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Investigation of chemical composition and evaluation of antioxidant, antibacterial and antifungal activities of ethanol extract from *Bidens pilosa* L.

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Abstract

Bidens pilosa L. is a species of plant that grows wild. It is commonly found in abundance in the Mekong Delta region of Vietnam. This study aimed to investigate the chemical composition, *in vitro* and *in vivo* antioxidant activity, the antimicrobial activity against some aquatic pathogenic bacteria and antifungal activity against plant pathogenic bacteria of *Bidens pilosa* L. extract. The results showed that *Bidens pilosa* L. extract had good antioxidant capacity through all four test methods of DPPH, $ABTS^{\bullet+}$, RP, and TAC with the EC_{50} values of 455.78 ± 3.28 µg/mL, 148.68 ± 2.02 µg/mL, 462.09 ± 12.57 µg/mL and 139.14 ± 4.34 µg/mL, respectively. Fruit flies fed on a diet supplemented with 0.5 g/mL of *Bidens pilosa* L. extract had an average lifespan of 2.15 times and 1.54 times longer under oxidative stress using 20 mM Paraquat and H_2O_2 10%, respectively. In addition, the total polyphenols and total flavonoids in the extract were also determined to be 107.49 ± 4.04 mg GAE/g and 165.63 ± 2.90 mg QE/g, respectively. Regarding the antimicrobial activity, the ethanol extract of *Bidens pilosa* L. showed stronger resistance to Gram (+) *S. agalactiae* than the tested Gram (-) bacteria, including *A. hydrophila, E. ictaluri,* and *A. dhakensis*. In addition, the ethanol extract from *Bidens pilosa* L. also showed the better ability to inhibit the growth of the fungus *Colletotrichum* sp. (MIC = 1250 µg/mL) than that of *Fusarium oxysporum* (MIC = 2500 µg/mL).

Keywords: antioxidant; antibacterial; antifungal activities; *Bidens pilosa* L.

Practical Application: The potential usage of *Bidens pilosa* L. extract in the development of functional foods with antioxidant effects or as an alternative antibiotic therapy.

1 Introduction

Oxidative stress is the primary cause of biochemical disorders associated with a deal great of different diseases such as aging, cancer, atherosclerosis, diabetes, Alzheimer's, and Parkinson's. Antioxidants can be considered an agent that can protect living cells from the aggression of free radicals and are the most important parts of human nutrition. Plants are crucial sources of antioxidant phytochemicals such as carotenoids, polyphenols, flavonoids, and alkaloids. Notably, diets rich in antioxidants have been reported to reduce the risk of chronic degenerative illnesses mediated by the production of free radicals. Nowadays, people have been increasingly leaning towards using plants for nutritional compositions, supplements, and pharmaceutical industry (Alaca et al., 2022; Esparza-Espinoza et al., 2022; Shafay et al., 2022; Sipahli et al., 2022).

Bidens pilosa L. belongs to the genus Bidens, family Asteraceae. According to Vietnamese traditional medicine, *Bidens pilosa* L. has a bitter taste. It is neutral and has the effect of clearing heat and detoxifying. The plant is often used to treat scabies, pain, injury, insect bites, or in combination to cure urinary retention. Many previous studies have demonstrated that the *Bidens pilosa* L. extract had antioxidant, antibacterial, anti-inflammatory, anti-cancer, and antiallergic properties (Rabe & Van Staden, 1997; Horiuchi & Seyama, 2006; Xin et al., 2021). Specifically, the methanol extract from *Bidens pilosa* L. was resistant to *S. aureus*, *S. epidermi,* and *B. subtilis* with MIC values determined at 2.0 mg/mL, 8.0 mg/mL, and 4.0 mg/mL, respectively (Rabe & Van Staden, 1997). Moreover, from ethanol extract of *Bidens pilosa* L., six flavonoid/flavonoid glycoside compounds were isolated, including isoquercitrin (1); vitexin (2); astragalin (3); 5,6,7,4'-tetramethoxylflavone (4); 5,3',4'-trihydroxy-3; 7-dimethoxylflavone (5); and quercetin (6). In particular, compound (6) showed an *in vitro* antioxidant effect on two test methods, including DPPH and $ABTS^{\bullet+}$. Compounds (4) and (5) showed significant growth inhibitory effect on RKO colorectal cancer cells with IC_{50} index of 39.08 µmol/L and 17.68 µmol/L ($p < 0.01$) (Yi et al., 2016). Besides, *Bidens pilosa* L. essential oil has also been shown to have antioxidant properties and is applied in food quality preservation in Northern Cameroon (Goudoum et al., 2016).

