Phytochemical Characterization of *Persicaria longiseta* Inflorescence Biomass and Its Biological Activities

Abeer S. Meganid,[a] and Mohamed A. Amin [b,*]

Nutritional values and biological activity of *Persicaria longiseta* inflorescence extract (PLE) were assessed relative to the antioxidant, anticancer, and antibacterial impact of ethanolic extracts from the plant's inflorescence. Using high-performance liquid chromatography (HPLC), the PLE ethanolic extract revealed that naringenin, chlorogenic acid, quercetin, rosmarinic acid, rutin, and gallic acid were all present in high concentrations (9780, 8250, 5190, 4320, 2520, and 2430 µg/g, respectively). Nutritional values assessment showed carbohydrates (166 mg/g), protein (53.2 mg/g), phenols (36.2 mg/g), and flavonoids (13.2 mg/g). When applied to pathogen species and cancer cells, the ethanolic extract of PLE showed inhibitory effects. Additionally, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were evaluated for the tested microorganisms. The *P. longiseta* extract exhibited significant inhibition of hemolysis, particularly in the presence of *B. subtilis* (77.7, 90.8, and 95.9%) compared to *K. pneumonia* (54.1 ± 1.16, 75.8, and 85.3%) at varying doses (25, 50, 75% MIC, respectively). These results represent the beginning of the use of this extract in advanced medical aspects as an antimicrobial, antioxidant, antihemolytic, and antitumor agent, with some confirmatory and accurate experiments *in vivo*.

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Keywords: Persicaria longiseta; Protein; Carbohydrates; Flavonoids; Phenols; Cancer; Bacteria; Antioxidants

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INTRODUCTION

Numerous research is being undertaken about the use of substances that come from plant or microbial origins for the treatment of various diseases in people, as well as animals (Alsolami *et al*. 2023; Al-Rajhi *et al*. 2024). The biochemical reactions of plant substances correlate with their active substances, including phenols as well flavonoids, antioxidants, and other metabolites. These chemical groupings may be present in several plant parts, such as seeds, shoots, and root system (Abdelghany *et al*. 2019; Almehayawi *et al*. 2024; Bakri *et al*. 2024).

Cancer has historically been regarded as being among the most perilous diseases, characterized by elevated global mortality rates. The International Agency for Research on Cancer predicts that there will be 24 million instances of cancer worldwide by 2040 (Sung, *et al*. 2021). Standard chemical-based therapies for cancer sufferers demonstrate insufficient selectivity and suboptimal efficacy. Consequently, numerous studies have concentrated on

leveraging natural resources to tackle this issue (Al-Rajhi and Abdelghany 2023; Selim *et al*. 2025). The synthetic and partially synthetic antimicrobial agents are extensively utilized worldwide for dedicated conditions; however, they can produce various side effects in certain patients, particularly with prolonged use. Consequently, the prevailing medical trend involves the utilization of natural antibiotics derived from microorganisms and plants, particularly those recognized for their curative effects, known as botanical medicine (Yahya *et al*. 2022; Selim *et al*. 2024). A diverse array of plants with medicinal properties is recognized and commonly utilized to these reasons (Almehayawi *et al.* 2024; Qanash *et al.* 2024), but several plants are not utilized for therapeutic applications to overcome the antibiotic resistance microorganisms.

In the current study, biological activities *Persicaria longiseta* were evaluated to overcome some heath disorders. *Persicaria senticosa*, in the family Polygonaceae, has been utilized in traditional healthcare for its beneficial role in treating different illnesses, including the reduction of swelling in wounds, carbuncles, and cellulitis, as well as promoting blood circulation and alleviating slow blood flow. Recent studies indicate that *Persicaria senticosa* exhibits anti-inflammatory properties (Jung *et al*. 2020). *Persicaria longiseta* extract (25%) decreased *Amaranthus spinosus* seed germination. As *P. longiseta* extract concentrations increased, the development of plant pathogenic fungus in the potato dextrose agar (PDA) medium exhibited an increasing trend toward inhibition. Particularly, the extract of *P. longiseta* had the most potent antibacterial action against *Pythium venterpoolii*, *Phytophthora infestans*, and *Phythium gravicola* (Choi *et al*. 2015). Choi *et al*. (2020) found that the methanolic extract of *Persicaria senticosa* exhibits antioxidant, anti-wrinkle, whitening, and anti-inflammatory properties. Aqueous *P. senticosa* (PS) extracts demonstrated the capacity to reduce cellular damage caused by ultraviolet exposure and decrease reactive oxygen species (ROS) generation in immortalized human keratinocyte cells. The antioxidative characteristics and importance in skin wellness are well recognized. The main bioactive components of PS are p-coumaric acid, isoquercitrin, quercetin-3-O-glucuronide, and quercetin (Hong *et al*. 2024). *Persicaria longiseta* has been described as an unused weed that is disposed of without benefit. Accordingly, this study looked into the phytochemical analysis of *P. longiseta* extract for the first time, with assessment of the nutrtional values (protein, carbohydrates, phenols and flavonoides) as well as antimicrobial, anticancer, and antioxidant activities of this extract.

EXPERIMENTAL

Source of *Persicaria longiseta* Inflorescence

All chemicals used in the work were from Sigma-Aldrich. *Persicaria longiseta* inflorescence was collected from the garden of the Faculty of Science, Al-Azhar University, Egypt. The plant has been identified by taxonomist Prof. Abdo A. Hamed of the Faculty of Science, Al-Azhar University, Egypt (Fig. S1). Herbarium specimen had been kept in the Al-Azhar University Faculty of Science Herbarium (Voucher no. 782). *Persicaria longiseta* inflorescence was thoroughly cleaned under running water, allowed to dry at room temperature in the shade, and then ground into a powder by a grinding machine. This powder was used to assess protein, carbohydrates, phenols, and flavonoids, as detailed in the supplementary material (see the Appendix).

