study. Briefly, fresh leaves of *G. philippicum* were collected, cleaned, and dried in a controlled drying cabinet at 40°C. The dried leaves were then ground into a fine powder and passed through a mesh no. 18 sieve. The powdered leaves were subjected to four different extraction techniques—

maceration, ultrasound-assisted extraction (UAE), reflux, and microwave-assisted extraction (MAE)—using four solvents: n-hexane, ethyl acetate, 70% ethanol, and distilled water. This process yielded 16 different extracts. Each extract was prepared by weighing 25 grams of sample and extracting it using one of the four different solvents. The extraction methods were conducted with specific durations and conditions tailored to each technique. For maceration, the process lasted 24 hours, with the first 8 hours involving continuous stirring using a magnetic stirrer (Thermo Scientific, Waltham, USA), followed by 16 hours of standing without agitation [11]. UAE was performed by mixing the sample with the solvent and placing it in an ultrasonic bath (Branson, Brookfield, USA) operating at a frequency of 40 kHz for 30 mins [12]. Reflux extraction involved combining the sample and solvent at a 1:20 ratio in a 1000 mL round-bottom flask, heating the mixture to 65°C on a heating mantle, and refluxing it for 2 hours before filtration [13,14]. MAE was carried out by irradiating the sample with a microwave (MG 2516, Modena, Italy) at 450 W for 10 mins, with the temperature monitored using an infrared thermometer. Following extraction, the mixtures were filtered, and the filtrates were concentrated using a rotary vacuum evaporator (Büchi, Flawil, Switzerland). The extraction yields were calculated and recorded [14,15]. FTIR spectra of all extracts were measured, and chemometric analyses were performed to cluster the extracts based on spectral similarities and to examine the relationships between functional groups and antioxidant activity. A schematic outlining the extraction and subsequent analyses is presented in **Figure 1**.

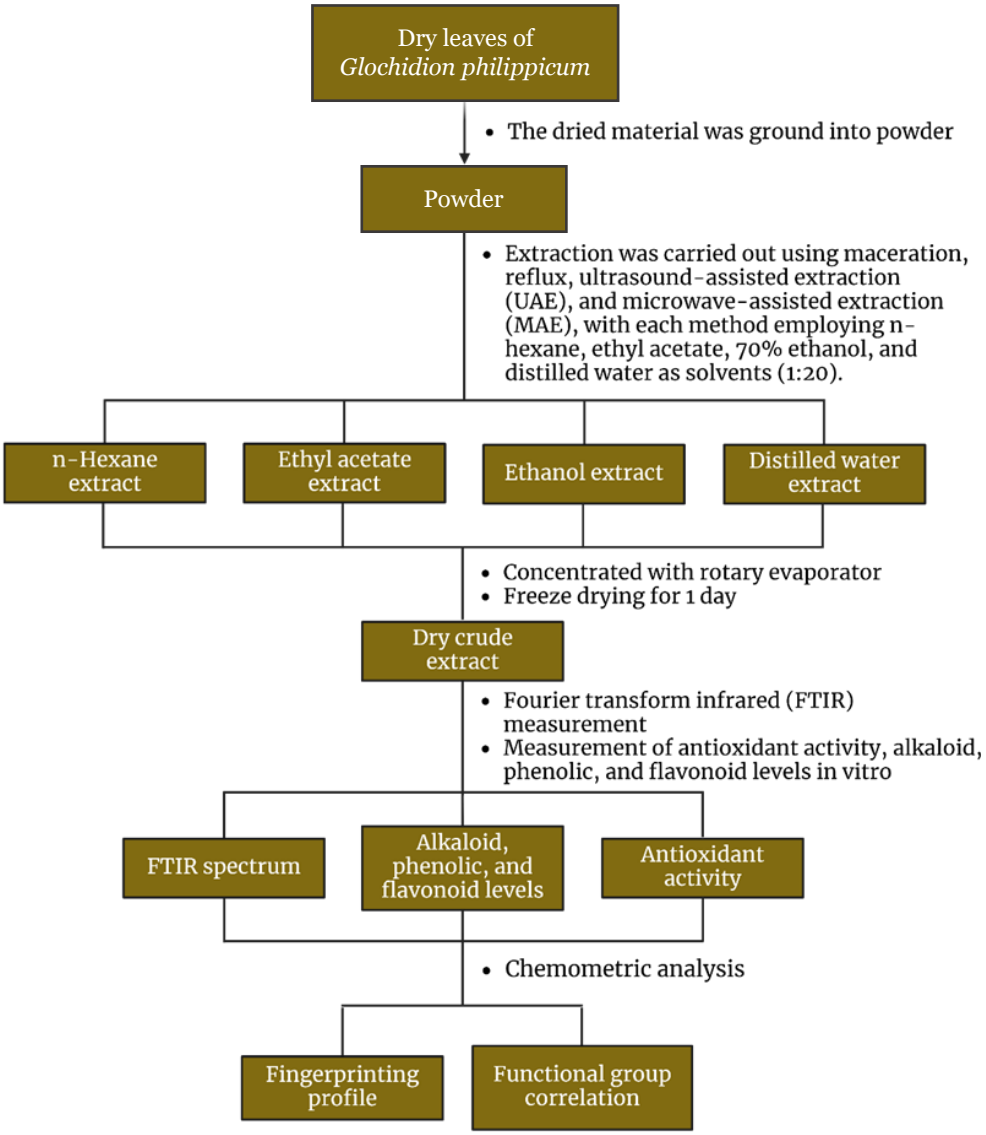

Figure 1. Schematic representation of the extract preparation process and subsequent analysis.

Determination of total phenolic and flavonoid content

For the determination of phenolic content, extracts were dissolved to a concentration of 1 mg/mL and mixed in a 96-well microplate. To each well, 50 µL of Folin-Ciocalteu reagent (1:10) (Sigma-Aldrich, St. Louis, USA) and 50 µL of Na_2CO_3 solution (7.5%) were added, followed by incubation for 30 mins in the dark. The total phenolic content was measured by reading the absorbance at 740 nm using a microplate reader (Agilent BioTek Epoch, Winooski, USA), and the results were calculated based on a gallic acid standard curve, reported in milligrams of gallic acid equivalents (mgGAE/g) [16]. For flavonoid determination, 50 µL of extract (1 mg/mL) or standard solution was added to a 96-well plate, followed by the addition of 10 µL of 10% $AlCl_3$, 150 µL of 96% ethanol, and 10 µL of 1M sodium acetate. The mixture was allowed to stand for 40 mins at room temperature and was shielded from sunlight. Absorbance was measured at 440 nm using the same microplate reader. The total flavonoid content was calculated from a quercetin standard curve and expressed as milligrams of quercetin equivalents (mgQE/g) [17]. For alkaloid determination, 50 mg of extract was dissolved in 5 mL of 2 N HCl and extracted with 10 mL of chloroform in a separatory funnel. After phase separation, the chloroform phase was discarded. To the remaining solution, 1 mL of 0.1 N NaOH, 2 mL of bromocresol green (BCG) solution, and 2 mL of phosphate buffer (pH 4.7) were added, followed by extraction with 5 mL of chloroform. This process was repeated twice. The final extract was evaporated, re-dissolved to 5 mL, and absorbance was measured at 273 nm. The total alkaloid content was determined using a caffeine standard curve and reported in milligrams of caffeine equivalents (mgCE/g) [18].

