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PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT, AND ANTIBACTERIAL ACTIVITIES OF CRUDE AND PARTIALLY PURIFIED EXTRACTS OF *Portulaca oleracea* LEAVES

Demy Valerie Chacon[1], Kiana Alika Co[1], Daphne Noreen Enriquez[1], Aubrey Love Labarda[1], Reanne Eden Manongsong[1], Edward Kevin B. Bragais[1,2,*]

[1]Department of Biology, School of Science and Engineering, Ateneo De Manila University, Quezon City, Philippines
[2]Department of Biochemistry and Molecular Biology, University of the Philippines Manila, Philippines

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ABSTRACT

The increasing antibiotic resistance among pathogens has driven the search for natural alternatives, particularly plant-based antimicrobials. This study investigates the antioxidant and antibacterial activities of *Portulaca oleracea*, a plant renowned for its polyphenolic constituents. The leaves were extracted using solvents of varying polarities to obtain different fractions for analysis. Qualitative phytochemical screening revealed a rich profile of polyphenolic compounds, including flavonoids, tannins, phenolic acids, and anthocyanins. The crude methanolic extract exhibited significant ($p < 0.05$) antioxidant activities against DPPH (50.08 ± 2.49%), superoxide (42.61 ± 0.56%), and hydroxyl (6.60 ± 0.94%). Furthermore, this extract demonstrated antibacterial efficacy, displaying zones of inhibition (ZOI) against *Staphylococcus aureus* (8.95 ± 0.19 mm) and *Escherichia coli* (7.18 ± 0.26 mm). Meanwhile, the polyphenol-rich aqueous fraction exhibited the highest antioxidant activities among the tested extracts ($p < 0.05$) with potent antibacterial activities against *S. aureus* (ZOI = 11.21 ± 0.11 mm) and *E. coli* (ZOI = 10.21 ± 0.18 mm). The bioactivities may be linked to polyphenolic compounds like quercetin, herbacetin, and rhamnetin, as identified by UPLC-MS. The high total phenolic content (181.58 ± 5.34 mg GAE/g) in the aqueous fraction aligns with its strong antioxidant and antibacterial effects ($p < 0.05$). These results suggest the potential of *P. oleracea* as a natural source of antibacterial agents and warrant further investigation into its mechanisms of action.

Keywords: Antibacterial activity, antioxidant capacity, liquid-liquid extraction, polyphenolic metabolites, *Portulaca oleracea.*

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[*]*Corresponding author email*: ebragais@ateneo.edu

INTRODUCTION

The rise of antimicrobial resistance (AMR) is an urgent global health crisis, with an estimated 700,000 deaths annually attributed to resistant infections a figure that could reach 10 million by 2050 if no new treatments are developed (World Health Organization, 2023). This alarming trend is largely driven by the overuse and misuse of antibiotics in human medicine and agriculture, compounded by social and economic factors, inadequate infection control measures, and poor community hygiene (Mancuso et al., 2021; Collignon & Beggs, 2019). As we face a future where many common infections could become untreatable, the need for alternative antimicrobial agents is more pressing than ever (Martinez & Baquero, 2014). Consequently, the discovery of alternative antimicrobial agents that can deal with antibiotic-resistant bacteria is prioritized in scientific research (Martinez & Baquero, 2014).

Natural products, particularly those derived from plants, offer a promising avenue for developing new therapeutic agents. Plant secondary metabolites have evolved to help plants respond to various environmental stressors, including herbivory, abiotic challenges, and interspecific competition (Yang et al., 2018). These plant metabolites have been used in traditional medicine for centuries, making plants valuable reservoirs for discovering new antimicrobial compounds (Álvarez-Martínez et al., 2021). These metabolites, including flavonoids, phenolic acids, and tannins, contribute to the plant's defense mechanisms and exhibit significant antimicrobial properties. Flavonoids, for instance, are known for their ability to scavenge reactive oxygen species (ROS), reducing oxidative stress and inflammation - factors implicated in chronic diseases like cardiovascular ailments and cancer (Barnham et al., 2004). Meanwhile, phenolic compounds have demonstrated broad-spectrum antibacterial activity, reinforcing the role of *Portulaca oleracea* in combating resistant pathogens. In recent years, the identification and characterization of various antimicrobial agents has been made possible by the rapid improvement of technology and the use of new, increasingly efficient methods (Katz & Baltz, 2016).

P. oleracea, also known as common purslane, is a warm-climate herbaceous succulent annual plant that grows worldwide and is one of the top eight plants on Earth (Srivastava et al., 2021). It is a member of the Portulacaceae family, which includes more than 120 species of succulent plants (Zhou et al., 2015). *P. oleracea* is highly valuable medicinally and has been identified by the World Health Organization as one of the most widely used medicinal plants worldwide (Sedaghati et al., 2019). Various studies have highlighted its pharmacological versatility, attributing to the numerous beneficial effects of *P. oleracea* extracts, including antibacterial properties, antioxidants, hypolipidemic, and anti-inflammatory activities (Khursheed & Jain, 2021; Erkan, 2012). More studies reported the significance of purslane consumption in lowering the risk of numerous illnesses, including cancer and heart disease. Furthermore, it is noted that the major phenolic compounds found in *P. oleracea* include flavonoids, alkaloids, monoterpene glycosides, tannins, and phenols (Xu et al., 2006; Dabbou et al., 2020). Due to its reported polyphenolic compounds of *P. oleracea*, the antioxidant potential is particularly significant in neutralizing reactive oxygen species (ROS) either via donating hydrogen or quenching singlet oxygen radicals (Chang et al., 2001). These highly reactive molecules can cause cellular damage, leading to oxidative stress, a critical factor in the pathogenesis of numerous chronic diseases, including cardiovascular diseases, neurodegenerative disorders, and cancer (Barnham et al., 2004). However, while the antibacterial properties of *P. oleracea* have been noted, there still needs to be a significant gap in the literature regarding a comprehensive assessment of the specific phytochemicals that contribute to these activities. Most existing studies have focused on broad biological activities and have not identified the potential phytochemicals responsible for such

bioactivities. Thus, this research assessed the phytochemical content, antibacterial and antioxidant efficacy of crude methanolic extracts and polyphenol-rich fraction derived from *P. oleracea* leaves.