Preparation of *Persicaria longiseta* Inflorescence Extract

For 72 h at 22 °C, a stoppered container containing 50 g of this powder was submerged in 0.5 L of 100% ethanol. After that, the extract was sonicated at 50 °C for 50 min. Filtration was used to create the crude PLE, and then the extracts were concentrated in a rotary evaporator at 45 °C under vacuum (Khowdiary *et al*. 2024). The final output was preserved for use in subsequent research.

HPLC Conditions

The assessment was carried out using high-performance liquid chromatography with an Agilent 1260 series system. The separation process employed a Zorbax Eclipse Plus C8 column with dimensions of 4.6 mm x 250 mm i.d. and a particle size of 5 µm. The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B), with a flow rate of 0.9 mL/min. The mobile phase was programmed sequentially in an even gradient as outlined below: 0 min (82% A); 0 to 1 min (82% A); 1 to 11 min (75% A); 11 to 18 min (60% A); 18 to 22 min (82% A); 22 to 24 min (82% A). The multi-wavelength detector exhibited observation at 280 nm. The injection volume for every specimen was 5 µL. The column's temperature was kept at 40 °C (Amin *et al*. 2024).

DPPH Free Radical Scavenging Activity Assays for Antioxidant Evaluation of *Persicaria longiseta* Inflorescence

Qualitative assessments were conducted following the technique outlined by Abdelghany *et al.* (2019). Ethanol extracts were prepared at levels between 1.95 and 1000 µg/mL. The control employed was ascorbic acid. One milliliter of the extract was combined with 1 mL of a 0.4 mmol/L ethanolic solution containing the stable 1,1-diphenyl-2-picryhydrazyl (DPPH) radical. Reliability was assessed by conducting each test for free radical scavenging activity three times using the same extract, and the standard deviation of the results was statistically calculated. The mixture was vortexed thoroughly and allowed to sit at room temperature in darkness for 30 min. The UV-VIS spectrophotometer (model 6305 Jenway, UK) was utilized to evaluate the reaction of the mixture at a wavelength of 515 nm. The percentage of DPPH radical scavenging activity of the extracts was assessed at these levels and throughout the dosage response range using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Abs control} - \text{Abs extract}}{\text{Abs control}} \times 100$$

Determination of Antimicrobial Activity of *Persicaria longiseta* Inflorescence

Microorganisms were collected from the culture collection of the microbiological lab at the Faculty of Science, Al-Azhar University, Egypt. Antibacterial ability was evaluated by diffusion on agar wells against Gram-positive (*Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633), Gram-negative (*Klebsiella pneumonia* ATCC 13883 and *Escherichia coli* ATCC 8739), and fungi (*Candida albicans* ATCC 10221 and *Aspergillus brasiliensis* ATCC 16888). Potato dextrose agar for fungi and nutrient agar to bacteria were added with the optimal inoculum size for the test microorganisms and put into plates to solidify, creating seeded plates. A sterile cork borer created 6 mm agar wells. Every well had 100 µL of extract, whereas others had 100 µL of conventional antibiotic (1.0 mg/mL). For bacteria and fungi, agar plates were cultivated at 37 °C for 24 h and 25 °C for 72 h, respectively. Zones of inhibition surrounding wells signify activity against microorganisms, but their absence denotes inactivity (Qanash *et al*. 2022).

Determination of Minimal inhibitory Concentration

The authors incubated a single culture of experimental bacteria for one night in trypsinized soy broth. To obtain a concentration of 1×10^5 to 1×10^6 CFU/mL, the bacterial suspension was thinned down in trypsinized soy broth. The concentration of the antibacterial material was about 100 times lower than the minimum inhibitory concentration (MIC) that has been achieved. The experiment was carried out in a 96-well microtiter plate using trypsinized soy broth and two different doses, ranging from 1000 to 1.95 µg/mL. The bacterial inoculum-free negative control was a series of twofold dilutions ranging from 1000 to 1.95 µg/mL. The bacterial suspension grown in the same broth but without the antimicrobial ingredient made up the positive control sample. For 16 to 20 h, the microtiter plates were kept at a temperature of 35 ± 2 °C. Using a Bio-Tek 800 TS microplate reader, the turbid progress's absorption wavelength was measured at 630 nm. To determine the minimum inhibitory concentration (MIC), colorimetry was used to compare bacterial growth in broth containing and lacking the antimicrobial agent (Al-Rajhi *et al*. 2024).

Determination of Minimal Bactericidal Concentration (MBC)

The MIC dilution technique used to calculate the MBC, which plated and counted the two greatest antimicrobial doses to determine CFU/mL. The MBC has been established by culturing 100 mL of microbiological culture from all the wells with complete growth repression, the final positive well, and the development control on Mueller Hinton agar plates was enriched with 10% sheep blood. The plates were microaerophilically incubated at 35 °C for 72 h. To evaluate the extracts' bactericidal or bacteriostatic properties, MBC/MIC ratios were calculated (French 2006).

Hemolysis Inhibition

Three mL of fresh blood was obtained in tubes containing heparin from healthy volunteers. The blood had been spun for 10 min at 3000 rpm following the dissolution of the gathered particles with a comparable quantity of typical saline solution to that of the supernatant, the resulting pellets were then suspended in a neutral buffer mixture composed of Na_3PO_4 buffer (10 mM) at pH 7.4, achieving a reconstitution quantity of 40% v/v. The water that was distilled acted as a hypotonic solution (HS) for dissolving the extract. The extract at different concentrations (100 to 1000 µg/mL) was added to 5 mL tubes that included HS (perdose). Erythrocyte (0.1 mL) was added with HS and extract in different tubes, merged carefully, and incubated at 37 °C for 1 h. The mixture underwent centrifugation at 1300 g for 3 min. The spectrophotometer (Milton Roy) was used to measure absorbance (OD) at 540 nm to determine hemoglobin content in the supernatant. The tube with 5 mL of distilled water was used as the control, while another containing 5 mL of 200 g/mL indomethacin operated as the positive control (Al-Rajhi *et al*. 2022). The equation was utilized to evaluate the percentage of hemolysis inhibition (HI) by *P. longiseta* inflorescence extract,

$$\mathrm{HI}(\%) = 1 - \frac{A_2 - A_1}{A_3 - A_1} \times 100 \quad (2)$$

where A_1 and A_2 are the extract's absorbance in IS and HS, respectively, while A_3 was the control absorbance in HS.