In vitro antioxidant activity assays

The in vitro antioxidant activity of the extracts was assessed using the ABTS method, with further validation through additional assays, DPPH and FRAP, conducted on the extracts exhibiting the highest antioxidant yields. All antioxidant evaluations were performed in triplicate. For the ABTS assay, the ABTS radical cation ($ABTS^{\bullet+}$) was prepared by combining 2 mM ABTS (Sigma-Aldrich, St. Louis, USA) with 2.45 mM $K_2S_2O_8$ in a 1:1 volume ratio, and the mixture was left to stand in the dark for 16 hours at room temperature. Before use, $ABTS^{\bullet+}$ was diluted with ethanol to achieve an absorbance of 0.70±0.05 at 734 nm. A 96-well plate was prepared by adding 20 µL of each sample and 180 µL of $ABTS^{\bullet+}$ solution, followed by incubation at room temperature for 6 mins, after which the absorbance was measured at 734 nm. Radical scavenging activity was calculated as the concentration required to inhibit 50% of the radicals [19]. For the DPPH assay, 160 µL of 1.5×10^{-4} M DPPH solution (Sigma-Aldrich, St. Louis, USA) was mixed with 40 µL of sample solution and incubated at room temperature for 30 mins. Absorbance was then measured at 540 nm using a microplate reader, and radical scavenging activity was expressed as the concentration that inhibited 50% of the radicals [20]. In the FRAP assay, the FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 2,4,6-tripyridyl-s-triazine (TPTZ) (Sigma-Aldrich, St. Louis, USA) (40 mM dissolved in 40 mM HCl), and 20 mM aqueous ferric chloride in a ratio of 10:1:1. Extracts (20 µL) were mixed with 280 µL of FRAP reagent, and after 30 mins of incubation in the dark, absorbance was measured at 630 nm. The results were expressed as mmol.Fe(II)/g [16].

Fourier-transform infrared spectroscopy (FTIR) profile

FTIR spectra were measured in an FTIR spectrophotometer (Shimadzu Type IRPrestige-21, Kyoto, Japan). The samples were prepared by mixing 2 mg of extract with 180 mg of potassium bromide (KBr) thoroughly to achieve a homogeneous blend. Pellets were then formed by applying a pressure of 8 tons for 15 mins using manual compression. The resulting pellets were placed in the sample compartment for analysis. FTIR measurements were performed within the 400–4,000 cm^{-1} wavenumber range, with a resolution of 4 cm^{-1} and 32 scans/min. The obtained FTIR spectra were stored as a data point table for subsequent analysis [21].

In vivo study

Drosophila stock

In this study, the *D. melanogaster* mutant line deficient in peptidoglycan recognition protein (PGRP)-LB protein, referred to as *PGRP-LB*$^{\Delta}$ flies, was used. This mutant line was originally

obtained from the Host Defense and Responses Laboratory, Kanazawa University, Kanazawa, Japan. It has been maintained for over 20 generations under standard laboratory conditions in the Laboratory of Pharmacology and Toxicology, Faculty of Pharmacy, Universitas Hasanuddin, Makassar, Indonesia. Flies were reared on a standard cornmeal-based diet at 25°C. Adult *D. melanogaster* flies aged 3–5 days were utilized. The *Drosophila* food was prepared by mixing corn, yeast, sugar, and agar, which was then supplemented with heat-killed *Escherichia coli*. This mixture was heated to 100°C with constant stirring until a thickened consistency was achieved.

Heat-killed E. coli preparation

The *E. coli* FNCC 0091 strain was cultured in luria-bertani (LB) broth medium at 37°C for 24 hours with agitation. Following incubation, the culture was autoclaved at 121°C and 2 atm pressure for 30 mins to eliminate bacterial viability.

Study setting

The extract solution was prepared using 70% ethanol as the solvent, given that the in vitro antioxidant activity was attributed to the ethanol-soluble fraction obtained through microwave-assisted extraction (MAEEO). To test its effects in vivo, MAEEO at concentrations of 0.625%, 0.312%, and 0.156% were prepared by pipetting the calculated volumes of the extract into the fly food, followed by thorough homogenization. The mixture was then carefully transferred into vials to create a controlled environment for subsequent experiments. Survival assays were conducted using 3-day-old second-instar larvae, and the effects of MAEEO on endogenous antioxidant-related genes—*sod1* (encoding superoxide dismutase 1), *sod2* (encoding superoxide dismutase 1), and *cat* (encoding catalase)—as well as ROS levels, were analyzed. The experimental design for the in vivo assays is depicted in **Figure 2**.

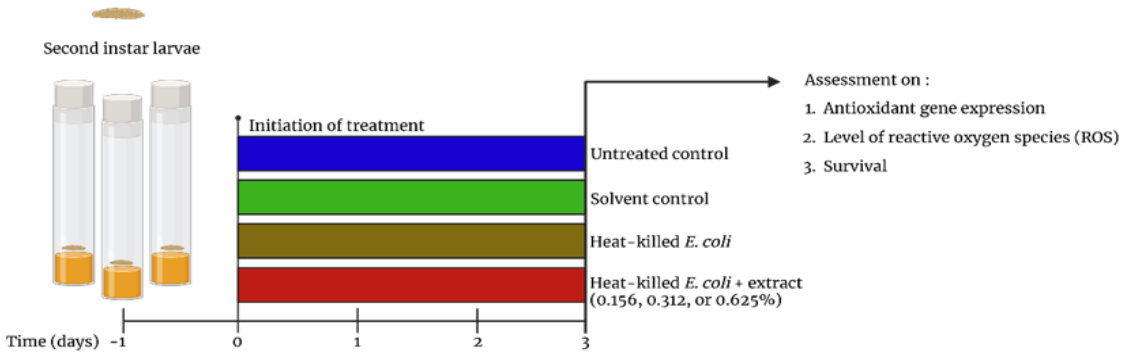

Figure 2. Schematic representation of in vivo assays used to evaluate the antioxidant and immunomodulatory activities of *Glochidion philippicum* extract in *Drosophila melanogaster*.

Survival analysis

A survival test was conducted to evaluate the ability of *D. melanogaster* larvae to survive under specific treatments. Second-instar larvae of *D. melanogaster* were exposed to various treatments incorporated into their fly food to evaluate the effects of the extracts. The treatments included: normal fly food as a control, fly food supplemented with heat-killed *E. coli* and 70% ethanol, fly food supplemented with heat-killed *E. coli* and 0.625% MAEEO extract, fly food supplemented with heat-killed *E. coli* and 0.312% MAEEO extract, and fly food supplemented with heat-killed *E. coli* and 0.156% MAEEO extract. The number of larvae that survived or died was recorded daily, with observations continuing until completion. The development of fruit flies from larvae to pupae, and from pupae to adults in each treatment group was observed.

Nitroblue tetrazolium reduction assay

Following the established protocol [22], the nitroblue tetrazolium (NBT) reduction assay was conducted to quantify ROS levels in the hemolymph of larvae *D. melanogaster*. The concentration of ROS was determined by measuring the absorbance at 595 nm. For statistical validity, a sample size of 50 larvae was used. Larvae were collected and rinsed in phosphate-buffered saline (PBS) to remove any residual food. Hemolymph was then extracted on ice to

prevent melanization. To the hemolymph, 200 µL of 1×PBS and an equal volume of NBT solution (Himedia by Laboratories, Kennett Square, USA) were added, resulting in a final volume of 300 µL. The mixture was incubated in the dark at room temperature for one hour, followed by the addition of 300 µL of 100% glacial acetic acid to terminate the reaction. After centrifugation at full speed at 16,000 rpm for one mins, the absorbance was measured using a spectrophotometer (Shimadzu UV-Vis 1800, Kyoto, Japan) at 595 nm after the addition of 50% acetic acid [22].

Gene expression analysis

Ten larvae *D. melanogaster* that had previously received different treatments incorporated into their food (**Figure 2**) were placed in Treff tubes for total RNA isolation using the PureLink RNA Mini Kit (Invitrogen, Thermo Fisher Scientific Inc, Massachusetts, USA), according to the manufacturer's protocol. Reverse transcriptase quantitative PCR (RT-qPCR) was then performed using the GoTaq 1-Step RT-qPCR System (Promega, Madison, USA) to evaluate the expression levels of target genes. The RT-qPCR reaction was conducted in a 10 µL volume, starting with an initial cycle at 37°C for 15 minutes, followed by 95°C for 10 minutes. This was followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. Each RT-qPCR run included standard melt curve analysis to confirm accurate amplification of the expected products. Ribosomal protein gene (*rp49*) was used as an internal control. The primer sequences used in this assay are listed in **Table 1**.