In general, the experiments of *in vivo* activity of *Bidens pilosa* L. are still limited. In addition to testing antioxidant capacity *in vitro* by four methods DPPH, $ABTS^{\bullet+}$, RP, and TAC, the study also used fruit fly model to test *in vivo* antioxidants, at the same time evaluated the antibacterial activity of pathogenic bacteria in aquatic animals, including *A. dhakensis, A. hydrophila, E. ictaluri* and *S. agalactiae*, and against the plant pathogenic

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fungus *Colletotrichum* sp. and *Fusarium oxysporum* to provide more scientific information, as well as to orient the application of this plant in practice.

2 Materials and methods

2.1 Plant material

Bidens pilosa L. was collected in Kien Giang province and identified by Dr. Nguyen Thi Kim Hue, Department of Biology, Faculty of Natural Sciences, Can Tho University, according to the Vietnamese plant taxonomy system.

2.2 Pathogenic microorganisms and Drosophila melanogaster

Aquatic pathogenic bacteria such as *Aeromonas dhakensis* (*A. dhakensis*), *Aeromonas hydrophila* (*A. hydrophila*), *Edwardsiella ictaluri* (*E. ictaluri*), *and Streptococcus agalactiae* (*S. agalactiae*) were provided by the Research Institute For Aquaculture No 2. Plant pathogenic fungus strains such as *Colletotrichum* sp. and *Fusarium oxysporum* were provided by the Faculty of Agriculture, Can Tho University. Wild fruit fly *Drosophila melanogaster* strain Canton S (CS) was sent from the Biofunctional Chemistry laboratory, Kyoto Institute of Technology, Japan.

2.3 Extract preparation

After being collected, the whole part of *Bidens pilosa* L. was removed, washed, dried, crushed, and soaked in ethanol 99° five times (each time lasting for 24 h). The ethanol extracts were combined, solvent distilled under low pressure to obtain the corresponding ethanol extracts, and used for investigating chemical composition and assessing bioactivity.

2.4 Investigation of chemical composition and quantification of total polyphenol and flavonoid

Phytochemicals constituents

The chemical composition of *Bidens pilosa* L. extract was determined as described by Jasuja et al. (2013) with some adjustments. The test sample was diluted in absolute ethanol or distilled water (depending on the extract) at 10 mg/mL. The test procedure is shown in Table 1.

Determination of total polyphenol content

The polyphenol content was determined according to the method of Singleton et al. (1999) with some adjustments. The Folin-Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate with a yellow color. It is used for the quantification of compounds containing phenols or polyphenols. In this experiment, the reaction mixture consisting of 250 µL of extract (or standard), 250 µL of water, and 250 µL of Folin-Ciocalteu reagent was mixed well. Na_2CO_3 10% was added and incubated for 30 min at 40 °C in a thermostat. The spectral absorbance of the reaction mixture was measured at 765 nm. The total polyphenol content in the extract was determined based on the standard curve equation of gallic acid $y = 0.0403x - 0.0033$ with the coefficient $R^2 = 0.9988$ (where: *y*-axis referred to the spectral absorbance value (Abs), the *x*-axis corresponds to gallic acid standard concentration).

