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Phytochemical composition, antioxidant and antibacterial activity of the Philippine copperleaf (*Acalypha wilkesiana*) ethanolic extract

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Abstract

The Philippine copper leaf (*Acalypha wilkesiana*) is a relatively untapped plant with notable ethnopharmacological properties. This study investigated the phytochemical composition, antioxidant and antimicrobial activity of the leaf ethanolic extract of *A. wilkesiana* collected at Central Philippine University Gardens in Iloilo City, Philippines. Qualitative phytochemical analysis revealed the presence of bioactive compounds, including alkaloids, tannins, saponins, proteins, phenols, flavonoids, glycosides, carbohydrates, and terpenoids. Quantitatively, the total phenolics and flavonoid contents of the extract were 1,203.7±0.33 mg gallic acid equivalent (GAE)/g and 235.36±2.0 mg of quercetin equivalent (QE)/g, respectively. The antioxidant activity, assessed using the 1,1-diphenyl-1-picrylhydrazyl (DPPH) assay, demonstrated a concentration-dependent radical scavenging capacity. The ethanolic extract exhibited strong antibacterial effects against *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Streptococcus agalactiae*, *Escherichia coli*, *Edwardsiella tarda*, *Pseudomonas aeruginosa*, and *Salmonella typhi*, with significant inhibition observed at 100 mg/mL. Resolute results from this first study on the phytochemicals, antioxidants, and antimicrobial activity of the Philippine copperleaf clearly demonstrate that it contains potent bioactive compounds with notable antibacterial and antioxidant properties. This underscores its potential for developing new therapeutic agents.

Key words: *Acalypha wilkesiana*, antimicrobial activity, antioxidant, phytochemical compounds, *Staphylococcus aureus*, *Pseudomonas aeruginosa,* copperleaf

1. Introduction

Acalypha wilkesiana, commonly known as the copperleaf or Jacob's coat, is a tropical shrub in the Euphorbiaceae family (Ibrahim et al., 2021), renowned for its vibrant foliage and ornamental value. In recent years, this plant has garnered increasing attention in scientific

circles for its potential health benefits, particularly its rich phytochemical composition, which includes a diverse array of secondary metabolites such as flavonoids, alkaloids, tannins, and terpenoids (Dada et al., 2019; Ibrahim et al., 2021). Among the various bioactive compounds identified in *A. wilkesiana*, its antioxidant and antibacterial properties stand out as areas of significant interest (Anokwuru et al., 2015; Didunyemi et al., 2020). Antioxidants in the plant play a critical role in neutralizing reactive oxygen species, thereby protecting cells from oxidative stress and damage. This suggests exciting prospects for the integration of *A. wilkesiana* into functional foods, dietary supplements, or pharmaceutical formulations. However, despite the promising antioxidant potential, there remain significant gaps in our understanding of the specific mechanisms of action, optimal extraction methods, and the stability of these compounds under different environmental conditions. In addition to its antioxidant potential, *A. wilkesiana* has demonstrated significant antibacterial activity, enhancing its biomedical relevance (Anokwuru et al., 2015). Extracts from this plant have shown inhibitory effects against various pathogenic bacteria, indicating their potential role in combating infectious diseases (Goptep et al., 2010; Oluduro et al., 2011). However, research in this area is still in its early stages, with many unanswered questions, including the identification of specific antibacterial compounds, the elucidation of underlying mechanisms, and the exploration of the plant's efficacy against a broader spectrum of bacterial strains.

This growing interest in medicinal plants, particularly in developing countries, is largely driven by the reported safety and lack of adverse side effects of herbal medicines compared to synthetic drugs (Helmstädter and Staiger, 2014). The global prevalence of multi-drug-resistant bacterial strains, which are becoming increasingly less susceptible to conventional antibiotics (Urban-Chmiel et al., 2022), has spurred research into endemic plants like *A. wilkesiana* as sources of novel antimicrobial agents. While commercial antibiotics are commonly used to treat bacterial infections, they are often associated with unfavorable side effects, such as allergic reactions and hypersensitivity. As a result, the search for safer, more effective alternatives in traditional and endemic medicinal plants has intensified (Pratap et al., 2012), with nearly 80% of the world's population relying on these plants for basic healthcare needs due to their availability and efficacy. Addressing the existing research gaps in understanding *A. wilkesiana*'s therapeutic potential will not only enhance our knowledge but also pave the way for practical applications in medicine and healthcare.