Table 1. Primers used in the RT-qPCR assay to assess the expression of endogenous antioxidant-related genes

Genes	Forward primer	Reverse primer
sod1	5'-AGGTCAA*CAT* CACCGACTCC-3'	5'-GTTGACTTGCTCAGCTCGTG-3'
sod2	5'-TGGCCA*CAT* CAACCACAC-3'	5'-TTCCACTGCGACTCGATG-3'
cat	5'-TTCCTGGATGAGATGTCGCACT-3'	5'-TTCTGGGTGTGAATGAAGGTGG-3'
rp49	5'-GACGCTTCAAGGGACAGTATCTG-3'	5'-AAACGCGGTTCTG*CAT* GAG-3'

Statistical analysis

The results of the antioxidant assay, along with the levels of phenolic, flavonoid, and alkaloid contents, were analyzed using Welch test (non-parametric) for pairwise comparisons using R Software (https://www.R-project.org/). Multivariate data analysis (MVDA) was performed using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/) to analyze FTIR wavenumber data, as well as the phenolic, flavonoid, and alkaloid contents, and antioxidant activity [23]. The sample grouping and differences were evaluated using principal component analysis (PCA). Potential functional groups associated with antioxidant activity were also analyzed to identify correlations. Survival assay data were presented as bar graphs, with statistical significance determined using the Log Rank test. The mRNA levels for all treatment groups were quantified and displayed in bar graphs. Statistical analysis of gene expression data was conducted using one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test. All datasets from the survival and gene expression studies were processed using GraphPad Prism 9 (GraphPad Software, Boston, USA). Data were expressed as mean ± standard deviation (SD), with statistical significance set at $p<0.05$.

Results

Extract yield

The resulting extract is characterized by a brown color and a viscous texture, reflecting its distinct consistency. The extraction yield varied significantly depending on the methods and solvents employed (**Figure 3**). This variation underscores the critical influence of both the extraction technique and solvent selection on the efficiency of extraction and the concentration of bioactive compounds within the extract.

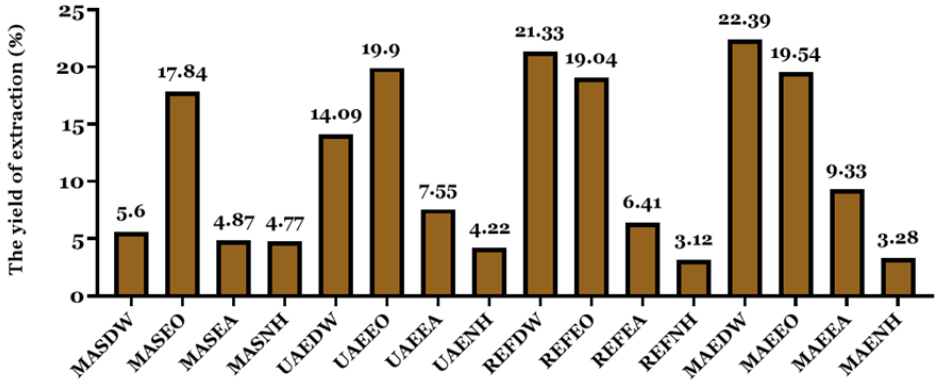

Figure 3. Comparisons of extraction yield (%) of different types of *Glochidion philippicum* extracts. MAEDW: microwave-assisted extraction with distilled water; MAEEA: microwave-assisted extraction with ethyl acetate; MAEEO: microwave-assisted extraction with ethanol; MAENH: microwave-assisted extraction with n-hexane; MASDW: maceration with distilled water; MASEA: maceration with ethyl acetate; MASEO: maceration with ethanol; MASNH: maceration with n-hexane; REFDW: reflux extraction with distilled water; REFEA: reflux extraction with ethyl acetate; REFEO: reflux extraction with ethanol; REFNH: reflux extraction with n-hexane; UAEDW: ultrasonic-assisted extraction with distilled water; UAEEA: ultrasonic-assisted extraction with ethyl acetate; UAEEO: ultrasonic-assisted extraction with ethanol; UAENH: ultrasonic-assisted extraction with n-hexane.

Total phenol content and total flavonoid content

This study quantitatively assessed the total phenolic and flavonoid contents across 16 extracts, with all analyses performed in triplicate and the results are presented in **Table 2**. Among these, the MAEEO extract demonstrated notably high levels of bioactive compounds, particularly phenolics and flavonoids.

In vitro antioxidant assay

The in vitro antioxidant activity of *G. philippicum* leaf extracts was assessed using the ABTS method, revealing notable antioxidant potential. Among the samples, the MAEEO extract demonstrated the most potent activity, with a value of 38.950±0.302 (**Table 3**). To corroborate these findings, additional assays were conducted using the DPPH and FRAP methods, which further confirmed the strong antioxidant capacity of the MAEEO extract.

Fourier-transform infrared spectroscopy (FTIR) results

The capability of rapid, nondestructive, reliable, and robust FTIR spectroscopy coupled with multivariate analyses for discrimination of *G. philippicum* leaf extract was evaluated in this study. The typical representative of FTIR spectra of *G. philippicum* leaf extract are presented in **Figure 4**. The figure is an infrared (IR) spectrum showing % transmittance versus wave number (cm^{-1}) for several samples. Each peak corresponded to specific functional groups associated with the infrared absorption of metabolites in *G. philippicum*. Notable peaks in the 1820–1660 cm^{-1} range corresponded to strong C=O group absorption, while the broad absorption band between 3400–2400 cm^{-1} was attributed to –OH groups. Medium to strong absorption at 1650–1450 cm^{-1} indicated aromatic C=C groups, and absorptions in the 1300–1000 cm^{-1} region suggested C–O (ester) groups. The fingerprint region (1000–400 cm^{-1}) exhibited consistent patterns, though variations in absorbance intensity were observed. The percentage of transmittance provides insight into how molecular groups absorb radiation at specific wavelengths, contributing to the differences in patterns across samples.

Table 2. Quantification of phenolic, flavonoid, and alkaloid contents in *Glochidion philippicum* leaf extract

Sample	Total phenolics content (mgGAE/g)	Total flavonoids content (mgQE/g)	Total alkaloid content (mgCE/g)
	Mean±SD	Mean±SD	Mean±SD
MASDW	273.306±5.006^{f,g}	2.672±0.158^{a,b}	0.794±0.072^{d,e}
MASEO	287.834±5.734^{h}	2.858±0.171^{b}	0.853±0.061^{e,f}
MASEA	11.698±0.367^{a,b}	7.288±0.621^{c}	0.699±0.019^{b,c,d}
MASNH	6.169±0.025^{a,b}	2.739±0.478^{b}	0.566±0.083^{a}
UAEDW	161.122±9.936^{c}	2.761±0.026^{b}	0.716±0.055^{c,d}
UAEEO	271.099±2.722^{f}	3.037±0.531^{b}	0.551±0.028^{a}
UAEEA	9.804±0.110^{a,b}	5.963±0.972^{c}	0.960±0.005^{f}
UAENH	3.428±0.061^{a,b}	2.292±0.273^{a,b}	0.506±0.008^{a}
REFDW	201.582±9.813^{d}	2.374±0.077^{a,b}	0.798±0.078^{d,e}
REFEO	236.892±5.126^{e}	2.754±0.254^{b}	1.327±0.025^{g}
REFEA	15.855±0.359^{a,b}	5.896±0.595^{c}	0.932±0.014^{f}
REFNH	1.380±0.017^{a}	3.044±1.017^{b}	0.574±0.018^{a,b}
MAEDW	273.674±3.674^{f,g}	2.449±0.068^{a,b}	0.535±0.006^{a}
MAEEO	261.903±10.526^{f}	2.344±0.034^{a,b}	0.490±0.012^{a}
MAEEA	17.878±0.314^{b}	6.819±0.542^{c}	1.638±0.036^{h}
MAENH	15.763±0.221^{a,b}	1.272±0.078^{a}	0.611±0.008^{a,b,c}

CE: caffeine equivalents; GAE: gallic acid equivalents; MAEDW: microwave-assisted extraction with distilled water; MAEEA: microwave-assisted extraction with ethyl acetate; MAEEO: microwave-assisted extraction with ethanol; MASDW: maceration with distilled water; MAENH: microwave-assisted extraction with n-hexane; MASEA: maceration with ethyl acetate; MASEO: maceration with ethanol; MASNH: maceration with n-hexane; QE: quercetin equivalents; REFDW: reflux extraction with distilled water; REFEA: reflux extraction with ethyl acetate; REFEO: reflux extraction with ethanol; REFNH: reflux extraction with n-hexane; UAEDW: ultrasonic-assisted extraction with distilled water; UAEEA: ultrasonic-assisted extraction with ethyl acetate; UAEEO: ultrasonic-assisted extraction with ethanol; UAENH: ultrasonic-assisted extraction with n-hexane