MATERIALS AND METHODS

Equipment, reagents and bacterial cultures

A laboratory oven was used to dry the collected plant materials. Then, the extracts were concentrated using a rotary evaporator (Buchi R114). A microplate reader was used for the antioxidant assays. Muller-Hinton agar (MHA) and nutrient agar (bacteriological) from Hi-Media prepared the bacterial culture. Solvents such as absolute ethanol and methanol were obtained from Scharlau. The Gram-positive *Staphylococcus aureus* (BIOTECH 1582) and Gram-negative *Escherichia coli* (BIOTECH 1634) were purchased from the Philippine National Collection of Microorganisms (PNCM) BIOTECH University of the Philippines Los Baños (UPLB).

Plant preparation

The whole plant of *P. oleracea* was obtained from the National Capital Region (NCR), Quezon City, Philippines. Quezon City is located in the northern part of Metro Manila, Philippines, at an elevation of approximately 17 meters above sea level. It has a tropical monsoon climate characterized by a dry and wet season. The plant was cultivated in an alluvial and clay soil, suitable for commercial and agricultural purposes. The leaves were collected from the plant and washed thoroughly with distilled water to remove dirt and necrotic parts. Subsequently, the leaves were oven-dried at 40 ^{o}C before grinding using a laboratory blender.

Extraction of *Portulaca oleracea* leaves

The leaf extract was prepared following Afsar et al. (2018) with minor modifications. Specifically, 500 grams of powdered plant material were macerated in 1 L of 85% methanol (Merck, Germany) acidified with 0.1% HCl (Merck, Germany) for three hours at room temperature (25−27 ^{o}C) with intermittent shaking. The mixture was filtered (Whatman No. 1, England) to have the residue re-extracted with the same volume of acidified 85% methanol. This process was repeated three times until a faint-colored extract was obtained. The filtrate was then concentrated using the rotary evaporator at 40 ^{o}C at 200 mbar and 200 rpm. Then, it was freeze-dried for 12−18 hours and stored at -18 ^{o}C for further analysis. The freeze-dried crude methanolic extract was reconstituted using 2 mL of 0.1% DMSO for the antioxidant and antibacterial assays and prepared in concentrations of 100 µg/mL, 50 µg/mL, and 10 µg/mL.

Preparation of polyphenol-rich leaf extract

The freeze-dried crude methanolic extract (10.26 grams) was added to a separatory funnel with 500 mL of distilled water (aqueous fraction). Liquid-liquid extraction (LLE) was performed thrice using n-hexane, ethyl acetate, and chloroform with 500 mL of the solvent. Each fraction was filtered using Whatman No.1 filter paper and concentrated via a rotary evaporator at 40 ^{o}C and 200 rpm. The obtained semi-liquid extract was dried in a laboratory oven before the dry weight was recorded. The remaining crude methanolic extract and four fractions, specifically aqueous, n-hexane, chloroform, and ethyl acetate, were stored at -18 ^{o}C until further use. Reconstitution of the extract on the assay used 2 mL of 0.1% DMSO. The summarized scheme from extraction to the preparation of fractions is provided in Figure 1.

Qualitative phytochemical screening

Crude and fractionated extracts underwent qualitative phytochemical screening according to the protocols outlined by Lawal et al. (2019). The Liebermann-Burchard test was employed to detect terpenes and terpenoids, while anthocyanin presence was verified through color changes induced by sodium hydroxide and HCl at varying pH levels. The $FeCl_3$ test identified tannins and phenolic compounds, saponins were detected using the froth test, and alkaloids were confirmed via Wagner's test. Flavonoids were verified using the lead acetate test.

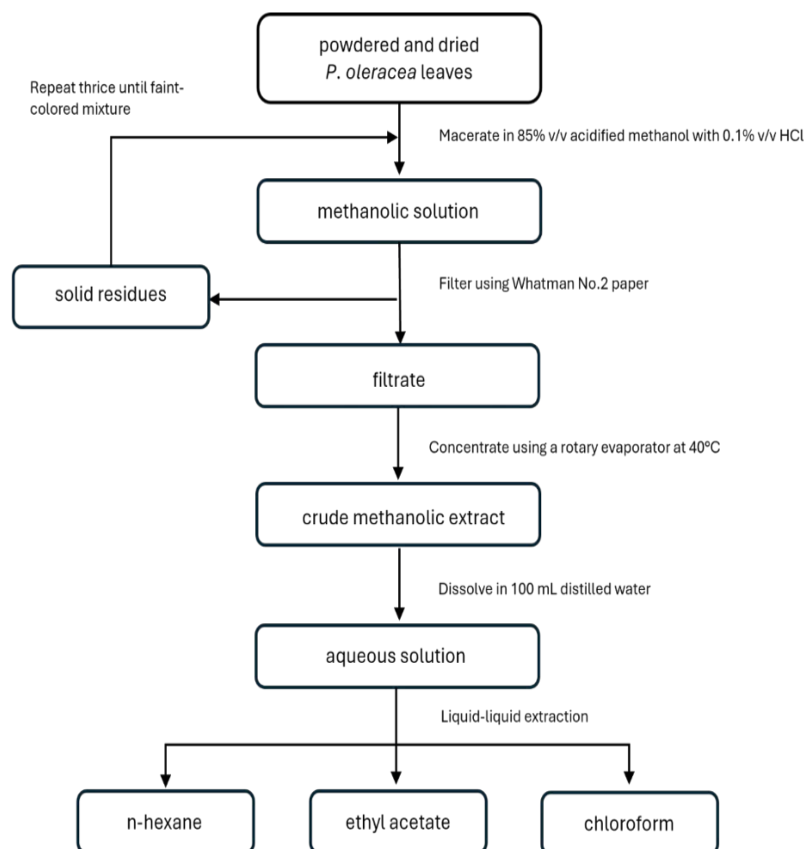

Figure 1. Schematic illustration of the extraction and preparation of polyphenol-rich fraction of *Portulaca olarecea* leaves