Determination of total flavonoid content

Total flavonoid content was determined according to the description of Bag et al. (2015). The reaction mixture consisted of 200 µL of extract or standard, 200 µL of water, and 40 µL of $NaNO_2$ 5% was shaken and allowed to stand for 5 min. Then, 40 µL of $AlCl_3$ 10% was added to the mixture and shaken well. After incubation for 6 min, the reaction mixture was added 400 µL of 1 M NaOH and water to make 1 mL. The reaction mixture was measured absorbance spectrophotometrically at 510 nm. The total flavonoid content in the extract was determined based on the standard curve equation of quercetin $y = 0.006x - 0.0235$ with the coefficient $R^2 = 0.9985$ (where: the *y*-axis referred to the value of spectral absorbance (Abs), the *x*-axis referred to the concentration of the quercetin standard).

2.5 In vitro antioxidant activity

Investigation of the free radical scavenging effect of 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH free radical neutralization ability of the extract was determined according to the method of Sharma & Bhat (2009) with some adjustments. The reaction mixture consisting of 100 µL DPPH and 100 µL of *Bidens pilosa* L. extract at different concentrations of 100, 200, 400, 800, and 1600 µg/mL was incubated in the dark at room temperature for 60 min. The post-reaction mixture was measured for absorbance at λ = 517 nm. The experiment was repeated 3 times. Gallic acid was used as a positive control.

The investigation by $ABTS^{\bullet+}$ method

The ability of the extract to neutralize $ABTS^{\bullet+}$ free radicals was determined according to the method of Nenadis et al. (2004)

Table 1. Qualitative experimental procedure for natural compounds.

Chemical compounds	Reagents	Identification
Alkaloids	Dragendorff	Orange-brown to red precipitate
Flavonoids	Concentrated H_2SO_4	Dark yellow to orange, red precipitate
Steroids and Triterpenoids	Liebermann Burchard	Change to blue, green, orange, or red of solution
Saponins	HCl 0.1N and NaOH 0.1N	Durable foaming
Tanins	Gelatin 1%	White cotton precipitate
Phenolics	$FeCl_3$ 10%	Blue-black or red-orange precipitate

with adjustments. Specifically, the reaction mixture of 10 µL of *Bidens pilosa* extract at different concentrations of 80, 100, 120, 160, 200, and 240 µg/mL and 990 µL of $ABTS^{\bullet+}$ free radicals, was incubated for 6 min at room temperature. After incubation, the absorbance was measured at 734 nm. The experiment was repeated 3 times. Ascorbic acid was used as a positive control.

Investigation of iron-reducing power (RP: reducing power)

The iron reduction capacity of the extract was determined according to the method of Oyaizu (1986) and Padma et al. (2013) with corrections. 500 µL of *Lumnitzera racemosa* extract at the concentrations from 50, 100, 200, 300, 400, and 500 µg/mL was added to 500 µL phosphate buffer (0.2 M, pH 6.6-7.2), continued to add 500 µL $K_3Fe(CN)_6$ 1% to the mixture, then kept for 20 min at 50 °C. Finally, 500 µL of CCl_3COOH 10% was supplemented and centrifuged at 3000 rpm for 10 min. 500 µL of the upper layer was taken into Eppendorf; added 500 µL of distilled water and 100 µL of $FeCl_3$ 0,1%, and measured the absorbance at 700 nm. The positive control used was gallic acid.

Phosphomolybdenum method

Total antioxidants were determined according to the method of Prieto et al. (1999). The *Bidens pilosa* L. extract was diluted in methanol to a concentration range of 0, 14, 27, 41, 55, 68, and 82 µg/mL. 100 µL of the above solution was added to the well containing 900 µL of solution A which was the combination of 0.6 M H_2SO_4, sodium phosphate 28 mM, and ammonium molybdate 4 mM. The mixture was incubated at 95 °C for 90 min. Then the absorbance was measured at 695 nm. The positive control used was gallic acid.