$^{a-h}$Different lowercase superscript letters within the same column indicate significant differences as determined by Welch test ($p<0.05$)

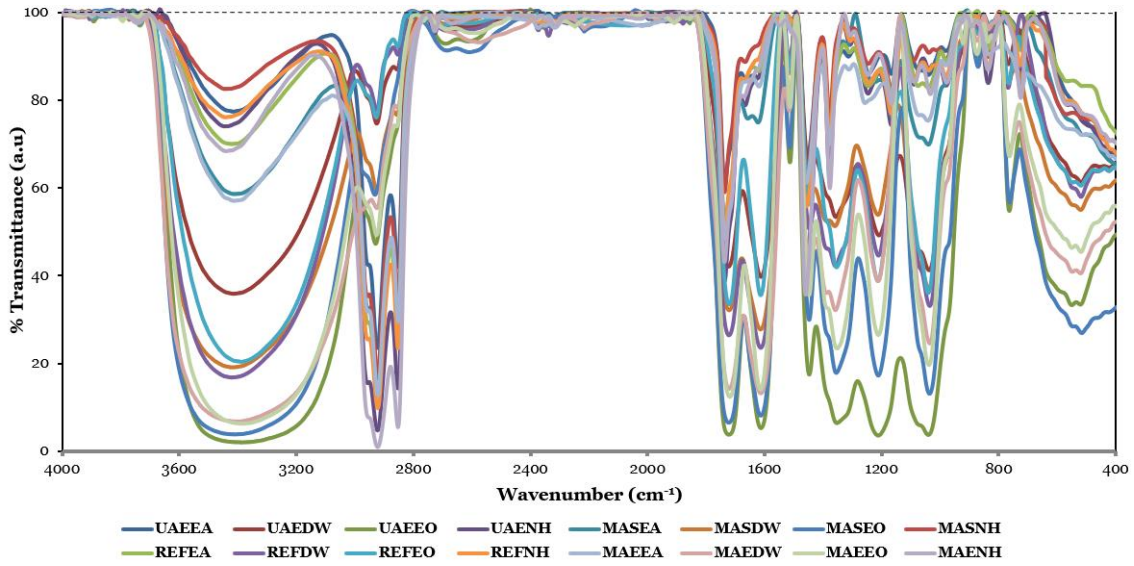

Figure 4. Fourier-transform infrared spectroscopy (FTIR) spectra of *Glochidion philippicum* leaf extracts in the 400–4,000 cm^{-1} region. Different colors represent the FTIR profiles of the 16 extracts. MAEDW: microwave-assisted extraction with distilled water; MAEEA: microwave-assisted extraction with ethyl acetate; MAEEO: microwave-assisted extraction with ethanol; MAENH: microwave-assisted extraction with n-hexane; MASDW: maceration with distilled water; MASEA: maceration with ethyl acetate; MASEO: maceration with ethanol; MASNH: maceration with n-hexane; REFDW: reflux extraction with distilled water; REFEA: reflux extraction with ethyl acetate; REFEO: reflux extraction with ethanol; REFNH: reflux extraction with n-hexane; UAEDW: ultrasonic-assisted extraction with distilled water; UAEEA: ultrasonic-assisted extraction with ethyl acetate; UAEEO: ultrasonic-assisted extraction with ethanol; UAENH: ultrasonic-assisted extraction with n-hexane.

Table 3. Antioxidant activity of *Glochidion philippicum* leaf extract using ABTS, DPPH, and FRAP methods

Sample	ABTS IC_{50} (µg/mL)	DPPH IC_{50} (µg/mL)	FRAP (mmol.Fe(II)/g)
	Mean±SD	Mean±SD	Mean±SD
MASDW	54.392±1.204^{a,b}	8.377±0.538^{a}	647.363±18.662^{g}
MASEO	63.482±1.287^{a,b}	15.519±1.454^{a}	516±7.512^{f}
MASEA	397.142±14.786^{d,e}	57.737±5.632^{a}	257.761±9.170^{d}
MASNH	264.984±13.503^{c}	1354.124±22.602^{f}	96.940±18.469^{b}
UAEDW	80.952±1.882^{b}	13.844±0.327^{a}	461.791±22.388^{e}
UAEEO	61.981±0.868^{a,b}	16.770±4.564^{a}	514.030±11.657^{f}
UAEEA	446.404±17.970^{e,f}	391.578±18.833^{c}	142.836±10.993^{c}
UAENH	492.763±25.882^{f}	900.144±33.537^{d}	41.716±11.044^{a}
REFDW	66.010±0.365^{b}	5.874±0.749^{a}	519.005±15.098^{f}
REFEO	55.119±1.293^{a,b}	7.223±0.204^{a}	527.463±6.840^{f}
REFEA	354.246±22.446^{d}	447.917±38.630^{c}	164.478±21.252^{c}
REFNH	647.954±59.846^{g}	1051.616±31.687^{e}	21.194±7.898^{a}
MAEDW	47.580±0.413^{a,b}	8.847±0.099^{a}	679.204±16.643^{g}
MAEEO	38.950±0.302^{a,b}	16.521±3.211^{a}	639.900±8.745^{g}
MAEEA	239.863±14.671^{c}	54.169±3.488^{a}	150.547±13.968^{c}
MAENH	349.826±4.156^{d}	300.162±65.565^{b}	45.821±4.222^{a}
Quercetin	7.674±0.263^{a}	3.914±0.317^{a}	-

ABTS: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power; IC_{50}: concentration to inhibit the 50% of ABTS or DPPH radical; MAEDW: microwave-assisted extraction with distilled water; MAEEA: microwave-assisted extraction with ethyl acetate; MAEEO: microwave-assisted extraction with ethanol; MASDW: maceration with distilled water; MAENH: microwave-assisted extraction with n-hexane; MASEA: maceration with ethyl acetate; MASEO: maceration with ethanol; MASNH: maceration with n-hexane; mmol.Fe(II)/g: mmol of Fe(II) equivalent per gram extract; REFDW: reflux extraction with distilled water; REFEA: reflux extraction with ethyl acetate; REFEO: reflux extraction with ethanol; REFNH: reflux extraction with n-hexane; UAEDW: ultrasonic-assisted extraction with distilled water; UAEEA: ultrasonic-assisted extraction with ethyl acetate; UAEEO: ultrasonic-assisted extraction with ethanol; UAENH: ultrasonic-assisted extraction with n-hexane
$^{a-g}$Different lowercase superscript letters within the same column indicate significant differences as determined by Welch test ($p<0.05$)

The functional groups and their corresponding transmittance values were further analyzed using hierarchical cluster analysis (HCA), and the results are displayed as a heatmap in **Figure 5**, which summarizes the functional group abundance of each sample with different solvents and extraction methods. The functional groups C–H (methyl), C–Br, N=O (nitro) 2, –CH (aromatic), C≡C, O–H (acid), C=C (aromatic), C=O, N=O (nitro) 1, C–O (ester) 2, C–O (ester) 1, and C–H (aldehyde) had high abundance in the samples, namely ultrasonic-assisted extraction with n-hexane (UAENH), maceration with n-hexane (MASNH), reflux extraction with ethyl acetate (REFEA), reflux extraction with n-hexane (REFNH), maceration with ethyl acetate (MASEA), ultrasonic-assisted extraction with ethyl acetate (UAEEA), microwave-assisted extraction with ethyl acetate (MAEEA), and microwave-assisted extraction with n-hexane (MAENH), while C–H (alkane) and C–H (aldehyde) 2 groups were abundant in samples of ultrasonic-assisted extraction with distilled water (UAEDW), microwave-assisted extraction with distilled water (MAEDW), MAEEO, reflux extraction with distilled water (REFDW), reflux extraction with ethanol (REFEO), ultrasonic-assisted extraction with ethanol (UAEEO), maceration with ethanol (MASEO), and maceration with distilled water (MASDW). Two principal components (PCs), which are principal component 1 (PC-1) at 86.1% and principal component 2 (PC-2) at 9.7%, accounted for 95.8% of the total variation (wavenumber data), as presented in the PCA score plot derived from the FTIR spectra data in **Figure 6**. The maximum total variance is considered by PC 1, the maximum residual variance is considered by PC 2, and so on. The PCA score plot, derived from FTIR spectra data, illustrated the clustering patterns and the differences and similarities between plant species and their components (**Figure 6A**). The samples near the central coordinate (0, 0) exhibited similarities, while those farther from the origin displayed greater differences, as indicated by the principal component (PC) values. Four functional groups C=C (aromatic), O–H (acid), N=O (nitro), and C–O (ester)—were identified as significant for differentiating the 16 extracts, with VIP values exceeding 1.0 (**Figure 6B**).