Quantification of total phenolic content

The total phenolic contents of the *P. olarecea* leaf extracts were assessed using the modified Folin-Ciocalteu method using gallic acid as the standard. Approximately 15.4 µL of the crude methanolic extract and four fractions were diluted with 61.5 µL of Folin-Ciocalteu reagent (diluted 1:10 with deionized water) and mixed with 123 µL of 7.5% sodium carbonate to neutralize the pH. The reaction mixture was incubated in the dark for 30 minutes at room temperature. Absorbance was read at 595 nm against a blank. Total phenolic contents were measured as milligrams of gallic acid equivalent (GAE) per 100 grams of dry matter (mg GAE/100 g dry matter).

Putative determination of polyphenols

The putative identification of polyphenols in the aqueous fraction was achieved using ultrahigh-performance liquid chromatography coupled with electrospray ionization and quadrupole time-of-flight mass spectrometry (UPLC-ESI-QTOF-MS). The aqueous fraction (1,000 mg/mL) was prepared by dissolving it in 0.1% DMSO in Type I water. The resulting mixture was diluted using Type I water and LC-MS grade methanol in a 50:50 ratio, yielding a final 20 mg/mL concentration. Before analysis, the solution was filtered through a 0.2 µm PTFE syringe filter. Data were processed using Waters UNIFI Scientific Information System v1.8.1.073 alongside the Waters Traditional Chinese Medicine Library. Compounds were considered good matches if the mass accuracy error was within ± 5 mDa and the precursor ion response was ≥ 2,000. Poor matches were reported for compounds with mass accuracy errors > 5 mDa, even if the precursor ion

response was ≥ 2,000. Leucine enkephalin served as a reference for mass correction throughout the analysis. The putative identification of compounds was based on their retention times, mass-to-charge ratios (m/z), precursor ion responses, and mass accuracy errors.

Determination of antioxidant activities of leaf extract

The antioxidant activities of crude methanolic extract of *P. olarecea* leaves and the four fractions were determined by scavenging three free radicals, specifically 2,2'-diphenyl-1-picrylhydrazyl (DPPH), superoxide, and hydroxyl radicals.

DPPH radical inhibition assay

About ten (10) µL of varying concentrations of the crude methanolic extract (10, 50, and 100 µg/mL) and fractions (100 µg/mL) were added to a 96-well microplate reader (Multiskan^{TM} FC Microplate photometer) along with 10.0 µL of ascorbic acid (100 µg/mL) standard and 140 µL of 68.5 µM DPPH solution. The microplate was incubated in the dark at room temperature for 30 minutes, and absorbance was measured at 517 nm against a blank using a UV-vis spectrophotometer. The assay was conducted in three technical replicates. The results were expressed as mean ± SEM. The formula below was used to compute the DPPH radical scavenging activities.

$$\%\ \text{DPPH radical scavenging activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

A_{sample} is the absorbance of the test sample/control while $A_{control}$ is the reference absorbance (DPPH without sample or standard).

Superoxide radical inhibition assay

Superoxide radical inhibition assays for crude and fractionated extracts were conducted according to Dasgupta et al. (2014) with some modifications. Ascorbic acid (100 µg/mL) and ten (10) µL of test samples, including increasing concentrations of the crude methanolic extract (10, 50, and 100 µg/mL) and four fractions (100 µg/mL), were added to the microplate reader. Subsequently, 100 µL of 465 µM NADH, 100 µL of 156 µM nitroblue tetrazolium (NBT), and 50 µL of 60 µM phenazine methosulfate (PMS) were pipetted to each well. After a five-minute incubation in the dark at room temperature, absorbance was measured against 0.1% DMSO as the blank. The assay was conducted in three technical replicates. The results were expressed as mean ± SEM. The following formula was used to compute the percentage inhibition:

$$\%\ \text{Superoxide radical scavenging activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Hydroxyl radical inhibition assay

The mixture was brought to a final volume of 1 mL to include the following: 100 µL of 2-deoxy-2 ribose (28 mM in 20 mM KH_2PO_4 buffer, pH 7.4), 500 µL of the leaf extract at various concentrations, 200 µL of 1.04 mM EDTA and 200 µM $FeCl_3$ (1:1 v/v), 100 µL of 1.0 mM hydrogen peroxide (H_2O_2), and 100 µL of ascorbic acid standard (100 µg/mL). After incubation at 37 ^oC for 1 hour, the free radical damage on deoxyribose was assessed using the thiobarbituric acid (TBA) test, and results are expressed as percent free radical inhibition. Samples were added with 1% TBA and 1.0 mL of 2.8% trichloroacetic acid (TCA) and incubated at 100 ^oC for 20 minutes. Absorbance was measured at 532 nm with deoxyribose and buffer as the blank. Ascorbic acid and DMSO served as positive and negative controls, respectively. The following formula was used to compute the percentage inhibition:

$$\% \text{ Hydroxyl radical scavenging activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Evaluation of antibacterial activity

Bacterial strains, culture media, and growth conditions

Muller-Hinton broth culture was adjusted to obtain the optical density of 0.5 McFarland standard. Growth media was prepared by dissolving 38 grams of MHA in 1.0 L double distilled water and autoclaving at 121 °C for 15 minutes. After plating, bacterial suspension was entirely streaked over the surface using sterile cotton swabs.