Anticancer Potential of *Persicaria longiseta* Inflorescence

The effectiveness of PLE in cancer prevention was assessed using HCT116 cancer cell lines and the WI38 cell line, comprising normal human fetal lung fibroblasts, sourced from Vacsera, Giza, Egypt. Cells (5×10^4 cells/well) were cultured in 96-well plates utilizing RPMI 1640 medium enriched with 10% heat-inactivated fetal bovine serum (FBS) and antibiotics, namely penicillin and streptomycin, at concentrations of 100 U/mL and 100 µg/mL, respectively. The cells were incubated for a full day in a humidified atmosphere containing 5% CO_2, after which 100 µL of excess medium was removed. Different concentrations of the plant extract, from 31.25 to 1000 µg/mL, were introduced to the microplate and incubated at 37 °C for 24 h. Subsequently, 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) at a stock concentration of 5 mg/mL was introduced, and the microplate was incubated at 37 °C for an additional 4 h. Dimethyl sulfoxide (DMSO) was added to each well in a volume of 150 µL to dissolve the formazan crystals. Absorbance was quantified at 490 nm utilizing a microplate reader (Selim *et al*. 2024). Unexposed cells were incubated under identical conditions to those of the exposed cells, serving as a control group. The morphological characteristics of cells subjected to the plant extract were examined using a phase contrast light microscope (Nikon Corporation).

Statistical Assessment

The results are reported as mean ± SE, with each test performed three times in total. The results of a one-way analysis of variance (ANOVA) were evaluated using GraphPad Prism V5 software from San Diego, CA, USA.

RESULTS and DISCUSSION

Proteins, Carbohydrates, Phenols, Flavonoids, and Polyphenolic Compounds of *Persicaria longiseta* Inflorescence

Carbohydrates (166 mg/g), protein (53.2 mg/g), phenols (36.2 mg/g), and flavonoids (13.2 mg/g) were revealed by the nutritional value assessment of *P. longiseta* inflorescence (Fig. S4).

Table 1 and the HPLC chromatogram (Fig. S2 and S3) reflect the investigation of phenols and flavonoids in the *P. longiseta* inflorescence extract (PLE) and the standard curves of phenolic and flavonoid compounds, respectively. The high content of naringenin, chlorogenic acid, quercetin, rosmarinic acid, rutin, and gallic acid (9780, 8250, 5190, 4320, 2520, and 2430 µg/g, respectively) was associated with PLE. Moreover, ferulic acid, caffeic acid, vanillin, hesperetin, and coumaric acid, were detected in the following concentrations 1220, 530, 441, 378, and 324 µg/g, respectively. In contrast, a low quantity (54.9 µg/g) of cinnamic acid followed by syringic acid (59.6 µg/g), then ellagic acid 79.0 µg/g and kaempferol 96.7 µg/g were recognized in PLE (Table 1). Additionally, catechin was utilized as a standard in HPLC; however, it was not detected in the extract. Flavonoid and phenol substances in *P. longiseta* extract play a crucial role in its medicinal properties. These substances enhance its antimicrobial, anti-inflammatory, and antioxidant capacities (Ashibuogwu *et al.* 2022; Amin *et al*. 2024, 2025). From *Persicaria* species, a variety of phytochemical compounds have been identified and isolated, including flavonoids, terpenoids, and anthraquinones. These potent biochemical components have enhanced the species' antileukemic, antioxidant, analgesic, and anticancer properties (Tonny *et al.* 2017).

Rich in antioxidants and other components, *Persicaria minor*, another species of *Persicaria*, has a range of pharmacological characteristics, such as antibacterial, and anti-inflammatory (Varghese *et al*. 2024). The total phenolic content, an allelopathic molecule, was found in *P. longiseta* in lower concentrations in the roots (228.6 mg/L) and stems (207.8 mg/L) but higher in the leaves (1080 mg/L). Compared to native plants, *P. longiseta* has a competitive advantage because it releases phenolic chemicals that have an allelopathic impact and influence underground flora's germination, development, and fungal growth (Choi *et al*. 2015).

Table 1. HPLC Analysis of *P. longiseta* Inflorescence Extract

Constituent	Compound Type	RT	Area (mAU*s)	Area (%)	Conc. (µg/g)
Gallic acid	Phenolics	3.534	664.44	8.84	2432.44
Chlorogenic acid		4.177	1184.66	15.77	8253.92
Catechin		4.422	0.00	0.00	0.00
Methyl gallate		5.599	62.75	0.83	175.55
Caffeic acid		5.779	206.71	2.75	530.24
Syringic acid		6.262	20.28	0.27	59.63
Ellagic acid		7.227	15.55	0.20	78.98
Coumaric acid		8.401	180.21	2.39	323.84
Ferulic acid		9.401	420.48	5.59	1220.43
Vanillin		8.892	242.93	3.23	440.55
Rosmarinic acid		11.639	888.72	11.83	4315.59
Cinnamic acid		19.356	56.64	0.75	54.89
Kaempferol	flavonoids	20.397	76.94	1.02	96.71
Hesperetin		21.375	161.57	2.15	378.28
Rutin		6.869	336.83	4.48	2521.03
Naringenin		10.403	2119.23	28.21	9779.60
Daidzein		16.122	39.11	0.52	111.97
Querectin		17.430	833.27	11.09	5188.96