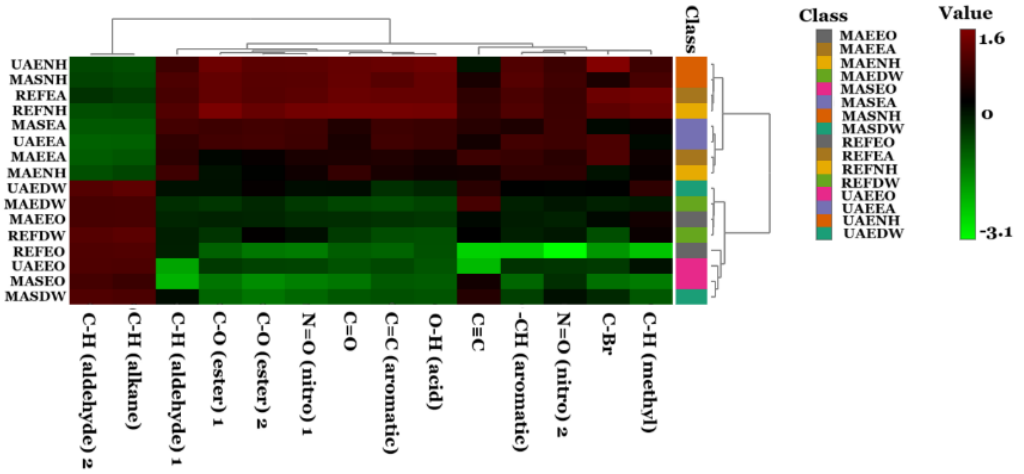

Figure 5. Heatmap representing the functional groups in *Glochidion philippicum* leaf extracts. The color scale illustrates the relative abundance of each functional group. Rows correspond to specific functional groups, while columns represent the various extraction samples. MAEDW: microwave-assisted extraction with distillation water; MAEEA: microwave-assisted extraction with ethyl acetate; MAEEO: microwave-assisted extraction with ethanol; MAENH: microwave-assisted extraction with n-hexane; MASDW: maceration with distillation water; MASEA: maceration with ethyl acetate; MASEO: maceration with ethanol; MASNH: maceration with n-hexane; REFDW: reflux with distillation water; REFEA: reflux with ethyl acetate; REFEO: reflux with ethanol; REFNH: reflux with n-hexane; UAEDW: ultrasonic-assisted extraction with distillation water; UAEEA: ultrasonic-assisted extraction with ethyl acetate; UAEEO: ultrasonic-assisted extraction with ethanol; UAENH: ultrasonic-assisted extraction with n-hexane.

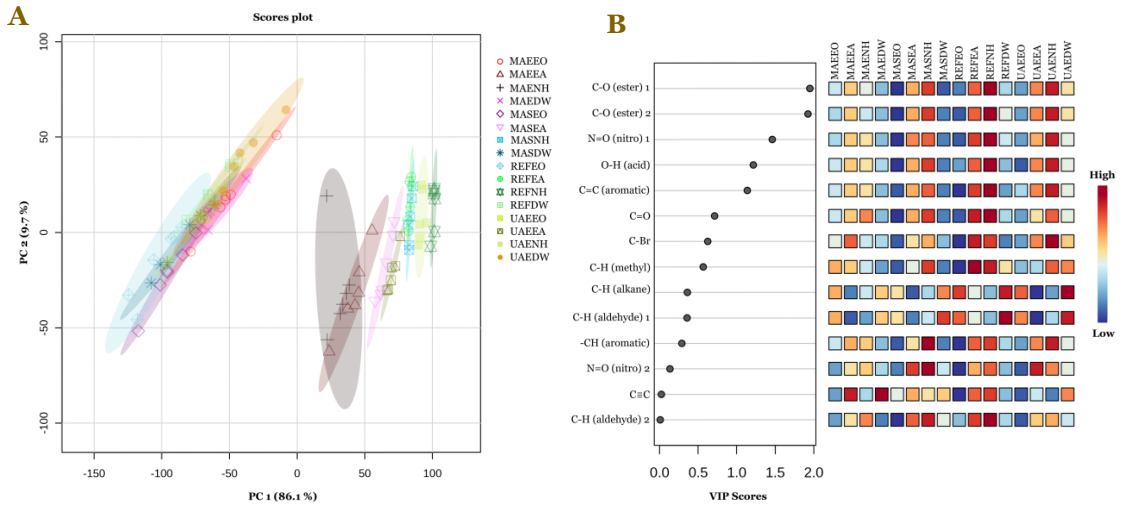

Figure 6. Clustering patterns and key bioactive metabolites of *Glochidion philippicum* leaf extracts. (A) Principal component analysis (PCA) score plots for the main components (PC-1) and (PC-2) on all samples of *G. philippicum* generated by FTIR analysis and the separation of clusters. (B) Variables importance in projection (VIP) scores of bioactive metabolites from partial least squares discriminant analysis (PLS-DA), highlighting compounds with high influence values on the sample variations. The variable scores, ranging from low to high, correspond to their importance. Colored boxes on the right indicate the relative concentration of the corresponding metabolites, with red representing high levels and blue indicating low levels. MAEDW: microwave-assisted extraction with distillation water; MAEEA: microwave-assisted extraction with ethyl acetate; MAEEO: microwave-assisted extraction with ethanol; MAENH: microwave-assisted extraction with n-hexane; MASDW: maceration with distillation water; MASEA: maceration with ethyl acetate; MASEO: maceration with ethanol; MASNH: maceration with n-hexane; REFDW: reflux with distillation water; REFEA: reflux with ethyl acetate; REFEO: reflux with ethanol; REFNH: reflux with n-hexane; UAEDW: ultrasonic-assisted extraction with distillation water; UAEEA: ultrasonic-assisted extraction with ethyl acetate; UAEEO: ultrasonic-assisted extraction with ethanol; UAENH: ultrasonic-assisted extraction with n-hexane.

The correlations between specific functional group compounds and their antioxidant potential, as assessed through the ABTS, DPPH, and FRAP assays using a combination of FTIR data and PLS-DA techniques, are presented in **Figure 7**. The antioxidant value in ABTS and DPPH methods refers to the IC_{50} value; the smaller the value, the better the antioxidant activity. While FRAP refers to its reduction ability; the greater the reduction value, the greater the antioxidant capacity. A strong positive correlation with ABTS activity was observed for the aromatic C=C, O–H, and C–O (ester) functional groups. Additionally, DPPH scavenging activity exhibited a positive correlation with the C=O, C=C (aromatic), and O–H groups. The C–H groups, presented in alkanes or aldehydes, also demonstrated a positive correlation with ABTS activity. Furthermore, the antioxidant activity of these metabolites was influenced by both their concentration and their reactivity with ROS.