Antibacterial susceptibility assay

Antibacterial activity was assessed using the Kirby-Bauer method performed. Extracts were prepared at 10, 50, and 100 µg/mL concentrations in 0.1% DMSO for crude methanolic and aqueous fractions. Positive controls included ten (10) µg/mL gentamicin and streptomycin, while the negative control was double distilled water, with 0.1% DMSO serving as the vehicle control. A 100 µL aliquot of each test sample was loaded onto 6-mm diameter sterile discs. After incubation at 37 °C for 24 hours, the zone of inhibition (ZOI) around each disc was measured in millimeters (mm). The ZOI was interpreted as follows according to Liasi et al. (2009): 0−5 mm-no inhibition; 6−9 mm-moderate inhibition; 10−14 mm-strong inhibition; and 15 mm or more -very strong inhibition. The antibacterial assay was conducted in triplicates. The ZOIs were expressed as means ± SEM.

Statistical data treatment

The effects of the extract and controls were analyzed statistically via one-way analysis of variance (ANOVA). The Tukey HSD test was then used to compare the means, with statistical significance set at a p-value < 0.05. Post-hoc two-tailed t-test was also performed in antioxidant and antibacterial assays.

RESULTS

Phytochemical screening

Preliminary qualitative phytochemical screening was performed in all *P. oleracea* extracts to determine the phytochemical constituents. Results showed varying quantities of flavonoids and tannins in all extracts. Notably, large amounts of flavonoids were detected in polar (methanol and aqueous) and semi-polar (ethyl acetate) fractions. Anthocyanins were present in low quantities in all plant extracts but were absent in the aqueous fraction. Furthermore, phenolics were absent in non-polar (hexane and chloroform) extracts but in large quantities in aqueous fractions. Both saponin and terpenoid were only detected in methanol, hexane, and chloroform fractions. Moreover, large amounts of alkaloids were present in the hexane and ethyl acetate fractions, while they were absent in the chloroform and aqueous fractions, as indicated in Table 1. The phytochemical constituents in all *P. oleracea* extracts upon screening were summarized in Table 1.

Total phenolic content quantification

The total phenolic content of the *P. oleracea* fractions was determined quantitatively using the Folin Ciocalteu method. The results for the total phenolic content assay are given in Figure 2. The values were based on a calibration curve of gallic acid (0−500 mg/mL) represented as gallic acid equivalents (GAE) per gram of dry plant extract. The findings indicate that the aqueous fraction had the highest total phenolic content with a concentration of 181.58 ± 5.34 mg GAE/g. The measurement of the aqueous fraction was higher than the crude methanolic extract (142.65 ± 4.30 mg GAE/g). Meanwhile, the hexane fraction had the lowest total phenolic content with a concentration of 68.39±6.05 mg GAE/g.

Additionally, the total phenolic contents of the ethyl acetate and chloroform fractions were found to be 128.89 ± 2.03 and 104.84 ± 5.20 mg GAE/g, respectively.

Table 1. Summary of phytochemical constituents present in *Portulaca oleracea* extracts

Phytochemical constituents	Extracts				
	Crude	Hexane	Chloroform	Ethyl acetate	Aqueous
Terpenoids	+	+	+	-	-
Phenolics	+	-	-	+	++
Anthocyanins	+	+	+	+	-
Tannins	+	+	+	++	++
Saponins	++	+	+	-	-
Alkaloids	+	++	-	++	-
Flavonoids	++	+	+	++	++

Note: (-) not detected; (+) detected in low quantities with low color intensity; (++) detected in large quantities with high color intensity.

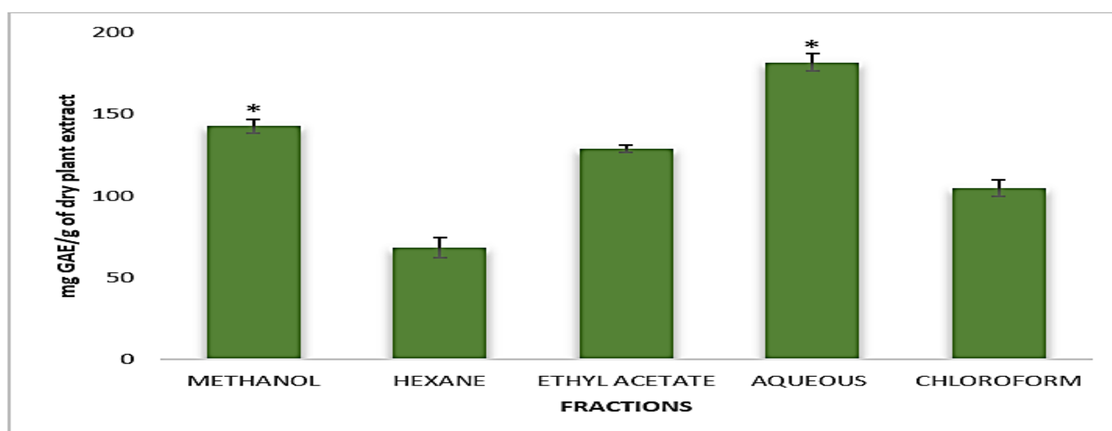

Figure 2. The total phenolic content of crude methanolic *Portulaca oleracea* extract and fractions. Data points indicate the mean±SEM of three trials. ANOVA and post hoc Tukey HSD statistics with $*p < 0.05$ were used in the comparison of the means and the crude methanolic extract

Putative determination of polyphenols

The analysis of the aqueous fraction of the sample revealed the presence of several polyphenols using ultrahigh-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC-ESI-QTOF-MS). The putative identification of compounds was based on their retention times, mass-to-charge ratios (m/z), precursor ion responses, and mass accuracy errors, as summarized in Table 2. The results indicate the presence of diverse polyphenolic compounds with reported antibacterial and antioxidant activities, such as *herbacetin, isoetin, quercetin, 6-hydroxykaemferol, robinetin, viscidulin, capillarisin*, and *rhamnetin*. Keser et al., 2021 reported the presence of quercetin (0.05 µg/g) and kaempferol (0.05 µg/g). Moreover, the isorhamnetin and quercetin were detected in raw and steamed *P. oleracea* (Fernandez-Poyatos et al., 2021). In another study by Ao (2019), bioactive fatty acids were determined using gas chromatography-tandem mass spectrometry (GC-MS) analysis. Likewise,

kaempferol, apigenin, luteolin, myricetin, quercetin, genistin, genistein, and Portulacanones A to D were isolated from *P. oleracea* leaf and stem extracts (Xu et al., 2006). However, the other five (herbacetin, isoetin, robinetin, viscidulin, and capillarisin) putatively determined polyphenolic compounds were not reported elsewhere. The identification of these compounds was confirmed by their retention times, accurate mass measurements, and comparison with known databases.