2.6 In vivo antioxidant activity in fruit fly model

The *in vivo* antioxidant activity of *Bidens pilosa* L. fruit extract was tested in the fruit fly model as described by Etuh et al. (2019). Newly hatched male flies 1-2 days old were reared in three different media, including standard medium, medium supplemented with a concentration of 0.5 mg/mL *Bidens pilosa* L. extract, and medium supplemented with gallic acid at a concentration of 0.05 mg/mL feed. Gallic acid was used as a positive control. The feed was renewed every 2 days. On day 20, all flies were kept under starvation for 3 h, then placed in glass vials having absorbent paper with PQ 20 mM or H_2O_2 10% (each treatment included 25 flies/vial). The number of live flies was recorded after 4 h of observation. Each treatment was repeated 5 times. Antioxidant capacity was determined based on mean lifespan (h), 50% survival time (h), and maximum lifespan (h).

2.7 Investigation of antimicrobial activity in aquatic animals

The antibacterial activity of the extract was determined by the method of agar plate diffusion as described by Oonmetta-aree et al. (2006) and Das et al. (2013) with adjustments. Spread 200 µL of 10^6 CFU/mL testing bacteria solution evenly over the surface of a petri dish containing TSB medium and allowed to dry. Created a small well with a diameter of 9 mm and 100 µL of the test sample was added to the wells. The sample was incubated for 24 h, then observed and recorded the results. DMSO 10% was the sample diluent and used as a negative control. The inhibitory activity was assessed by measuring the diameter of the inhibitory ring of the microorganism. The MIC value (minimum inhibitory concentration) was determined as the lowest concentration of extract capable of inhibiting the growth of bacteria. The experiment was repeated 3 times.

2.8 Investigate antifungal activity against plant pathogenic bacteria

The antifungal activity of the extract was performed according to the method of Gakuubi et al. (2017) with some corrections (the poisoned food technique). PDA medium (Potato D-glucose agar) was supplemented with *Bidens pilosa* L. extract to achieve the concentrations of 312.5; 625; 1250; 2500 and 5000 µg/mL, respectively. The pathogenic fungal strains were prepared on agar plates then chiseled into small pieces with a diameter of 9 mm. Sterile forceps were used to pick up the fungal plaques, placed between the above Petri dishes, and incubated at room temperature (25 ± 2 °C) within 7 days. The control sample was a mycelium grown on a standard PDA medium. The experiment was repeated 3 times. The percentage of inhibition of mycelium growth was calculated using the formula of Philippe et al. (2012) (Equation 1).

$$Percentage\ of\ inhibition\ of\ fungal\ growth\ (\%) = \frac{D_C - D_T}{D_C} \times 100 \quad (1)$$

Where: D_C referred to the diameter of mycelium growing in the control sample (mm), D_T referred to the diameter of mycelium growing in the experimental treatment (mm). The minimum inhibitory concentration (MIC) was determined as described by Adjou et al. (2012).

2.9 Statistical analysis

Experiments were repeated three to five times, with average and error results. Experimental data were processed, and the graphs were drawn on Microsoft Excel and Minitab (version 16.0) software.

3 Results and discussion

3.1 Chemical composition, total polyphenol, and total flavonoid content

Determination of chemical composition is the first and the most important stage in studying the activity of plant extracts. The analysis result of chemical composition helps guide further experiments. In this study, the chemical composition of *Bidens pilosa* L. was determined through specific reagents. Research results showed that most of the important groups of secondary metabolites such as polyphenols, flavonoids, alkaloids, tannins, glycosides were present in the ethanol extract of *Bidens pilosa* L. contained; whereas saponins were absent (Table 2). The results are consistent with Ajanaku et al. (2018), in the fractional extracts of hexane, dichloromethane, ethyl acetate, and methanol of *Bidens*

pilosa L., there was no presence of saponins compounds. It may be because the saponin content is not enough for chemical detection.