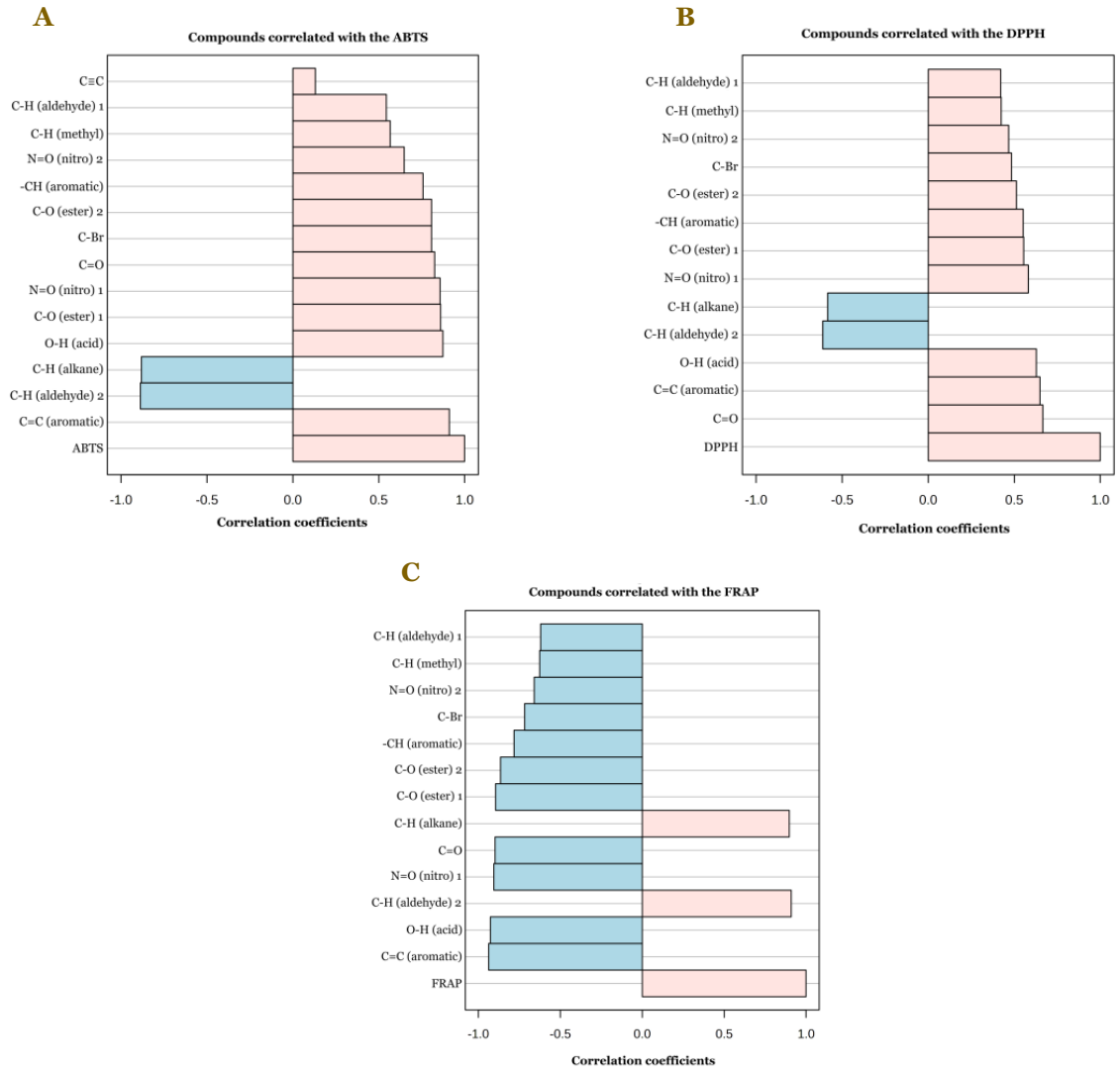

Figure 7. Correlation of functional group of *Glochidion philippicum* leaf extract and antioxidant activity. (A) 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS); (B) 1,1-diphenyl-2-picrylhydrazyl (DPPH); (C) ferric reducing antioxidant power (FRAP).

Effects of *Glochidion philippicum* extract on survival rate and ROS levels in *Drosophila* autoinflammation model

PGRP-LB$^{\Delta}$ mutant of *D. melanogaster* in a survival assay to assess the safety of MAEEO extract and investigate potential phenotypic effects in an autoinflammatory fly model was utilized. The comparison between untreated and heat-killed *E. coli* control groups revealed a reduced three-day survival rate in second instar larvae of the *PGRP-LB*$^{\Delta}$ mutant larvae (**Figure 8A**). Notably, no significant difference was observed between the solvent control (70% ethanol) and heat-killed *E. coli*. The increased production of ROS in *PGRP-LB*$^{\Delta}$ flies was attributed to the overactivation

of immune signaling in response to heat-killed *E. coli*. However, treatment with *G. philippicum* extract at all concentrations significantly improved the survival rate of the autoinflammatory flies, which serve as a model for chronic oxidative stress (**Figure 8B**). The treatment of *PGRP-LB*$^{\Delta}$ mutant larvae with heat-killed *E. coli,* followed by supplementation with MAEEO extract, led to a significant reduction in ROS levels, even at lower extract concentrations (**Figure 8C**). The concentrations of 0.312% and 0.156% of the extract significantly reduced ROS levels, a key marker of oxidative cellular damage (**Figure 8**).

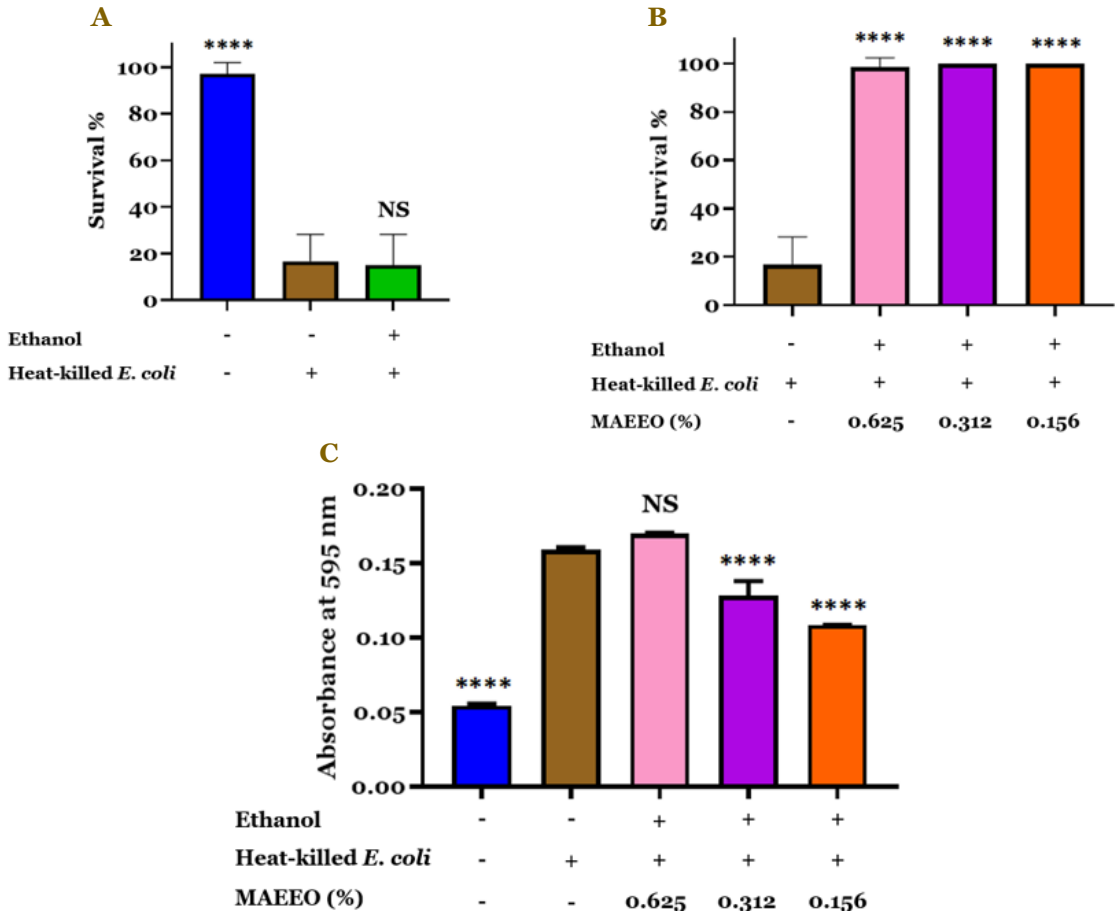

Figure 8. Pro-survival effect of microwave-assisted extraction with ethanol (MAEEO) of *Glochidion philippicum* on the lifespan of *Drosophila melanogaster PGRP-LB*$^{\Delta}$ treated with heat-killed *Escherichia coli*. (A) The survival of *D. melanogaster PGRP-LB*$^{\Delta}$ larvae was decreased following the treatment with heat-killed *E. coli*. (B) The survival of *D. melanogaster PGRP-LB*$^{\Delta}$ larvae treated with MAEEO extract at all concentrations was longer following exposure to heat-killed *E. coli*. (C) Treatment of *PGRP-LB*$^{\Delta}$ larvae with heat-killed *E. coli* increased reactive oxygen species (ROS) levels, while supplementation with MAEEO extract at concentrations of 0.312% and 0.156% significantly reduced ROS levels. ROS levels were assessed using the nitroblue tetrazolium (NBT) reduction assay coupled with spectrophotometric analysis. Data derived from each group were compared to that of the heat-killed *E. coli* control group. NS: not significant; ****significant at $p<0.0001$.