Antioxidant activity of *Portulaca oleracea*

The potential of crude methanolic extracts from *P. oleracea* in varying dilutions to neutralize DPPH, superoxide, and hydroxyl radicals was assessed spectrophotometrically. The results of the analyses are illustrated in Figure 3. The crude methanolic extract exhibited dose-dependent DPPH radical scavenging activity ranging from 9.58 ± 1.80% to 50.08 ± 2.49%. Analysis of dose-dependent superoxide scavenging activity revealed that inhibition activity increased with higher concentrations of crude methanolic extracts ($p < 0.05$). Specifically, superoxide scavenging activities of 21.42 ± 1.41%, 31.07 ± 1.79%, and 42.61 ± 0.56% were detected in 10 µg/mL, 50 µg/mL, and 100 µg/mL crude methanolic concentrations, respectively, showing statistical significance ($p < 0.05$) against the positive control (ascorbic acid). Moreover, the dose-dependent hydroxyl inhibition analysis of crude methanolic extracts showed inhibitions ranging from 5.24 ± 1.49% to 17.60 ± 1.66%, which are significantly lower than the observed hydroxyl radical inhibition of ascorbic acid ($p < 0.05$).

Table 2. Summary of polyphenolic compounds present in aqueous fraction with reported antibacterial and antioxidant activities

Component name	Chemical formula	Retention time (mins)	Observed m/z	Mass error (mDa)	References
Herbacetin	$C_{15}H_{10}O_7$	6.27	303.05	1.83	Li et al., 2016; Sedik et al., 2024
Isoetin	$C_{15}H_{11}O_5$	4.98	302.04	1.78	Shi et al., 2008; Gawrońska-Grzywacz et al., 2011
Quercetin	$C_{15}H_{10}O_7$	5.44	302.24	1.22	Cushnie et al., 2005; David et al., 2016
6-hydroxykaemferol	$C_{15}H_{10}O_7$	6.00	303.05	1.83	Periferakis et al., 2022; Sharma et al., 2021
Robinetin	$C_{15}H_{10}$O6	6.50	301.05	0.48	Manrique-de-la-Cuba et al., 2019; Cushnie et al., 2005
Viscidulin	$C_{19}H_{24}O_8$	9.10	368.17	1.10	Fu et al., 2008
Capillarisin	$C_{17}H_{18}O_8$	8.00	346.10	1.89	Kim et al., 2017; Seo et al., 2010
Rhamnetin	$C_{16}H_{10}O_7$	7.20	302.24	0.60	Lee et al., 2022

The scavenging activities of varying LLE fractions against DDPH, superoxide, and hydroxyl were analyzed, as shown in Table 3. The LLE fractions showed varying DPPH scavenging activities at 100 µg/mL. Mainly, n-hexane, chloroform, ethyl acetate, and aqueous fractions had DPPH radical inhibitions of 13.60 ± 1.46%, 23.62 ± 1.409%, 30.84 ± 1.86%, and 66.12 ± 0.76%, respectively. Notably, the aqueous fractions exhibited the highest DPPH radical scavenging activity, whereas the hexane fractions showed the lowest activity ($p < 0.01$).

The dose-dependent superoxide scavenging activity analysis revealed that the aqueous fraction showed 60.90 ± 1.22% inhibition, the highest scavenging activity among the LLE fractions at 100 µg/mL

(*p* < *0.01*). The chloroform fraction demonstrated the lowest superoxide scavenging activity with 5.56 ± 2.11% inhibition, whereas hexane and ethyl acetate fractions exhibited 20.60 ± 5.62% and 23.20 ± 2.21% inhibition, respectively (*p* < *0.05*). Despite the potent superoxide radical scavenging activity of aqueous fraction, it is still lower than the radical inhibition capacity of ascorbic acid (*p* < *0.05*).

Table 3. Antioxidant activities (DPPH, Superoxide, and Hydroxyl radical scavenging) of varying LLE fractions of *Portulaca oleracea* (100 µg/mL)

Fraction	DPPH	Superoxide	Hydroxyl
Aqueous	66.12 ± 0.76$^{a,□}$	60.90 ± 1.22$^{a,□}$	45.77 ± 3.79$^{a,□}$
Ethyl acetate	30.84 ± 1.86^{b,*}	23.20 ± 2.21^{b,*}	5.08 ± 0.97^{b,*}
Chloroform	23.62 ± 1.41^{c}	5.56 ± 2.12^{c}	5.37 ± 0.41^{b}
Hexane	13.60 ± 1.45^{d}	20.60 ± 5.62^{d,*}	2.58 ± 1.41^{c}

Note: Data presented are means ± SEM of three replicates (n = 3). Different superscript letters (a, b, c, d) within a column signifies statistically significant between fractions as described by one-way ANOVA and subsequent post-hoc Tukey HSD test ($p < 0.05$). Superscript symbols indicate levels of significance (*$p < 0.05$, □$p < 0.01$) between groups where applicable.

Dose-dependent hydroxyl scavenging activity was also detected in LLE fractions at 100 µg/mL, with the aqueous fraction exhibiting the highest hydroxyl scavenging activity of 45.77 ± 3.79%, showing statistically significant radical inhibition against the other fractions (*p* < *0.01*). This result was consistent with previously detected scavenging activities against DPPH and superoxide radicals, where the aqueous fraction showed the highest activity. In contrast, the lowest hydroxyl scavenging activity was observed in hexane with 2.58 ± 1.41% inhibition. The chloroform and ethyl acetate fractions also demonstrated hydroxyl scavenging activities of 5.37 ± 0.41% and 5.08 ± 0.97%, respectively.