While published reports have highlighted the antioxidant and antibacterial properties of *A. wilkesiana* against various bacterial pathogens, there is still a notable gap in the literature that needs to be addressed. To date, no comprehensive study has evaluated the antibacterial efficacy of *A. wilkesiana* extract specifically against human pathogenic bacteria in the Philippines. This underscores the urgent need for in-depth research into the antimicrobial attributes of the Philippine copperleaf. Such an investigation should include a thorough analysis of its phytochemical composition and antioxidant activity. Given the bioactive compounds with promising antibacterial activities possessed by *A. wilkesiana*, a thorough investigation of its antimicrobial characteristics is imperative. This study focused on the first evaluation of the phytochemical constituents, antioxidant potential, and antibacterial activity of the Philippine copperleaf *A. wilkesiana* against selected human bacterial pathogens, including those implicated in zoonoses. Resolute results generated from this pilot study are pivotal not only for enhancing our understanding of the therapeutic properties of *A. wilkesiana* against human pathogenic bacteria but also for paving the way for future research focusing on the isolation of novel bioactive compounds in concomitance with the development of effective interventions for managing infectious diseases.

2. Materials and Methods

Collection of plant material

The leaves of copperleaf plants were collected from Central Philippine University Gardens (10° 43' 29.39" N, 122° 32' 33.59" E) in January 2024 at daytime with ambient temperature ranging from 29-32°C. The plant samples were washed thrice with tap water and once with distilled water and air dried. The leaves were then dried in a drying oven at 45°C for 72 hours following a modified method adapted from Goptep et al. (2010). The plant samples were cut into small pieces and powdered using a mixer grinder. The powdered samples were collected in sterile amber bottles and stored at -20°C until used (Pakingking et al., 2022).

Preparation of *A. wilkesiana* extract

Utilizing a modified method adapted from Pakingking et al. (2022), ethanol was employed to extract the powdered leaves. The plant extraction process involved a 1:3 dilution ratio, with 150 grams of dried plant powder soaked in 450 mL of 80% ethanol for 72 hours at room temperature (28°C). The resulting extracts were filtered through sterile Whatman filter paper No. 1 (320 mm, 11 µm). This extraction procedure was repeated for an additional 72 hours. Subsequently, the two filtrates were combined and concentrated using a rotary evaporator under reduced pressure at 45°C. The concentrated extracts were then stored at -20°C until needed for various analyses.

Phytochemical testing

The *A. wilkesiana* leaf ethanolic extract was subjected to a series of assays to determine the presence of phytochemical compounds present based on the standard procedures outlined by Harborne (1998), Evans (2009), and Silva et al. (2017). The qualitative detection of alkaloids present in *A. wilkesiana* ethanolic extract was carried out using Mayer's and Wagner's test. Additionally, the presence of flavonoids (Shinoda test), glycosides (Keller-Killiani test), phenols and tannins (ferric chloride test), saponin (foam's test), proteins (Biuret test), carbohydrates (Molisch's test), and terpenoids (Salkowski test), were likewise qualitatively examined.

Quantitative Analysis

Total phenolic content

Following Singleton's method (Singleton et al., 1999) outlined by Stankovic et al. (2011), the quantification of total soluble phenolic compounds in the *A. wilkesiana* extract was conducted utilizing the Folin-Ciocalteu reagent, with gallic acid serving as the reference standard (Singleton et al., 1999). The extract was dissolved in ethanol, attaining a concentration of 1 mg/mL for analysis. In summary, 2.5 mL of 10-fold diluted Folin-Ciocalteu reagent, along with 2 mL of 7.5% $NaHCO_3$, was combined with 0.5 mL of the ethanolic extract. The resultant reaction mixture was thoroughly mixed and allowed to stand for approximately 45 minutes at 45°C. Subsequently, the absorbance was measured at 765 nm against a blank sample using a spectrophotometer. Utilizing a standard curve generated with gallic acid, the total phenolic content was determined and expressed as milligrams of gallic acid equivalent per gram (mg GAE/g) of extract.

Total flavonoid content

The flavonoid content in *A. wilkesiana* was determined using the Dowd method (Arvouet-Grand et al., 1994). A 1 mL aliquot of extract solution (ranging from 25 to 200 µg/mL) or quercetin (25 to 200 µg/mL) was mixed with 0.2 mL of 10% (w/v) aluminum chloride solution

in methanol, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water. This mixture was incubated at room temperature for 30 minutes, after which the absorbance was measured at 415 nm against a blank. The results were expressed as milligrams of quercetin equivalents per gram (mg QE/g) of dry extract.