Effect of *Glochidion philippicum* extract on antioxidant-related gene expression in *Drosophila* autoinflammation model

The administration of MAEEO extract led to a significant reduction in ROS levels. Furthermore, the expression analysis of endogenous antioxidant genes, including *sod1*, *sod2*, and *cat*, revealed notable changes in their expression levels following MAEEO treatment in the *Drosophila* autoinflammation model (**Figure 9**). These findings indicate that the reduction in ROS levels induced by the extract may be due to its antioxidant activity, which could result from its inherent antioxidant potential or the modulation of genetic pathways involved in the endogenous antioxidant system.

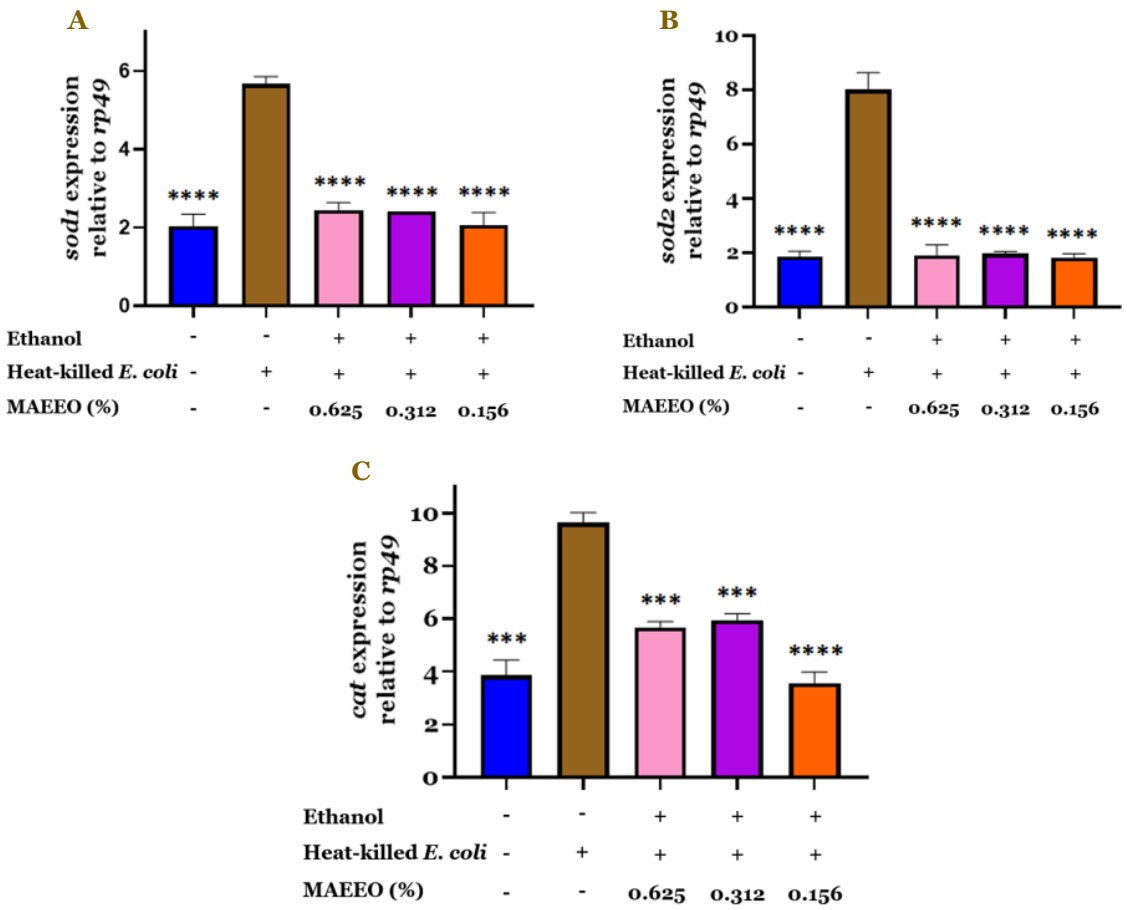

Figure 9. Expression of endogenous antioxidant genes *sod1* (A), *sod2* (B), and *cat* (C) in *PGRP-LB*$^{\Delta}$ mutant larvae treated with microwave-assisted extraction with ethanol (MAEEO) extract. *PGRP-LB*$^{\Delta}$ larvae were pretreated with heat-killed *Escherichia coli* before receiving MAEEO extract and subjected to molecular analysis using RT-qPCR. Data derived from each group were compared to that of the heat-killed *E. coli* control group. ***significant at $p<0.001$; ****significant at $p<0.0001$.

Discussion

This study elucidated the chemical fingerprint of *G. philippicum* through a comprehensive analysis of various parameters, including FTIR profiles, phenolic content, flavonoids, alkaloids, and antioxidant activity, both in vitro and in vivo. The extraction method and its specific conditions had a significant impact on the yield of biomass extract obtained (**Figure 3**). Notably, distilled water consistently produced higher extract yields across all extraction techniques, indicating that it is a more efficient solvent under the tested conditions. Furthermore, 70% ethanol generally produced higher yields than ethyl acetate and n-hexane, emphasizing the importance of solvent choice in optimizing extraction efficiency. Our data also indicated that the solvent, extraction duration, and temperature were critical factors in influencing yield, with methods involving heating (MAE and reflux) further enhancing extraction efficiency. The results underscored the potential for adapting the extraction parameters of distilled water and 70% ethanol to optimize yield across various extraction methods. By fine-tuning the extraction conditions, particularly for solvents such as distilled water and 70% ethanol, the overall efficiency of the extraction process can be significantly improved, enabling more effective utilization of bioactive compounds in further applications.

The highest phenolic contents were observed in samples extracted using maceration with ethanol and MAEDW, demonstrating the effectiveness of both maceration and MAEs with ethanol and water as solvents. These results suggested that ethanol and water are particularly efficient in extracting phenolic compounds due to their polarity, which facilitates the release of active molecules from the plant matrix. Additionally, the use of microwave heating in the MAE

method enhanced the release of phenolic compounds by reducing extraction time, thus improving efficiency compared to conventional methods. This is attributed to the rapid heating and pressure created within the plant matrix, which enhances solvent penetration and facilitates the release of bioactive compounds [24]. Semi-polar solvents, such as ethyl acetate, are more effective at extracting flavonoids, as indicated by the high total flavonoid contents (TFC) in samples extracted with ethyl acetate, such as MASEA and MAEEA. Ethyl acetate's ability to dissolve non-polar polyphenolic substances makes it highly effective for flavonoid extraction [25]. Furthermore, the use of microwave and ultrasonic energy enhances solvent diffusion into the plant matrix, thereby further boosting flavonoid yields. Ethyl acetate's ability to dissolve non-polar polyphenolic substances makes it highly effective for flavonoid extraction [25]. The highest alkaloid concentrations were found in MAEEA and REFEO, highlighting the efficiency of the MAE and reflux techniques with ethyl acetate as the solvent for alkaloid extraction. Ethanol and ethyl acetate are particularly suited for alkaloid extraction due to their ability to dissolve semi-polar compounds [26]. Among the solvents tested, ethanol and ethyl acetate were the most effective in extracting phenolic compounds, flavonoids, and alkaloids, as their polarity aligns well with the characteristics of these bioactive compounds. Water was also effective, especially when used in the MAE method, further indicating that polar solvents enhance the extraction of bioactive compounds. In contrast, n-hexane was less effective for extracting phenolic compounds and flavonoids, which are more polar, although it could still extract alkaloids under certain conditions.