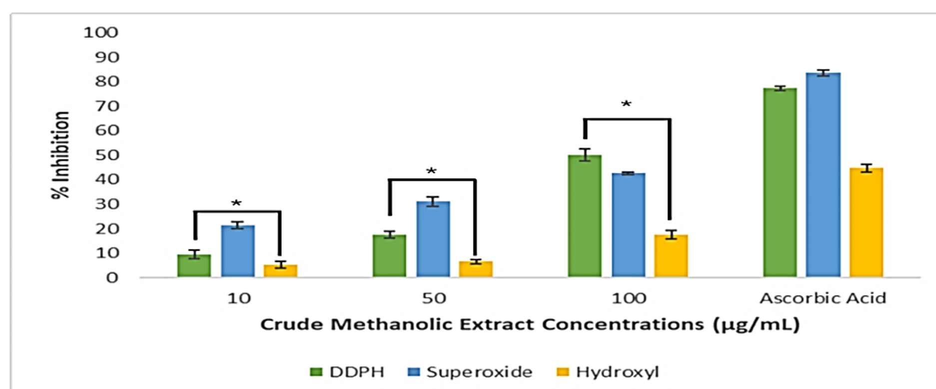

Figure 3. Antioxidant activities of crude methanolic extract of *Portulaca oleracea* in varying dilutions. Data are expressed as mean ± SEM. Significant differences from the positive control (ascorbic acid at 100 µg/mL) were determined using one-way ANOVA followed by post-hoc Tukey HSD test, with *$p < 0.05$ indicating statistical significance

Antibacterial activity of *Portulaca oleracea*

The *P. oleracea* aqueous fraction was used in the antibacterial assay as it demonstrated the highest radical scavenging activities. The Kirby-Bauer disk diffusion method was applied to assess the antibacterial properties of crude methanolic *P. oleracea* extract against Gram-negative *E. coli* and Gram-positive *S. aureus* with 10 µg/mL of

gentamicin and streptomycin antibiotics as the positive controls. No zone of inhibition (ZOI) was recorded for double distilled water with 0.1% DMSO as the negative control. However, the ZOIs of the positive controls (streptomycin and gentamicin) were significantly ($p < 0.05$) higher than the ZOIs of the crude methanolic *P. oleracea* extract and aqueous fraction for both *E. coli* and *S. aureus*. It was observed that the growth inhibition (ZOI) of both bacterial strains increases with increased concentrations of the crude methanolic *P. oleracea* extract (1.21 ± 0.18 mm to 7.18 ± 0.26 for *E. coli* and 2.28 ± 0.04 to 8.95 ± 0.19 mm for *S. aureus*) and aqueous fraction (3.41 ± 0.41 mm to 10.21 ± 0.18 for *E. coli* and 3.43 ± 0.17 to 11.21 ± 0.11 mm for *S. aureus*). It is important to note that the ZOI exhibited by the highest concentration (100 µg/mL) of both crude methanolic extract and aqueous fraction were comparable and not statistically significant to the ZOI of positive controls for both bacterial strains ($p < 0.05$). Moreover, the results show that the antibacterial activity of the aqueous fraction is higher than the crude methanolic *P. oleracea* leaf extract ($p < 0.05$), and the highest concentration of aqueous fraction (100 µg/mL) is closer to the positive control measurements ($p < 0.05$). Additionally, it was observed that ZOIs measured for all treatments are higher on *S. aureus* than on *E. coli*. Overall, the data suggests an effective antibacterial activity of the 100 µg/mL of crude methanolic extract and aqueous fraction against both bacterial strains. The results of the antibacterial assay are summarized in Table 4.

Table 4. Zone of inhibition of the crude methanolic *Portulaca oleracea* extract and aqueous fraction against *Escherichia coli* and *Staphylococcus aureus* bacteria

Treatments	Concentration	Bacterial Strains (ZOI in mm)	
		Escherichia coli	*Staphylococcus aureus*
Crude Methanolic	10 µg/mL	1.21 ± 0.18^{c}	2.28 ± 0.04^{c}
	50 µg/mL	3.28 ± 0.01^{c}	4.29 ± 0.29^{c}
	100 µg/mL	$7.18 \pm 0.26^{c,b}$	$8.95 \pm 0.19^{c,b}$
Aqueous Fraction	10 µg/mL	3.41 ± 0.41^{c}	3.43 ± 0.17^{c}
	50 µg/mL	7.62 ± 0.13^{c}	8.24 ± 0.29
	100 µg/mL	10.21 ± 0.18^{a}	11.21 ± 0.11^{a}
Gentamicin	10 µg/mL	14.21 ± 0.06	15.33 ± 0.10
Streptomycin	10 µg/mL	13.53 ± 0.15	11.28 ± 0.01

Note: Values indicate means±SEM of three replicates. *Escherichia coli*, *Staphylococcus aureus*; $p < 0.05$. [a]shows significant differences in inhibition between the same extract against varied strains; [b]shows significant differences in inhibition between varied extracts against the same strain; [c]shows significant differences in inhibition based on respective control.

DISCUSSION

The search for antibiotic alternatives has been increasingly important due to the rising antimicrobial resistance. Consequently, plants have been examined because of their use in traditional medicine, and over 1,300 plants have been documented to possess antimicrobial properties (Vaou et al., 2021). Specifically, plants contain varied phytochemical compounds or secondary metabolites that enhance their environmental fitness through certain functions, including producing compounds against pathogens and pests (Teoh, 2015). Some essential phytochemicals in plants are polyphenols, which include terpenoids, phenolics, flavonoids, and alkaloids, that serve as significant sources of bioactive compounds used in nutraceuticals and modern medicines (Velu et al., 2018). These phytochemicals are naturally occurring compounds found predominantly in plants. They have been demonstrated to exhibit antioxidant activity that reduces oxidative stress and neutralizes

free radicals (Suriyaprom et al., 2022). They also showed potent antibacterial activity against various pathogenic bacteria and fungi. Other bioactivities include anti-inflammatory, anti-cancer, and cardio-protective effects, highlighting its enormous potential for preventive medicine (Kanner, 2023).