1,1-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The assessment of *A. wilkesiana* extract's capacity to neutralize 1,1-diphenyl-1-picrylhydrazyl (DPPH) free radicals was conducted in accordance with the procedure outlined by Cho et al. (2010), with minor modifications. Briefly, ethanol was utilized to prepare stock solutions of the extracts, yielding concentrations of 2.5, 2.0, 1.0, 1.50, 0.75, 0.50, and 0.25 mg/mL. Subsequently, 100 microliters of each diluted solution were combined with 100 µL of 0.1 mM DPPH, which was previously prepared in ethanol. The resulting mixture underwent a 30-minute incubation period in darkness at room temperature (28°C), following which the absorbance was recorded at 515 nm using a microplate reader. The blank utilized in this study was ethanol, while the control consisted of 100 µL of ethanol mixed with 100 µL of DPPH. Additionally, ascorbic acid (20 µg/mL), known as vitamin C, served as the positive reference (Cho et al., 2010). The percentage of DPPH scavenged was determined using the formula:

$$\text{DPPH Scavenging activity (\%)} = ([\text{ACon} - \text{ATest}]/\text{ACon}) \times 100,$$

where ACon represents the absorbance of the control, and ATest represents the absorbance of the sample. The crucial half-maximal inhibitory concentration (IC_{50}) was determined through rigorous linear regression analysis, averaging results from three determinations.

Antibacterial Assay

Bacterial strains

The bacterial isolates used in the assay include *Staphylococcus aureus* (ATCC 25923), *S. aureus* (BIOTECH 1582), *Streptococcus agalactiae* (TMD10206) (Pakingking et al., 2022), methicillin resistant *S. aureus* (RMC0224), *Edwardsiella tarda* (TK2014) (Pakingking and Nguyen, 2022), *Escherichia coli* (BIOTECH 1634), *Pseudomonas aeruginosa* (ATCC 27853), *P. aeruginosa* (BIOTECH 1335), *and Salmonella typhi* (RMC 0324) (Table 3). All microorganisms used in the assay were maintained in trypticase soy broth (TSB; Merck) supplemented with 15% glycerol at -80°C (Pakingking et al., 2022).

Agar Well Diffusion Method

The antibacterial activity of the *A. wilkesiana* ethanolic extract was examined using the modified agar well diffusion method of Mattana et al. (2010) as described by Pakingking et al. (2022). Briefly, the *A. wilkesiana* extract was dissolved in sterile NSS to obtain an initial concentration of 1000 mg/ mL and sterilized by filtration through 0.45 µm membrane filter (Millipore). All tests were conducted in triplicate using different concentrations of the *A. wilkesiana* extracts diluted in NSS. Amoxicillin (0.025 mg/mL) was used as the standard antimicrobial agent.

The bacterial isolates utilized in the antibacterial assay were cultured following the methodology outlined by Pakingking et al. (2015). Briefly, bacterial isolates were inoculated into tryptic soy broth (TSB) and were incubated for 18-24 hours at 37°C. The concentration of the cultures was standardized, by adjusting their turbidity to match the 0.5 McFarland standard using sterile normal saline solution (NSS), resulting in approximately 1×10^8 colony forming units per mL (CFU/mL). The bacterial suspensions were then evenly spread on Mueller-Hinton agar (MHA; Merck) plates (150 mm), each containing 75 mL of solidified MHA, and subsequently punctured with wells measuring 7 mm in diameter at suitable

intervals using a sterile cork borer. Each well was loaded with 100 µL of *A. wilkesiana* extract at concentrations ranging from 1.562 to 100 mg/mL (Table 3). Simultaneously, wells containing 100 µL of amoxicillin (0.025 mg/mL) and NSS were used as positive and negative controls, respectively. The plates were then incubated at 37°C for 24 hours. Following incubation, the plates were retrieved, and the zones of growth inhibitions surrounding the wells were measured. The presence of clear zones around the wells indicated the occurrence of antibacterial activity.

Statistical Analysis

All experiments were conducted in triplicate and one-way ANOVA was employed to compare the mean values of each treatment. Significant differences among the bacterial isolates tested per treatment or concentration of *A. wilkesiana* extract were compared using Duncan test ($P< 0.05$).

3. Results

Yield of *A. wilkesiana* extract

In the current study, 20.28 grams of crude *A. wilkesiana* ethanolic extract were obtained, resulting in a yield of 13.52%. This outcome is notably consistent with the findings of Ogbonna et al. (2021), who reported a yield of approximately 12.05% using 70% ethanol as the extraction solvent from 100 grams of powdered *A. wilkesiana*. While our yield is slightly higher, it concurs closely with their results, suggesting comparable efficiency in the extraction process.

Phytochemical screening

As presented in Table 1, the qualitative phytochemical analysis of various aliquots of the crude *A. wilkesiana* ethanolic extract revealed the presence of several bioactive compounds. These compounds include alkaloids, tannins, saponins, proteins, phenols, flavonoids, glycosides, carbohydrates, and terpenoids.