Antioxidant Activities of *P. longiseta* Inflorescence Extract

Increasing in the antioxidant activity of *P. longiseta* extract was observed parallel with its concentration up to 1000 µg/mL reached to 93.3 ± 1.25% of DPPH scavenging (Table 2). Compared to ascorbic acid as standard antioxidant, the *P. longiseta* extract is considered a promising antioxidant agent, where at 500, 125, and 7.81 µg/mL, the DPPH scavenging was 87.3 ± 0.36, 75.8 ± 2.01, and 45.1 ± 1.36% using *P. longiseta* extract, while it was 94.6 ± 1.02, 88.5 ± 1.36, and 56.8 ± 3.21% using ascorbic acid, respectively. Additionally, the measured IC_{50} (12.61 ± 2.3 µg/mL) validated the antioxidant properties of *P. longiseta* extract compared to 3.07 ± 2.10 µg/mL of ascorbic acid. The present extract was rich with phenols and flavonoids, namely chlorogenic acid, querectin, rosmarinic acid, naringenin, rutin, chlorogenic acid, and gallic acid, which possess antioxidant activity as reported in another studies (Alawlaqi *et al*. 2023; Al-Rajhi *et al*. 2023a,b). Gallic acid, naringenin, and quercetin existed in *Pleurotus ostreatus* extract, which revealed antioxidant activity with 16.17 µg/mL as IC_{50} value (Binsaleh *et al*. 2025). Another study found that

Persicaria japonica, *Persicaria viscofera*, *Persicaria conspicua*, *Persicaria sieboldin*, *Persicaria perfoliata*, and *Persicaria lapathifolia* have antioxidant potential *via* DPPH with IC_{50} 24.4 ± 0.7, 39.1 ± 0.6, 48.1 ± 0.6, 80.3 ± 0.8, 68.8 ± 0.5, and 24.5 ± 0.8 µg/mL, respectively (Choi *et al*. 2020). All these studies showed the promising role of PLE as a more effective antioxidant than other species, which have low IC_{50}.

Table 2. Antioxidative Activity of *P. longiseta* Inflorescence Extract (µg/mL) by DPPH Free Radical Scavenging Assay

Concentration (µg/mL)	DPPH Scavenging (%)		HSD
	Extract	Ascorbic acid	
1000	93.3 ± 1.25	98.0 ± 0.65	2.32
500	87.3 ± 0.36	94.6 ± 1.02	3.69
250	82.6 ± 1.02	92.3 ± 2.32	5.23
125	75.8 ± 2.01	88.5 ± 1.36	5.32
62.50	68.2 ± 0.85	80.6 ± 2.32	4.65
31.25	61.0 ± 1.36	73.6 ± 1.03	5.36
15.63	53.6 ± 2.03	65.5 ± 2.36	6.32
7.81	45.1 ± 1.36	56.8 ± 3.21	4.32
3.90	36.8 ± 0.84	51.5 ± 1.32	3.21
1.95	28.2 ± 0.56	43.2 ± 2.01	4.31
IC_{50}	12.61 ± 2.3	3.07 ± 2.10	5.12

Antimicrobial and Antibiofilm Activities of *P. longiseta* Inflorescence Extract

Inhibition of tested microorganisms was recorded using *P. longiseta* extract with different levels according to tested microbes (Table 3 and Fig. 1). *C. albicans* was the most sensitive with an inhibition zone 24 ± 0.49 mm, followed by *Bacillus subtilis* (19 ± 0.84 mm), *K. pneumonia* (19 ± 0.65 mm), *S. aureus* (16 ± 0.53mm), and *S. typhi* (16 ± 0.45 mm). Unfortunately, the antimicrobial potential result of extract was lower than that using standard Erythromycin/Nystatin compound. *A. niger* was resistant to the present extract, wuch that the growth was not inhibited. Moreover, MIC and MBC were investigated for tested microorganisms; for example 15.62 ± 0.87 and 31.25 ± 1.37 µg/mL were recorded for *C. albicans*, whereas 62.5 ± 0.45 and 125 ± 2.37 were recorded for *S. aureus*, respectively.

The antibacterial properties were attributed to some flavonoids and phenolic acids because they can react with cell proteins to create complex constituents that injure the membrane integrity of cells (Qanash *et al*. 2023). Additionally, as reported previously, the compounds related to phenols possess antibacterial activities because of their alkaline properties and capability to modify osmotic tension, which can damage bacteria's cell walls (Selim *et al*. 2024). In the present investigation, *P. longiseta* extract exhibited destructive effects on the biofilm construction of the tested bacteria. At different doses of MBC 25, 50, and 75% the biofilm inhibition was 58.44 ± 0.45, 86.44 ± 1.23, and 93.88 ± 0.03% for *S. typhi* while it was 49.62 ± 0.65, 74.58 ± 0.32, and 92.86 ± 0.16% for *K. pneumonia* (Table 4 and Fig. 2). The mechanism of the current extract to inhibit the biofilm inhibition may be due to the prevention the creation of polymeric ingredients, which contribute to the formation of biofilm.

Table 3. Antimicrobial Activity of *P. longiseta* Inflorescence Extract Against Different Microorganisms

Tested Microorganisms	Inhibition Zones (mm)		MIC(µg/mL)	MBC(µg/mL)
	Extract	Positive control (Erythromycin/Nystatin)		
B. subtilis	19 ± 0.84	29 ± 1.54	31.25 ± 2.25	62.5 ± 3.35
S.aureus	16 ± 0.53	25 ± 1.36	62.5 ± 0.45	125 ± 2.37
K. pneumonia	19 ± 0.65	20 ± 0.85	31.25 ± 2.36	62.5 ± 3.53
S. typhi	16 ± 0.45	22 ± 0.56	62.5 ± 3.65	125 ± 2.56
C. albicans	24 ± 0.49	32 ± 0.85	15.62 ± 0.87	31.25 ± 1.37
A. niger	--	34.50±0.18	--	--