Qualitative phytochemical screening

The initial qualitative screening of the phytochemical constituents of the *P. oleracea* leaf extracts revealed the presence of polyphenolic compounds, notably flavonoids, tannins, and phenolics. This screening employed colorimetric and chemical methods, which demonstrated positive reactions for flavonoids, tannins, and phenolic acids (Table 1).

Following the qualitative screening, UPLC-MS analysis was performed to further identify and characterize the polyphenolic compounds present in the aqueous fraction. The analysis revealed several key polyphenols, including specific flavonoids and phenolic acids, as summarized in Table 2. The UPLC-MS analysis confirmed the presence of various flavonoids, such as quercetin, herbacetin, and kaempferol derivatives. The putative identification was based on accurate mass measurements, retention times, and fragmentation patterns, which were consistent with known standards. The UPLC-MS data provided preliminary data allowing a comprehensive understanding of the polyphenolic constituents present in the aqueous fraction.

Zhuo et al. (2010) found that flavonoids are major practical components in *P. olarecea* roots, stems, and leaves. This was confirmed by the flavonoid content of the extracts across different extraction technologies, ranging from 1.76 mg/g to 11.36 mg/g in dry weight. The aerial parts are high in flavonoids due to various compounds such as quercetin, kaempferol, isorhamnetin, apigenin, and luteolin (Okafor & Ezejindu, 2014). Additionally, the study of Al-Quwaie et al. (2023) confirms high flavonoid content at 56 mg/g dry weight from the whole *P. oleracea* plant, accounting for 33% of its liquid chromatography-mass spectroscopy profile. In the same profile, 11% were found to be tannins. Furthermore, via ethanol extraction, Almashad et al. (2020) confirmed high tannin content in *P. oleracea* leaves.

Lastly, the present study confirmed a substantial concentration of total polyphenols by the total phenolic content of *P. olarecea* leaf extract, corroborating the findings from the qualitative and UPLC-MS analysis. The high TPC value suggests that the sample possesses significant antioxidant potential, which is attributed to the cumulative effects of the identified polyphenols. Additionally, this supplements the study of Aisyah et al. (2023) that compared extracts from roots and aerial parts of *P. olarecea* and *P. grandiflora,* wherein it was shown that *P. olarecea* has approximately 250 mg GAE per gram of plant extract compared to 200 mg GAE/g of *P. grandiflora*. The high TPC is particularly relevant given the increasing interest in the health benefits of polyphenolic compounds, including their potential roles in reducing oxidative stress (antioxidant activities) and preventing bacterial infection (antibacterial properties).

Antioxidant activity of *Portulaca oleracea*

The antioxidant activities of the crude methanolic leaf extract and its four fractions (n-hexane, chloroform, ethyl acetate, and aqueous) from *P. oleracea* were determined using DPPH, superoxide, and hydroxyl radical inhibition assays. The results demonstrated that the *P. oleracea* extracts, precisely the aqueous fraction, showed significant potential in neutralizing reactive oxygen species (ROS), highlighting the promising antioxidant activities of *P. oleracea*.

The crude methanolic extract showed notable DPPH radical scavenging activity, but the aqueous fraction stood out with the highest inhibition, suggesting the presence of highly potent water-soluble antioxidants. The consistent performance of the aqueous fraction in exhibiting the highest scavenging activities suggests that water-soluble polyphenolic compounds, such as phenols, flavonoids, and tannins, are primarily responsible for this

activity (Dabbou et al., 2020). Phytochemical screening revealed large quantities of flavonoids in polar and semi-polar fractions and phenolics in large quantities, specifically in the aqueous fraction. The total phenolic content quantification revealed that the aqueous fraction had the highest total phenolic content (181.58 ± 5.34 mg GAE/g), significantly higher than the crude methanolic extract (142.65 ± 4.30 mg GAE/g) and other fractions. These results verified that the *P. oleracea* aqueous extracts contained significant polyphenols, resulting in potent antioxidant activities. This is backed up by a study showing that the aqueous extract of *P. oleracea* garnered higher antioxidant activity and total phenolic content than its methanolic extract (Cai et al., 2004).

Data has shown that the aqueous fraction outperformed others, such as hexane, ethyl acetate, and chloroform. The differential solubility of polyphenolic compounds in various solvents and the resulting concentration of these compounds in each fraction likely influence this observed variance in activity. The aqueous fraction typically exhibits the highest activity, attributed to its greater polyphenolic content, including flavonoids, tannins, and phenolic acids, which are highly soluble in water (Yang et al., 2018; Álvarez-Martínez et al., 2021). These compounds are known for their potent radical-scavenging (antioxidant) and membrane-disrupting (antibacterial) properties (Barnham et al., 2004). Methanol, being slightly less polar, extracts both polar and moderately nonpolar compounds but generally yields a lower polyphenolic concentration than water, resulting in moderate activity (Xu et al., 2006). Ethyl acetate, chloroform, and hexane fractions progressively exhibit lower antioxidant and antibacterial effects, reflecting their reduced efficacy in extracting polyphenols; instead, they primarily contain semi-polar and nonpolar compounds like terpenoids and lipids, which have limited ROS-scavenging abilities and antibacterial efficacy (Zhou et al., 2015; Dabbou et al., 2020). These observations imply the influence of solvent polarity on extract bioactivity, with water-based extraction maximizing polyphenolic yield and enhancing activity potential.