Table 1. Qualitative phytochemical screening of the *Acalypha wilkesiana* ethanolic extract

Phytochemical compound	Test	Result		
		Aliquot 1	Aliquot 2	Aliquot 3
Alkaloids	Mayer's and Wagner's Test	+	+	+
Tannins	Ferric Chloride Test	+	+	+
Saponins	Foam Test	+	+	+
Proteins	Biuret Test	+	+	+
Phenols	Ferric Chloride Test	+	+	+
Flavonoids	Shinoda Test	+	+	+
Glycosides	Keller Killiani Test	+	+	+
Carbohydrates	Molisch's test	+	+	+
Terpenoids	Salkowski Test	+	+	+

(+) = present

Total phenolic content

As indicated in Table 2, the mean total phenolic content in the three aliquots of *A. wilkesiana* ethanolic extract was 1,203.7±0.33 mg GAE/g.

Total flavonoid content

The total flavonoid content in the crude *A. wilkesiana* ethanolic extract was measured using quercetin as the standard and expressed in terms of its equivalent (mg QE/g). The mean flavonoid content in the three aliquots of the *A. wilkesiana* ethanolic extract was found to be 235.36±2.0 mg QE/g (Table 2).

Table 2. Total phenolic and flavonoid contents of the *Acalypha wilkesiana* ethanolic extract

Test	Result*
Total phenolic content (mg of gallic acid equivalent per gram [mg GAE/ g] of extract)	1,203.7±0.33
Total flavonoid content (mg of quercetin equivalent per gram [mg QE/g] of extract)	235.36±2.0

*Data are presented as Mean ± SD of 3 aliquot samples.

DPPH radical scavenging activity

Figure 1 illustrates the DPPH radical scavenging activity of the *A. wilkesiana* ethanolic extract. This extract showed a concentration-dependent increase in scavenging capacity, ranging from 0.25 to 2.5 mg/mL. As the concentration increased, the recorded scavenging activities rose from 28.33±1.53% to 92.5±0.87%. The calculated IC_{50} value for the *A. wilkesiana* ethanolic extract was 0.86 mg/mL. Furthermore, the scavenging activity of the *A. wilkesiana* extract was comparable to that of the positive control, ascorbic acid.

Antibacterial activity

To further investigate the antibacterial activity of the *A. wilkesiana* ethanolic extract, the agar well diffusion method was employed. None of the NSS control wells produced zones of inhibition on any of the bacterial strains tested, while the antibiotic control wells showed inhibition zones of 30.3±0.6 mm for *S. aureus* ATCC25923. As illustrated in Figure 2 and detailed in Table 3, the *A. wilkesiana* ethanolic extract exhibited strong antibacterial activity against *S. aureus* ATCC25923, *S. agalactiae* TMD10206, and *E. tarda* K2014, producing inhibition zones of 28 mm, 28.7±1.2 mm, and 28.7±1.2 mm, respectively, after 24 hours of exposure to 100 mg/mL of the extract. A concentration-dependent decrease in antibacterial activity was observed, with the inhibition zone for *S. aureus* reducing to 12 mm at 1.562 mg/mL. Additionally, the extract demonstrated antibacterial activity against *E. coli* BIOTECH 1634, *P. aeruginosa* ATCC27853 and BIOTECH 1335, and *S. typhi* RMC 0324. The antibacterial activity at 100 mg/mL against *S. agalactiae, E. tarda, E. coli, and P. aeruginosa* were not significantly different ($P<0.05$). However, the *A. wilkesiana* extract showed antibacterial activity against MRSA only at the lowest tested concentration of 25 mg/mL (Table 3).

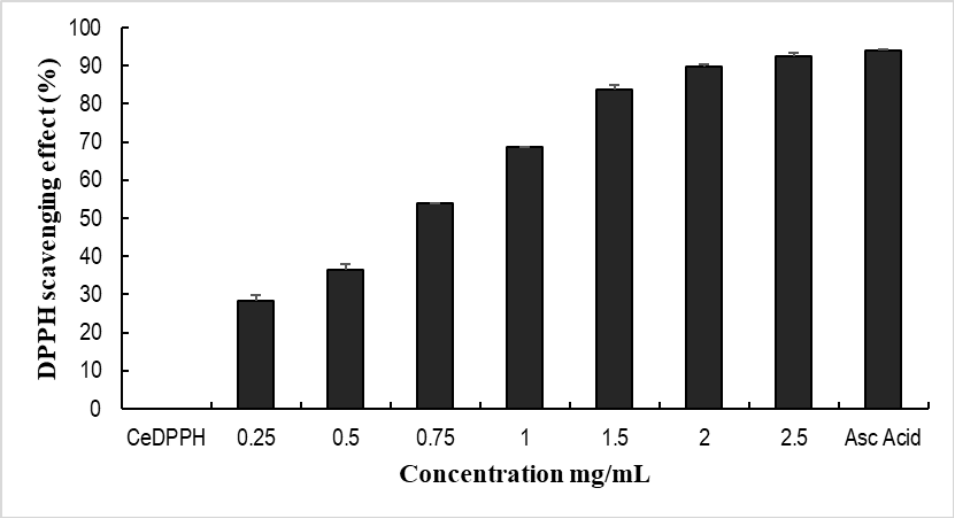

Figure 1. DPPH (1,1-Diphenyl-1-picrylhydrazyl) radical scavenging activity of the *Acalypha wilkesiana* ethanolic extract. Ethanol + DPPH (CeDPPH) and ascorbic acid (Asc) were used as negative and positive controls, respectively.