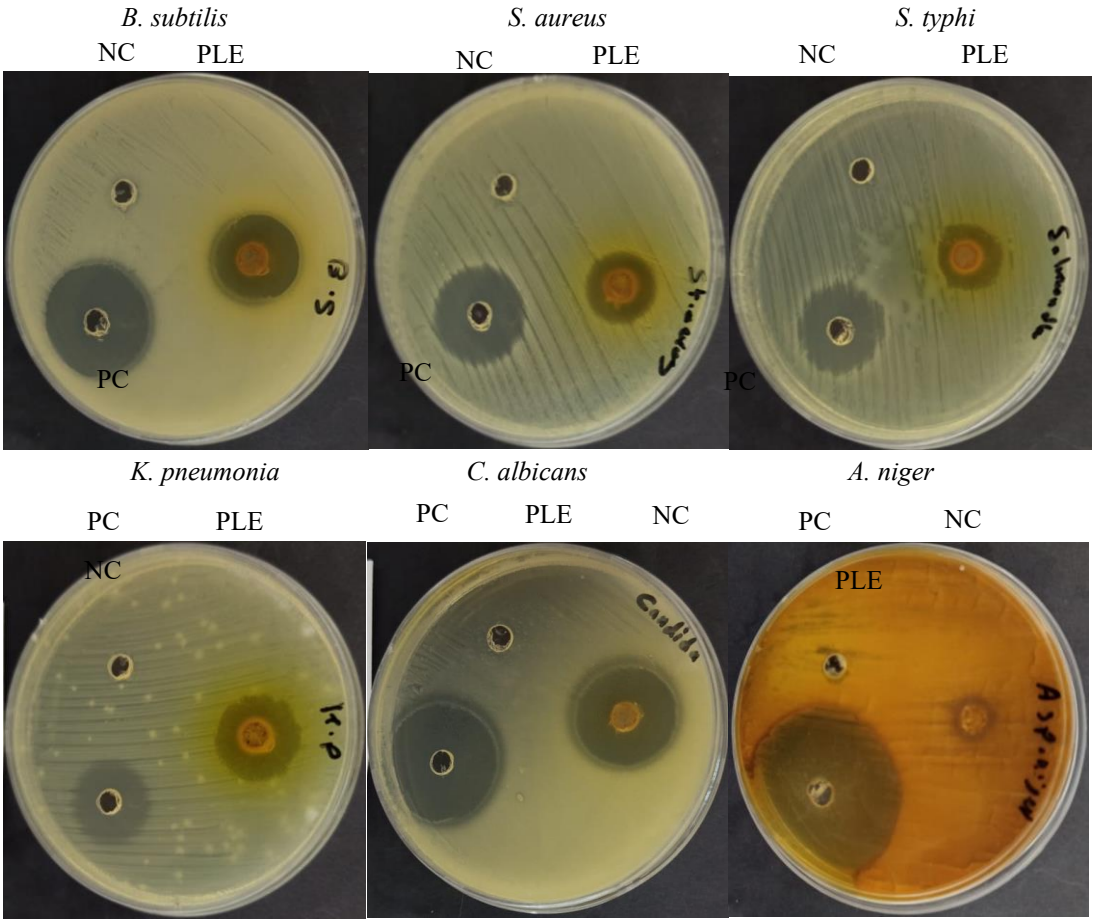

Fig. 1. Inhibition zones of *P. longiseta* inflorescence extract against different microorganisms

Table 4. Antibiofilm Activity of *P. longiseta* Inflorescence Extract Against Different Microorganisms

Concentration (% MBC)	*S. aureus*	*S. typhi*	*B. subtilis*	*K. pneumonia*
0.0	0	0	0	0
25	50.53 ± 0.85	58.44 ± 0.45	35.76 ± 1.02	49.62 ± 0.65
50	77.73 ± 1.05	86.44 ± 1.23	67.67 ± 0.69	74.58 ± 0.32
75	93.58 ± 0.14	93.88 ± 0.03	91.19 ± 0.12	92.86 ± 0.16

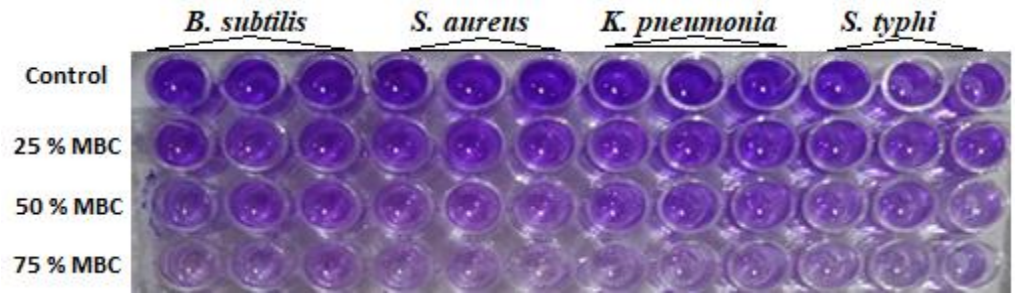

Fig. 2. Antibiofilm activity of *P. longiseta* inflorescence extract against different microorganisms

Hemolysis Activities of *P. longiseta* Extract

Sublethal doses of *P. longiseta* extract including 25, 50, and 75 MIC were tested to prevent hemolysis in the existence of tested bacteria (Table 5). From the obtained findings, the *P. longiseta* extract showed excellent inhibition for hemolysis, particularly in the presence of *B. subtilis* (77.7 ± 0.54, 90.8 ± 0.54, and 95.9 ± 0.56%), while it was less in *K. pneumonia* (54.1 ± 1.16, 75.8 ± 1.21, and 85.3 ± 0.36%) at different doses (25, 50, 75% MIC, respectively). Figure 3 visualizes the hemolysis inhibition in presence of tested bacteria compared with untreated and treated samples with control.

Table 5. Hemolysis Inhibition by Different Doses (MIC) of *P. longiseta* Inflorescence Extract in the Presence of Tested Bacteria

Concentration (% MIC)	*S. aureus*	*S. typhi*	*B. subtilis*	*K. pneumonia*
0.0	0	0	0	0
25	70.5 ± 1.25	65.1 ± 0.69	77.7 ± 0.54	54.1 ± 1.16
50	83.1 ± 0.23	81.1 ± 0.63	90.8 ± 0.54	75.8 ± 1.21
75	92.7 ± 0.36	93.2 ± 1.03	95.9 ± 0.56	85.3 ± 0.36