The total polyphenol content in the *Bidens pilosa* L. extract was determined based on the standard curve equation of gallic acid, $y = 0.0403x - 0.0033$, with the coefficient $R^2 = 0.9988$; and the total flavonoid content in the extract was identified based on the Quercetin's calibration curve, $y = 0.006x - 0.0235$, with the coefficient $R^2 = 0.9958$. The analysis results showed that the Abs value of the extract was 0.2395 ± 0.01, corresponding to the total polyphenol content of 107.49 ± 4.04 mg GAE/g of the extract. Similarly, the total flavonoid content was also determined as 165.63 ± 2.90 mg QE/g extract. The study results also showed that the total polyphenol content was 1.49 times higher, and the total flavonoid content was 1.34 times higher than that of the methanol extract of *Bidens pilosa* L. reported by Singh et al. (2017) (total polyphenols of 72 mg GAE/g and total flavonoids of 123.30 mg QE/g extract).

3.2 In vitro antioxidant activity

Quantitative results recorded that the ethanol extract of *Bidens pilosa* L. contained high levels of polyphenol and flavonoid compounds. In addition, previous studies have also defined that polyphenols and flavonoids are an important group of secondary metabolites with high biological activity, especially the ability to remove free radicals. Therefore, the study was conducted to investigate the *in vitro* antioxidant activity of *Bidens pilosa* L. extract by 4 methods of DPPH, $ABTS^{\bullet+}$, RP, and TAC. The study result presented in the Table 3 proved that antioxidant activity of extract from *Bidens pilosa* L. was increased in a concentration dependent manner.

The Table 4 showed that the ethanol extract of *Bidens pilosa* L. exhibited antioxidant capacity on the four test methods with EC_{50} or $Abs_{0,5}$ values of 455.78 ± 3.28 μg/mL (DPPH), 48.68 ± 2.02 μg/mL ($ABTS^{\bullet+}$), 462.09 ± 12.57 μg/mL (RP) and 139.14 ± 4.34 μg/mL (TAC), respectively. Accordingly, *Bidens pilosa* L. extract showed the strongest reducing activity for Mo (VI) to form Mo (V). However, the free radical scavenging ability of *Bidens pilosa* was still lower than that of the control agents of gallic acid or ascorbic acid. Specifically, in the TAC method, the EC_{50} value of *Bidens pilosa* L. was 139.14 ± 4.34 g/mL, which was higher than the EC_{50} value of gallic acid (EC_{50} = 14.14 ± 0.12 μg/mL). Therefore, the antioxidant activity of *Bidens pilosa* L. was 9.84 times lower than that of gallic acid. According to the research results of Singh et al. (2017), the methanol extract from *Bidens pilosa* L. had the ability to remove DPPH and $ABTS^{\bullet+}$ free radicals with EC_{50} values of 80.45 μg/mL and 171.6 μg/mL, respectively. Thus, when compared with the same DPPH test method, the ethanol extract in the current study showed lower activity than the corresponding methanol extract but higher for the $ABTS^{\bullet+}$ method.

3.3 In vivo antioxidant activity in fruit fly model

The fruit fly is an effective animal model for human pathological studies because its genome has been completely

Table 2. Chemical composition of the ethanol extract from *Bidens pilosa* L.

Chemical compounds	Alkaloids	Flavonoids	Steroids and Triterpenoids	Saponins	Tannins	Phenolics
Presence	+	+	+	-	+	+

(-) absent; (+) present.

Table 3. Free radical scavenging effect of ethanol extract from *Bidens pilosa* L.

DPPH		$ABTS^{\bullet+}$		RP		TAC	
Content (μg/mL)	Scavenge %	Content (μg/mL)	Scavenge %	Content (μg/mL)	Abs	Content (μg/mL)	Abs
100	4.14 ± 0.36	80	19.35 ± 0.58	200	0.23 ± 0.03	27	0.13 ± 0.03
200	12.60 ± 0.43	100	32.19 ± 0.73	300	0.30 ± 0.01	64	0.27 ± 0.05
400	19.32 ± 0.44	120	42.14 ± 1.03	400	0.41 ± 0.02	91	0.36 ± 0.03
800	28.61 ± 0.32	160	56.00 ± 1.95	500	0.55 ± 0.04	182	0.62 ± 0.02
1600	61.21 ± 0.37	200	65.99 ± 1.26	600	0.65 ± 0.04	273	0.94 ± 0.04
$y = 0.0786x + 1.2024$ ($R^2 = 0.9923$)		$y = 0.3298x - 1.8623$ ($R^2 = 0.9857$)		$y = 0.0011x - 0.0079$ ($R^2 = 0.9930$)		$y = 0.0032x + 0.0549$ ($R^2 = 0.9985$)	

x is the value of the extraction concentration; *y* is Abs value or free radical neutralization efficiency (%).