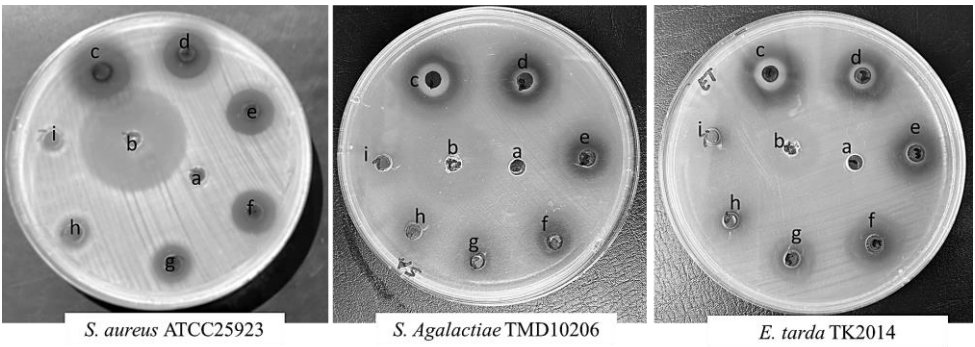

Figure 2. Representative plates for the agar well diffusion assay showing zones of inhibition produced by the varying concentrations of the *A. wilkensiana* ethanolic extract (AwEE) on *Staphylococcus aureus* ATCC25923, *Streptococcus agalactiae* TMD1026, and *Edwardsiella tarda* TK2014. Negative control (NSS) (a); Amoxicillin 0.025 mg/mL (b); 100 mg/mL AwEE (c); 50 mg/mL AwEE (d); 25 mg/mL AwEE (e); 12.5 mg/mL AwEE (f); 6.25 mg/mL AwEE (g); 3.125 mg/mL AwEE (h); 1.563 mg/mL AwEE (i).

Table 3. Antimicrobial activity of the *Acalypha wilkensiana* ethanolic extract against selected human bacterial pathogens

Microorganism	Inhibition Zone (mm±SD)								
	Amoxicillin 0.025 mg/mL	NSS	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.125 mg/mL	1.562 mg/mL
Gram-Positive									
Staphylococcus aureus ATCC 25923	30.3±0.6	–	28.0±0.0^{c}	25.3±0.6^{c}	23.7±0.6^{c}	21.7±1.2^{c}	18.0±0.0^{d}	13.7±0.6^{c}	12.0±0.0^{c}
Staphylococcus aureus BIOTECH 1582	27.7±0.6	–	31.0±1.0^{ab}	26.3±1.5^{bc}	24.3±1.2^{bc}	20.7±1.2^{c}	17.7±0.6^{d}	14.3±0.6^{c}	10.7±0.6^{d}
Streptococcus agalactiae TMD10206	31.3±0.6	–	28.7±1.2^{c}	25.0±0.0^{c}	20.3±0.6^{d}	16.7±0.6^{d}	14.3±0.6^{e}	12.0±0.0^{d}	–
Methicillin resistant *S. aureus* RMC0224	–	–	21.3±0.6^{d}	18.0±0.0^{d}	14.3±0.6^{e}	–	–	–	–
Gram-negative									
Edwardsiella tarda TK2014	22.7±0.6	–	28.7±1.2^{c}	26.7±1.2^{b}	24.3±0.6^{bc}	22.3±0.6^{bc}	19.3±0.6^{c}	14.3±0.6^{c}	11.3±0.6^{d}
Escherichia coli BIOTECH 1634	–	–	28.7±1.2^{c}	27.3±0.6^{ab}	25.3±0.6^{b}	23.7±0.6^{b}	21.0±1.0^{b}	16.3±0.6^{b}	15.0±0.0^{b}
Pseudomonas aeruginosa ATCC 27853	–	–	27.7±0.6^{c}	23.7±0.6^{c}	20.3±0.6^{d}	17.3±0.6^{d}	13.7±1.0^{e}	–	–
Pseudomonas aeruginosa BIOTECH 1335	–	–	29.3±1.2^{bc}	25.3±0.6^{c}	20.3±0.6^{d}	15.0±1.0^{e}	–	–	–
Salmonella typhi RMC 0324	29.7±0.6	–	31.7±1.5^{a}	28.3±0.6^{a}	27.3±0.6^{a}	25.3±0.6^{a}	24.0±0.0^{a}	22.0±0.0^{a}	17.7±0.6^{a}