Samples with high antioxidant activity in the ABTS and DPPH assays also exhibited elevated TPC, such as MAEDW (273.674 mg GAE/g) and MAEEO (261.903 mg GAE/g), supporting the correlation between phenolic content and antioxidant activity. These samples also showed significant flavonoid content (2.344–2.449 mg QE/g) and strong activity across all three assays (ABTS, DPPH, and FRAP). While MASEA had high flavonoid content (7.288 mg QE/g), its high IC_{50} values (397.142 and 57.737 µg/mL) suggested that phenolics may contribute more to its antioxidant activity. Indeed, a recent study suggested that flavonoids and phenolics acted as free radical inhibitors by donating electrons to radicals [27]. Alkaloids appear to play a lesser role in antioxidant activity compared to flavonoids and phenolics [28]. For example, MAEEA had the highest alkaloid concentration (1.638 mg CE/g), but a modest FRAP value (150.547 mmol.Fe (II)/g) (**Table 3**), which is in line with some literature that shows alkaloids have weaker antioxidant properties [29]. FTIR analysis may provide further insight into the bioactive compounds contributing to the observed antioxidant activity. Differences in antioxidant activity across assays arise from their distinct mechanisms. The ABTS assay detects both hydrophilic and lipophilic antioxidants, while the DPPH assay is more suited for lipophilic compounds [30]. FRAP measures the ability of compounds to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) under acidic conditions, which tends to reflect the total reduction capacity, not just the radical scavenging activity [30]. Complex compounds, such as plant extracts, contain a mixture of hydrophilic and lipophilic compounds with different antioxidant activities, leading to differences in the resulting activity.

The FTIR spectra of the 16 extracts showed similar patterns, with variations in the intensity of transmittance peaks (**Figure 4**). Notably, hydroxyl groups play a crucial role in antioxidant mechanisms by donating hydrogen atoms to neutralize free radicals. Carbonyl groups, especially when conjugated with aromatic systems, enhance radical stabilization by participating in electron delocalization and influencing the molecule's ability to chelate pro-oxidant metal ions [31, 32].

To classify the samples by organ type, species, and origin, unsupervised pattern recognition techniques, PCA and HCA, were employed [33]. Phenolic groups and conjugated electron systems, which tend to attract hydroxyl groups, play a crucial role in antioxidant properties by facilitating electron transfer between radicals, hydroxyl groups [34], and hydrogen atom transfer [35]. This indicates that the scavenging activity may be attributed to the active hydrogen donor ability of hydroxyl group substitution [36]. Based on these observations and the in vitro antioxidant activity data, the MAEEO extract was selected for further testing. The antioxidant activity of MAEEO extract most likely originates from hydrogen atom transfer from C–H groups, present in alkanes or aldehydes.

The PCA technique was also used in this study for the discrimination and/or grouping of samples based on the solvent and extraction method used. The total PCA value formed was 95.8%, which indicated that the samples were well discriminated/grouped. A total PCA value above 70% indicates good sample discrimination [37, 38]. In addition, the combination of FTIR and PLS-DA data was applied to find the functional groups that contribute majorly to the biological activity performed (ABTS, DPPH, and FRAP). This combination technique has also been applied to *Sonchus arvensis* as an antioxidant and xanthine oxidase inhibitor [39].

Based on these findings, the MAEEO extract, which exhibited consistent antioxidant activity, was selected for further in vivo testing using the autoinflammatory model *Drosophila PGRP-LB$^{\Delta}$*. The peptidoglycan recognition protein (PGRP) in *D. melanogaster*, specifically PGRP-LB, plays a crucial role in regulating the synthesis of antimicrobial peptides (AMPs) and inhibiting the immune deficiency (IMD) pathway [40]. In the absence of PGRP-LB, the presence of peptidoglycans from Gram-negative bacteria, such as *E. coli*, triggers a significant increase in the production of proinflammatory AMPs under the IMD pathway. A previous study has shown that continuous activation of the IMD pathway in *PGRP-LB$^{\Delta}$* mutant flies resulted in reduced survival rates [41]. Our data indicated that heat-killed *E. coli* exposure reduced the survival rate of *PGRP-LB$^{\Delta}$* larvae, while the solvent used to dissolve the extract had no adverse effect on survival (**Figure 8**). However, treatment with all concentrations of the MAEEO extract significantly improved the survival of heat-killed *E. coli*-treated *PGRP-LB$^{\Delta}$* larvae. Given that treatment with all concentrations of the MAEEO extract significantly improved survival without exhibiting a dose-dependent effect, it is possible that the protective effects of the extract reach their maximum efficacy at the lowest concentration tested. This suggests that the active compounds in the extract may be sufficient to activate the protective pathways even at lower concentrations, with higher doses failing to provide additional benefits. This could be due to the saturation of the biological target or signaling pathway responsible for the protective effect, where once the target is fully activated, further increases in concentration do not enhance the outcome.

Cell stress often leads to an increase in ROS levels [42], a phenomenon that could be triggered by the use of heat-killed *E. coli* and/or extracts. To explore this, NBT assay was used to measure ROS levels after administering heat-killed *E. coli*, in the presence or absence of *G. philippicum* leaf extract. Our data indicated that treatment of heat-killed *E. coli* increased the level of ROS in *PGRP-LB$^{\Delta}$* mutant larvae, similar to our previous observation [22]. However, treatment with 0.312% and 0.156% MAEEO extracts resulted in reduced ROS levels. These results suggested that the extract may help mitigate ROS effects, potentially by enhancing the expression of antioxidant enzymes such as SOD, which neutralize ROS and preserve cellular redox balance. To test this hypothesis, the expression of *sod1*, *sod2*, and *cat* using RT-qPCR was measured. Treatment with heat-killed *E. coli* was observed to upregulate the expression of *sod1*, *sod2*, and *cat* in *PGRP-LB$^{\Delta}$* mutant larvae. However, when heat-killed *E. coli* was administered in conjunction with MAEEO extract, the expression of *sod1*, *sod2*, and *cat* was found to be downregulated. These findings indicate that an excessive immune response may elevate ROS levels, resulting in oxidative stress. This oxidative stress likely stimulates the upregulation of SOD and CAT as a compensatory mechanism to mitigate ROS. Once ROS levels are effectively reduced, the expression of *sod1*, *sod2*, and *cat* returns to their basal levels, signifying the restoration of cellular redox homeostasis. Additionally, the results can be interpreted to suggest that the MAEEO extract may exert an antioxidant effect independently, without the need for upregulation in *sod1, sod2,* and *cat*. However, it is reasonable to assume that the MAEEO extract can reduce ROS either by directly exerting antioxidant effects or by modulating the expression of these key antioxidant genes, which play a critical role in neutralizing oxidative stress. These findings underscore the potential of the MAEEO extract as a therapeutic agent for managing conditions related to oxidative stress, possibly by activating endogenous antioxidant defense mechanisms.

Despite our findings, we acknowledge some limitations in our study. Notably, data supporting the conclusion of endogenous antioxidant activity in the *Drosophila* autoinflammation model is still lacking. Further investigation into the molecular mechanisms through which the extract confers protection against reduced survival due to heat-killed *E. coli* is necessary, including the analysis of other pro-survival-related cellular signaling pathways. This research will offer a more comprehensive understanding of how these extracts enhance the

survival of the affected host. Addressing these gaps will undoubtedly enhance our understanding and broaden the knowledge base regarding the potential of *G. philippicum* as an antioxidant.

Conclusion

The present study demonstrated the antioxidant properties of *G. philippicum* leaf extracts, confirmed through in vitro assays (ABTS, DPPH, FRAP) and in vivo testing. The antioxidant activity was influenced by extraction methods and solvents, as indicated by distinct fingerprint profiles. In *D. melanogaster*, the MAEEO extract, extracted using MAE method with ethanol as solvent, reduced ROS levels, suggesting its potential to mitigate oxidative stress. In addition, significant changes in antioxidant gene expression were observed. These findings highlight the potential of *G. philippicum* as a natural antioxidant for further exploration.

Ethics approval

Not required.

Acknowledgments

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

This study used artificial intelligence (AI) tools and methodologies in the following capacities. A manuscript writing support AI-based language model (ChatGPT) was employed for language refinement (improving grammar, sentence structure, and readability of the manuscript). We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

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