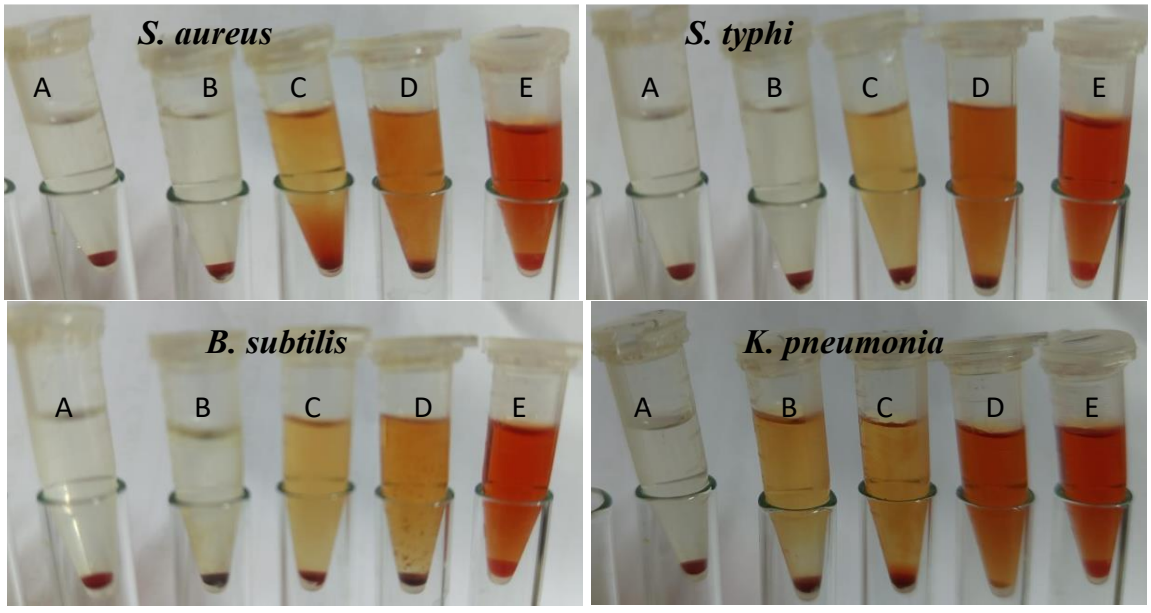

Fig. 3. Hemolysis inhibition by different doses (MIC) of *P. longiseta* inflorescence extract in the presence of tested bacteria: E, untreated; D, 25%; C, 50%; B, 75%; and A, treated with standard

Anti-Cancer Activities of *P. longiseta* Extract

The viability of both normal cells (Wi38) and cancer cells (HCT116) were decreased with the increasing concentration of *P. longiseta* extract but with different levels. At 62.5, 125, and 250 µg/mL of extract the viability was 99.95, 95.58, and 26.70% for Wi38, while it was 57.21, 22.10, and 6.04% for HCT116, respectively (Fig. 4), indicating the anticancer properties of the extract. Additionally, the obtained IC_{50} 199.62 ± 1.23 and 82.78 ± 1.6 µg/mL against the Wi38 and HCT116 confirmed the selective toxicity of the extract against cancer cells. Morphological changes of Wi38 (Fig. 5) and HCT116 (Fig. 6) tested cells reflected the efficacy of *P. longiseta* extract particularly against HCT116.

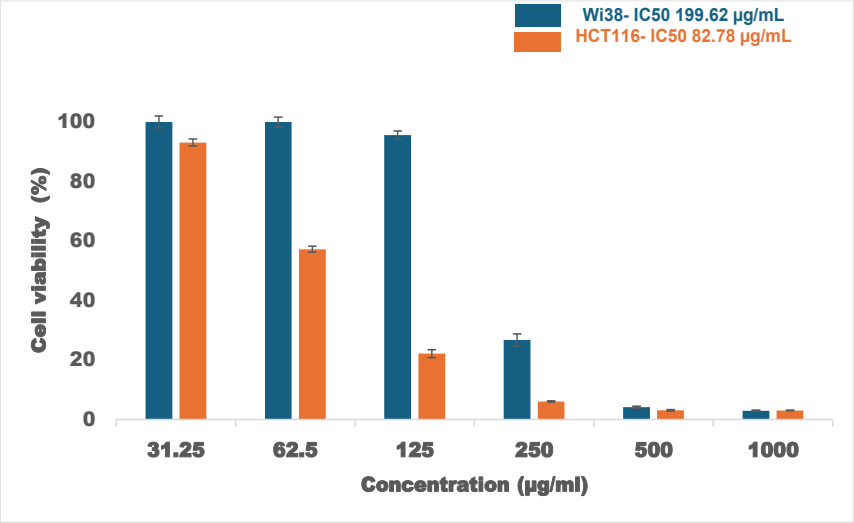

Fig. 4. The viability percentage of *P. longiseta* inflorescence against Wi38 and HCT116 cancer cell lines. Due to the disparate Tokay's letters at the time, the data were substantially different; identical concentration (n = 3; p < 0.05)