(–) no zone of inhibition. Data are presented as Mean ± SD. Values with different superscripts a, b, c, d, e within each column are significantly different as determined by Duncan test ($P < 0.05$)

4. Discussion

In concurrence with the previous report of Ogbonna et al. (2021), our current study also identified various bioactive compounds in the *A. wilkesiana* ethanolic extract, including alkaloids, flavonoids, phenols, saponins, tannins, glycosides, proteins, and terpenoids. However, unlike Ogbonna et al. (2021), we detected glycosides, proteins, and terpenoids, which were absent in their findings. This discrepancy could be attributed to differences in plant species, geographical region, seasonal variation, and extraction processes, which can significantly affect the chemical composition of plants. The bioactive compounds present in *A. wilkesiana* leaves are known to possess antioxidant and antibacterial properties, making them potential alternatives for treating bacterial infections in humans, including those inflicted by zoonotic pathogens. The phytochemicals identified in this study, such as terpenoids, alkaloids, and flavonoids, exhibit a range of bioactivities, including antimutagenic, anticarcinogenic, antioxidant, antimicrobial, and anti-inflammatory properties (Okarter and Liu, 2010). Notably, flavonoids are well documented for their antimicrobial and potent antiviral activities and are often referred to as biological response modifiers due to their ability

to alter the body's reactions to allergens and carcinogens (Dias et al., 2021). Numerous flavonoid derivatives have proven to be effective antimicrobial agents against various microorganisms. Their antimicrobial activity is likely due to their ability to form complexes with extracellular and soluble proteins as well as with bacterial cell walls. Additionally, the lipophilic nature of flavonoids allows them to disrupt microbial membranes (Dias et al., 2021). Alkaloids, on the other hand, are known for a broad range of pharmacological potentials, including antiasthma, antimalarial, and anticancer activities (Thawabteh et al., 2019). These complex heterocyclic nitrogenous compounds exhibit significant antimicrobial properties and are effective against viral and protozoan infections. The mechanism of action for alkaloids often involves their ability to intercalate with DNA, thereby disrupting vital cellular processes (Thawabteh et al., 2019). Saponins have been identified for their antidiabetic, antifungal, and anti-inflammatory properties. These amphipathic glycosides, which can be mono- or polydesmodic, are known for their immunostimulant and antinociceptive (pain-relieving) effects (Nah et al., 2000). Tannins, which are polyphenolic compounds, exhibit antioxidant, antimicrobial, and anti-inflammatory properties (Macáková et al., 2019). They are particularly noted for their role in wound healing, as they help repair underlying tissues (Macáková et al., 2019). Tannins possess astringent properties that facilitate the healing of wounds and inflamed mucous membranes and reduce bacterial cell proliferation by inhibiting key microbial enzymes and metabolic pathways (Rivière et al., 2009). Glycosides have been reported to exhibit anti-hyperglycemic activity. Terpenoids, another class of bioactive compounds, are recognized for their antiviral, antimalarial, antibacterial, and anti-inflammatory properties (Bergman et al., 2019). It is therefore apparent that the presence of these bioactive compounds in *A. wilkesiana* supports its potential use in developing natural treatments for bacterial infections and promoting overall health. Additionally, the diverse pharmacological activities of flavonoids, alkaloids, saponins, tannins, glycosides, and terpenoids highlights *A. wilkesiana*'s therapeutic potential and underscores the importance of further research into its medicinal properties.