The high radical scavenging activities observed in the aqueous fraction suggest its potential utility in combating oxidative stress-related diseases and conditions. Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, plays a pivotal role in the pathogenesis of various chronic diseases, including cardiovascular diseases, neurodegenerative disorders, and cancer (Barnham et al., 2004; Teleanu et al., 2022). By effectively neutralizing free radicals, particularly hydroxyl and superoxide radicals, the aqueous fraction of *P. oleracea* may offer therapeutic benefits in mitigating oxidative damage and inflammation associated with these diseases.

Polyphenols exhibit antioxidant properties primarily through their ability to donate hydrogen atoms and neutralize ROS (Zeb, 2020). They can directly scavenge free radicals, where polyphenols donate hydrogen atoms to stabilize free radicals and terminate the chain reactions that lead to oxidative damage (Di Meo et al., 2013). Additionally, polyphenols can chelate metal ions such as iron and copper, which catalyze the production of ROS via the Fenton reaction, thereby reducing ROS formation (Pan et al., 2022). Furthermore, polyphenols can upregulate the expression of endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, enhancing the body's antioxidant defenses (Zhang & Tsao, 2016). Through these combined actions, polyphenols help maintain cellular redox balance and protect against oxidative stress-related diseases.

Antibacterial activity of *Portulaca oleracea*

In the present study, the crude methanolic extract of *P. oleracea* exhibited moderate antibacterial activity. At the same time, the polyphenol-rich aqueous fraction revealed potent antibacterial activity against *E. coli* and *S. aureus*, comparable to two antibiotics (gentamicin and streptomycin). This suggests that the antibacterial activity of the aqueous solution may be due to the presence of large

amounts of phytochemical constituents, specifically phenolics, flavonoids, and tannins (as seen in Table 1). This agrees with the findings of Al-Quwaie et al. (2023) who reported *P. oleracea* methanolic extract's antibacterial efficacy against pathogenic bacteria, noting *S. aureus* as the most susceptible Gram-positive bacteria among the bacteria tested, while a study by Pilar et al. (2021) validates the high content of both phenolics and flavonoids, reporting that among the 24 compounds extracted from *P. oleracea*, 30% were phenolics and 25% were flavonoids.

Phenolics affect bacterial cells through membrane destabilization, enzyme inhibition, and nucleic acid synthesis inhibition (Borges et al., 2013). They alter the physicochemical surface properties of bacterial cells, such as hydrophobicity and surface charge, which leads to cytoplasmic leakage. A study by Borges et al. (2013) found that phenolic acids, a subclass of phenolics, caused damage to the membranes of both *S. aureus* and *E. coli*, evidenced by leakage of potassium and other intracellular components as well as a higher Propidium Iodide (PI) staining, indicating a high number of cells with compromised membrane integrity.

Tannins show antimicrobial activity due to their hydrophilic structure with multiple hydroxyl groups, allowing them to form complexes with microbial cell membrane proteins, affecting cell wall morphology and increasing membrane permeability (Ecevit et al., 2022; Huang et al., 2018). A study by Štumpf et al. (2020) validates tannins' antimicrobial activity against *E. coli*, exhibiting the lowest Minimum Inhibitory Concentration (MIC) values among the tested compounds with 180 µg/mL at double the concentration of *E. coli* growth medium (2×10^5 CFU/mL), indicating the highest antibacterial activity. The results suggest tannins' antibacterial activity may be due to chelation of microelements in the growth medium or interaction with essential proteins (Štumpf et al., 2020). Akiyama et al. (2001) confirmed that tannins affect *S. aureus* by inhibiting coagulation, enhancing the efficacy of β-lactam antibiotics (e.g., oxacillin and cefdinir), disrupting cell membranes, and reducing fibrin formation. This reduction in fibrin, a key component of *S. aureus* biofilms, can inhibit biofilm development and make the bacteria more susceptible to treatment. It was also shown that tannic acid was effective at concentrations below the MIC, impacting the clotting process crucial for bacterial adherence and biofilm formation (Akiyama et al., 2001).

Flavonoids act both bacteriostatic and bactericidal by disrupting bacterial cell walls, suppressing energy metabolism, nucleic acid synthesis, or membrane function (Ecevit et al., 2022). A 2018 study by Desta & Cherie aligns with this finding, showing that the methanol extract of *P. quadrifida*, a plant under the same family as *P. olereacea,* which had the highest flavonoid content (2.335 ± 0.0097 mgQE/g), exhibited the highest antimicrobial activity. It had the largest ZOIs against Gram-positive *S. aureus* (17.3 ± 2 mm) and Gram-negative *E. coli* (11.88 ± 1 mm) among other tested plants. Similarly, a study by Shamsudin et al. (2022) showed the ability of flavonoids to inhibit the growth of *E. coli* and *S. aureus*, with specific flavonoids like quercetin demonstrating higher antibacterial activity against the bacteria due to its structural feature of having multiple hydroxyl groups, leading to increased permeability of the cytoplasmic membrane, causing leakage of cellular contents and ultimately cell death.

Further research, including *in vitro* and *in vivo* studies, is required to fully understand the mechanisms underlying the antioxidant and antibacterial activities of the crude methanolic extract and polyphenol-rich fraction of *P. oleracea*.

CONCLUSION

The study investigated the phytochemical constituents, antioxidant properties, and antibacterial activities of *P. oleracea* leaf extracts and fractions. Qualitative screening revealed the presence of various phytochemicals, particularly polyphenolic compounds like flavonoids in the crude

methanolic extract, with varying amounts detected in the LLE fractions. The aqueous fraction exhibited the highest total phenolic content and demonstrated significant scavenging activities against DPPH, hydroxyl, and superoxide radicals, indicating the presence of potent water-soluble antioxidants. Additionally, the aqueous fractions showed effective antibacterial activity against gram-positive and gram-negative bacteria, comparable to standard antibiotics. Given the promising potential of the crude and aqueous fractions, further in vivo and in vitro studies are recommended to explore their antioxidant and antibacterial properties against a broader range of free radicals and pathogens. Overall, *P. oleracea* leaves are a viable option for pharmaceutical applications to combat antibiotic resistance and prevent oxidative damage.

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