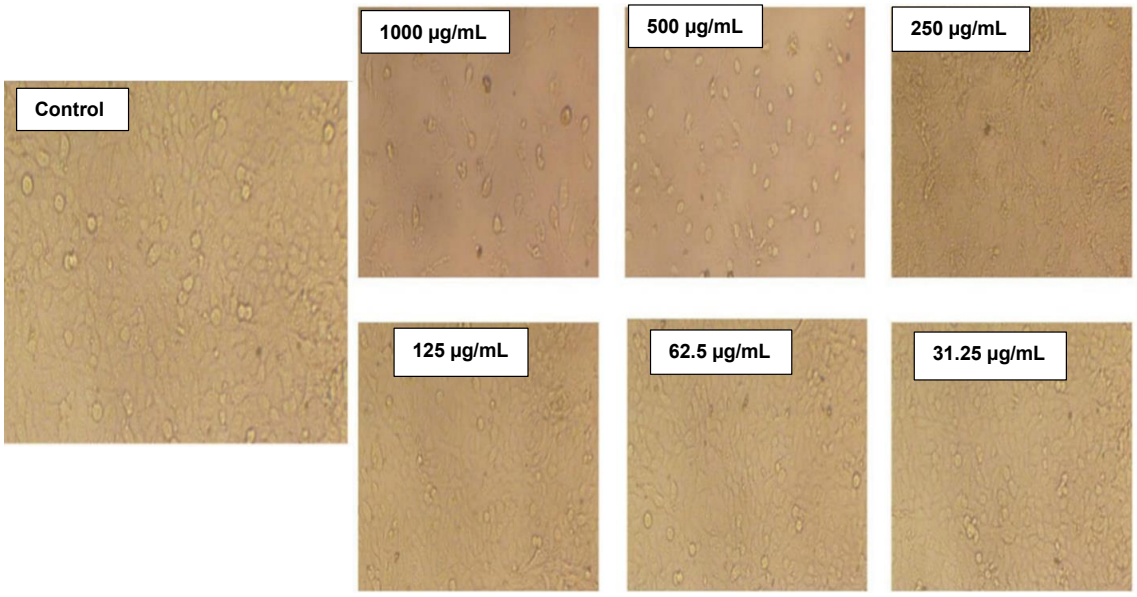

Fig. 5. Effect of different concentrations of *P. longiseta* inflorescence extract against Wi38 cell lines

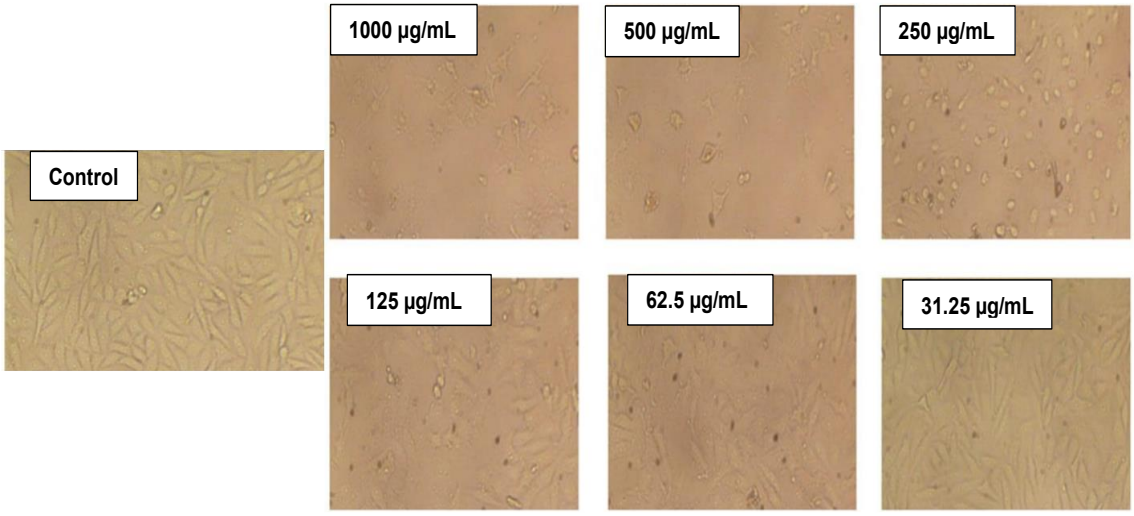

Fig. 6. Effect of different concentrations of *P. longiseta* inflorescence extract against HCT116 cancer cell lines

CONCLUSIONS

1. Phenolic and flavonoid active ingredients were present in the *Persicaria longiseta* inflorescence ethanol extract (PLE).
2. With an inhibition zone of 24 ± 0.49 mm, *Candida albicans* was the most sensitive, followed by *Bacillus subtilis* (19 ± 0.84 mm) to PLE.
3. The potential advantages of PLE as an antioxidant and anti-cancer drug were shown by *in vitro* tests.
4. In this study, it was determined that the ethanol extract from PLE was effective against colorectal cancer cells with IC50 82.78 µg/mL
5. Additionally, the measured IC_{50} (12.61 ± 2.3 µg/mL) validated the antioxidant properties of *P. longiseta* extract compared to 3.07 ± 2.10 µg/mL of ascorbic acid.
6. The IC50 values of 199.62 ± 1.23 and 82.78 ± 1.6 µg/mL for Wi38 and HCT116, respectively, confirm the selective toxicity of the extract towards cancer cells.

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APPENDIX

Methods

Measurement of soluble carbohydrates and protein in Persicaria longiseta inflorescence

To obtain carbohydrates, 5 mL of 2% phenol and 10 mL of 30% trichloroacetic acid were incorporated into 1 g of inflorescence powder. The mixture was allowed to sit overnight before filtration. Subsequently, the volume of the filtrate was completed to 50 mL (Said *et al.* 1964). The anthrone method for measuring carbohydrates. The protein content was determined following the methodology established by Lowry *et al.* (1950).

Phenol and flavonoid assessment in Persicaria longiseta inflorescence

Phenols (mg g^{-1} DW) were assessed using the Folin-Ciocalteu method, as reported by Cumplido-Nájera *et al*. (2019). In order to calculate the amount of total phenols in the dry inflorescences, 3.5 milliliters of plant extract were put to a test tube and combined with distilled water. 250µL of Folin's-phenol reagent was then added to the test tube to oxidize the contents. Following a five-minute incubation period, 1.25 mL of 20% Na_2CO_3 was used to neutralize the mixture. In comparison to the blank solution, the absorbance of the color that developed at 725 nm following 40 minutes of the reaction was determined. Using the gallic acid standard curve, the amount of phenols was determin ed. Flavonoids value is assessed by 0.5 mL of extract + 2.5 mL of distilled water. Next, 0.15 mL of 5% $NaNO_2$ was added. The mixture was maintained for 5 min; then 0.3 mL of 10% $AlCl_3$ was introduced and held for an additional 5 min. Subsequently, add 1 mL of 1 M NaOH and 0.55 mL of distilled water. The absorbance of the solution was measured at a wavelength of 510 nm. Each flavonoid in the extract will form a complex yellow color when treated with $AlCl_3$ solution. The content was expressed in equivalent milligrams of Quercetin /g of dry weight (mg EQ g^{-1} DW) (Zhishen *et al*. 1999).