Phenolic compounds are ubiquitous secondary metabolites commonly found in plants and are known for their diverse biological activities, including antioxidant and antibacterial effects. They are excellent scavengers of free radicals, which are implicated in the pathology of various human diseases (Zhang et al., 2022). The antioxidant activity of phenolic compounds is largely dependent on the structure of their hydrophobic benzenoid rings and the hydrogen-bonding potential of their phenolic hydroxyl groups. To measure the total phenolic content in the *A. wilkesiana* extract, we employed the Folin-Ciocalteu method. In this assay, phenols in the plant extract lose a proton to form a phenolate ion, which then reduces the Folin-Ciocalteu reagent, and the resulting changes are measured spectrophotometrically at 765 nm (Stankovic et al., 2011). The total phenolic content of the *A. wilkesiana* ethanolic extract obtained in this study was 1,203.7±0.33 mg GAE/g (Table 2). This value is slightly lower than the total phenolics quantified by Oyedemi et al. (2018) in *A. hispida*, which was approximately 3,600 mg GAE/g. However, it is important to note that the Folin-Ciocalteu reagent also reacts with other compounds such as ascorbic acid, amino acids, and sugars, which may result in an overestimation of the phenolic content in *A. hispida* leaves. Flavonoids, another significant group of phenolic compounds, are increasingly consumed in significant amounts in daily diets worldwide due to their numerous health benefits (Dias et al., 2021). In the total flavonoid assay, aluminum ions in the reacting mixture form complexes with the C-4 keto and either C-3 or C-5 hydroxyl, or with ortho-hydroxyl groups in the A or B ring of flavonoids detected in the plant extracts (Arvouet-Grand et al., 1994). As shown in Table 2, the total flavonoid content of the *A. wilkesiana* ethanolic extract in this study was 235.36 ± 2.0 mg QE/g. This is somewhat lower compared to the total flavonoids quantified by Oyedemi et al. (2018) in *A. hispida*, which was approximately 1,200 mg QE/g. The total phenolic content quantified in *A. wilkesiana* was higher compared to its total flavonoid content, which supports the fact that flavonoids are a subset of phenolic compounds.

Importantly, our current data clearly demonstrate that the *A. wilkesiana* ethanolic extract is a rich source of phenolic compounds. The differences in the quantities of total phenolics and flavonoids observed in *A. wilkesiana* compared to other published reports on *Acalypha* species can be attributed to several factors, including species variation, stage of maturity, plant part examined, harvesting time, geographical location, and the assay methods employed.

The skin and oral cavity are frequently exposed to various oxidants, including cigarette smoke, chemicals, UV light, nicotine, and alcohol. These exposures can lead to the production of reactive oxygen species (ROS), such as hydroxyl radicals and superoxide anions, which are highly reactive molecules derived from oxygen metabolism (Zhang et al., 2022). While ROS play crucial roles in biochemical, immunological, and physiological processes, their excessive accumulation can damage vital organs and therefore contribute to various human diseases. Recognizing the need to combat stress-induced diseases, the food and pharmaceutical industries have focused on medicinal plants rich in phenolic and flavonoid compounds (Zhang et al., 2022). These compounds possess significant antioxidant properties, neutralizing free radicals and protecting the body from oxidative stress. By scavenging these reactive species, phenols and flavonoids mitigate cellular and tissue damage, preventing or alleviating conditions linked to oxidative stress. In the current study, the *A. wilkesiana* extract exhibited a concentration-dependent scavenging effect, with its inhibitory effect increasing with higher concentrations of the extract (Fig. 1). The IC_{50} value for the *A. wilkesiana* ethanolic extract was determined to be 0.858 mg/mL, which is at par with the IC_{50} documented in the crude methanolic extract of *A. hispida* previously reported by Oyedemi et al. (2018). Also, consistent with published reports on *Acalypha* species (Anokwuru et al., 2015; Didunyem et al., 2020; Goptep et al., 2010; Ogbonna et al., 2021; Oluduro et al., 2011), the antioxidant activity of *A. wilkesiana* extract in this study correlated with its phenolic and flavonoid content. As pointed out earlier, free radicals, produced during normal metabolism (e.g., mitochondrial activity, inflammatory responses, phagocytosis, and physical activities), can be accelerated by external factors such as radiation, drugs, and pesticides. Overproduction of free radicals can damage all classes of biological molecules, including proteins, amino acids, nucleic acids, and carbohydrates (Rodríguez-Yoldi, 2021). Thus, counteracting oxidative stress through natural antioxidants, such as those derived from *A. wilkesiana*, is a practical strategy to protect organisms from oxidative damage (Anokwuru et al., 2015). This approach underscores the importance of leveraging natural compounds in developing effective therapeutic agents.

As illustrated in Table 3, the ethanolic extract of *A. wilkesiana* exhibits potent antibacterial activity against *S. aureus* strains ATCC 25923 and BIOTECH 1582. In a previous study, Ogbonna et al. (2021) documented that 125 mg/mL of the *A. wilkesiana* ethanolic extract induced a mean inhibition zone of 16.67±0.9 mm. Notably, our current study shows a higher antibacterial activity, with inhibition zones of 28 and 31±1.0 mm, respectively, for the *S. aureus* strains exposed to 100 mg/mL of the extract. Furthermore, a concentration-dependent reduction in antibacterial activity was observed; even at the lowest concentration of 1.562 mg/mL, the extract produced inhibition zones of 12 and 10.7±0.6 mm for *S. aureus* strains ATCC 25923 and BIOTECH 1582, respectively (Table 3). These findings concur with previous reports that highlight the sensitivity of gram-positive *S. aureus* strains to *Acalypha* spp. extracts, particularly *A. wilkesiana* (Haruna et al., 2013; Ogbonna et al., 2021; Oyedemi et al., 2018; Ureta et al., 2019). *S. aureus*, a common skin microbiota component, is notorious for causing skin infections due to its resistance to first-line antibiotics. Additionally, *S. aureus* is part of the natural microbiota in pond-reared fish and their environments (Pakingking et al., 2015). While not typically considered a fish pathogen, *S. aureus* is a leading cause of gastroenteritis from fish products contaminated with the bacterium and its enterotoxins (Arfatahery et al., 2015). High populations of *S. aureus* in harvested fish indicate significant spoilage. The *A. wilkesiana* extract also demonstrated potent antibacterial activity against *S. agalactiae* in a concentration-dependent manner. This finding is crucial given the emergence

of multidrug-resistant *S. agalactiae* strains in humans and fish, underscoring its zoonotic potential through the consumption of infected fish (Li et al., 2020). Moreover, the extract showed dose-dependent antibacterial activity against MRSA strain RMC0224 (Table 3). Our finding corroborates previous reports by Osibote et al. (2021) and Akenyemi et al. (2005) on the antibacterial efficacy of *A. wilkesiana* extract against MRSA. Considering that MRSA is a significant cause of bacteremia, endocarditis, skin and soft tissue infections, bone and joint infections, and hospital-acquired infections (Turner et al., 2019), the potential of *A. wilkesiana* extract against MRSA warrants further investigation.

Notably, the *A. wilkesiana* extract exhibited potent antibacterial activity against several gram-negative human pathogenic bacteria, including *E. tarda, E. coli, P. aeruginosa*, and *S. typhi*. At a concentration of 100 mg/mL, the zones of inhibition induced by the extract were significantly pronounced. Even at the lowest concentration tested (1.562 mg/mL), antibacterial activity was still evident for *E. tarda, E. coli*, and *S. typhi* (Table 3). The antibacterial efficacy of the *A. wilkesiana* ethanolic extract against *E. coli, P. aeruginosa*, and *S. typhi* corroborates previous studies of Haruna et al. (2013), Osibote et al. (2021), and El-Raey et al (2016). Our current data highlight the potential of *A. wilkesiana* extract as a valuable source of antibacterial compounds, offering a pragmatic strategy for combating multi-drugresistant bacterial pathogens (Parmanik et al., 2022). It is particularly noteworthy that this study is the first to report the antibacterial activity of *A. wilkesiana* crude ethanolic extract against *E. tarda*. Human infections with *E. tarda* often present as gastroenteritis but can escalate to systemic and potentially fatal conditions (Pakingking and Nguyen, 2022). Given these findings, the *A. wilkesiana* extract could play a crucial role in developing new treatments for such infections. Taken together, the significant antibacterial properties of *A. wilkesiana* ethanolic extract against both gram-positive and gram-negative bacteria, including antibiotic-resistant strains, underscore its potential as a source of novel antimicrobial agents and therefore reinforces the need for further research into the therapeutic applications of *A. wilkesiana*.

The ethanolic leaf extract of *A. wilkesiana* demonstrated significant antibacterial activity against selected gram-positive and gram-negative human pathogenic bacteria. The antibacterial effect was concentration-dependent, with higher concentrations of the extract producing larger zones of inhibition. This indicates that the efficacy of *A. wilkesiana* is directly related to its concentration. The potent antibacterial activity can be attributed to the presence of bioactive compounds in the *A. wilkesiana* ethanolic extract, which contain important functional groups. These compounds include alkaloids that can intercalate with bacterial DNA, disrupting their replication and function, and flavonoids that can complex with extracellular and soluble proteins as well as the bacterial cell wall, thereby inhibiting bacterial growth. Additionally, the lipophilic nature of flavonoids allows them to disrupt microbial membranes, further enhancing their antibacterial properties. To the best of our knowledge, this study is the first to report on the phytochemical composition, antioxidant, and antibacterial activities of the Philippine copperleaf (*A. wilkesiana*) ethanolic extract. Our findings are particularly significant in addressing the challenge posed by antibiotic-resistant bacteria such as MRSA. The investigation of this traditional medicinal plant has revealed its substantial potential for therapeutic applications. Future studies should therefore focus on isolating and characterizing the major bioactive components of *A. wilkesiana*. Furthermore, it is worth investigating the antiparasitic activity of *A. wilkensiana*, especially against *Entamoeba histolytica*, a significant protozoan pathogen associated with diarrheal outbreaks (Beup et al., 2024). Understanding their specific modes of action against clinically important bacterial pathogens, including multidrug-resistant strains, will be crucial for developing new antibacterial therapies. Finally, our study underscores the essential value of investigating traditional medicinal plants as a source of innovative solutions to address modern medical challenges.

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Conflict of interests

The authors declare that they have no competing interests.

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