Results

***Inflorescence of Persicaria longiseta* (Bruijn) Kitagawa**

Fig. S1. Photo of the plant, and full scientific name

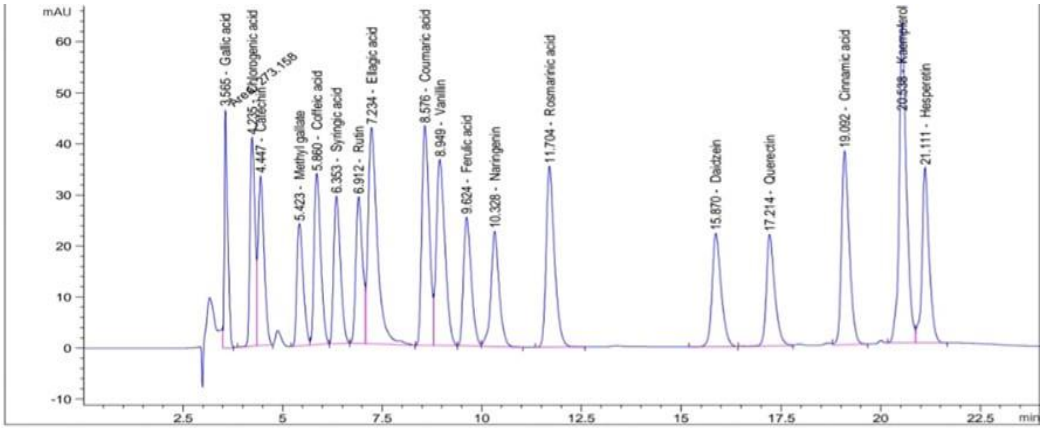

Fig. S2. HPLC chromatograms of the standard for the phenolic and flavonoids

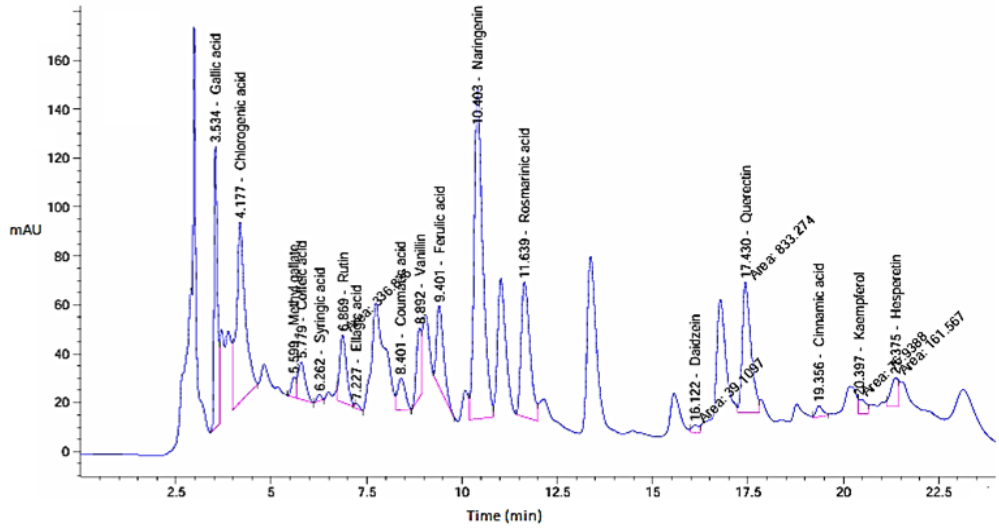

Fig. S3. HPLC chromatogram for the detection of phenolic compounds in *P. longiseta* inflorescence extract

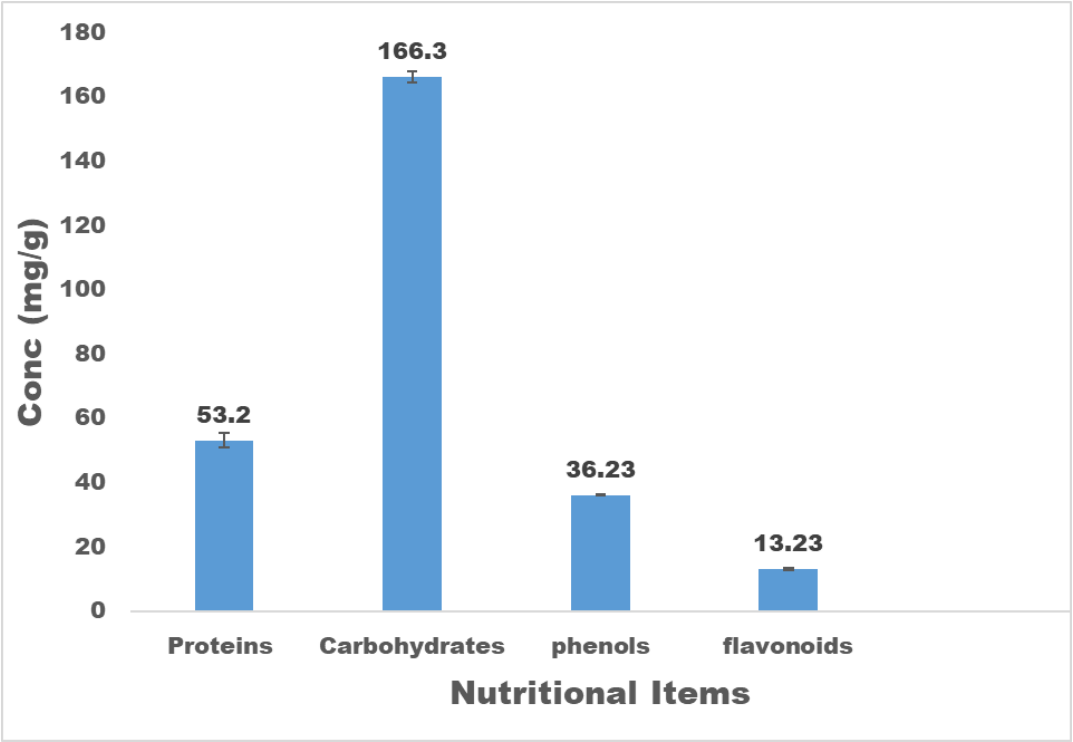

Fig. S4. Nutritional values of *P. longiseta* inflorescence extract

References for Supplementary Material

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