Table 4. EC_{50} or $Abs_{0,5}$ values of test methods.

Extract/compounds	EC_{50} or $Abs_{0,5}$ (μg/mL)			
	DPPH	$ABTS^{\bullet+}$	RP	TAC
Bidens pilosa L.	455.78 ± 3.28	148.68 ± 2.02	462.09 ± 12.57	139.14 ± 4.34
Gallic acid	3.92 ± 0.06	-	9.25 ± 0.03	14.14 ± 0.12
Ascorbic acid	-	2.86 ± 0.05	-	-

decoded, with 75% of the genes responsible for human disease (Bier, 2005). In addition, the fruit fly has a short life cycle and is easy to rear. In this study, fruit flies were selected to evaluate *in vivo* antioxidant capacity of the ethanol extract from *Bidens pilosa* L. Newly hatched male fruit flies were reared in conditions supplemented with *Bidens pilosa* L. extract at a 0.5 g/mL concentration of the feed. The control treatment used standard food, and the positive control used the feed supplemented with gallic acid at a concentration of 0.05 g/mL of feed. The results of the study are presented in Figure 1.

The study results showed that under the conditions of oxidative stress induced by Paraquat 20 mM, fruit flies reared in the medium supplemented with *Bidens pilosa* L. extract had a mean lifespan of 1,41 times longer than those reared in normal conditions; whereas that value in the method using H_2O_2 10% was 1.25 times longer. Although fruit flies reared in a diet supplemented with *Bidens pilosa* L. extract had a lower maximum lifespan than fruit flies supplemented with gallic acid, they lasted 2.15 times longer than fruit flies reared in normal conditions (Paraquat 20 mM) and 1.54 times (H_2O_2 10%). Thus, the results of *in vivo* antioxidant testing on fruit flies have contributed to further confirmation of the antioxidant potential of *Bidens pilosa* L. extract.

3.4 Antimicrobial activity of aquatic pathogens

According to the results of previous studies, in addition to the antioxidant ability, the *Bidens pilosa* L. extract also showed the ability to resist many different bacterial strains such as *P. Aeruginosa* (MIC = 220 ± 0.17 µg/mL), *C. Albicans* (MIC = 870 ± 0.25 µg/mL), *E. Coli* (MIC = 80 ± 0.05 µg/mL), *S. Aureus* (MIC = 110 ± 0.17 µg/mL), *B. Subtilis* (MIC = 380 ± 0.27 µg/mL), *M. Luteus* (MIC = 250 ± 0.15 µg/mL), *E. Faecalis* (MIC = 1250 µg/mL), *S. Epidermidis* (MIC = 630 ± 0.25 µg/mL) and *Rhizopus* (MIC = 3125 µg/mL) (Falowo et al., 2016; Singh et al., 2017; Ajanaku et al., 2018). However, the studies were mainly conducted on pathogenic bacteria in humans. There haven't been many publications on bacterial strains causing disease in aquatic animals. In this study, 04 pathogenic bacterial strains in aquatic animals were selected for antibacterial testing, including *A. dhakensis*, *A. hydrophila*, *E. ictaluri,* and *S. agalactiae*. The antibacterial ring diameters are presented in Figure 2, and the minimum inhibitory concentrations are presented in Table 5.

The study results indicated that the ethanol extract of *Bidens pilosa* L. expressed the ability to resist the four tested bacterial strains with MIC values in the range of 625-1250 µg/mL. In addition, the test results also showed that *Bidens pilosa* L. extract had better resistance on bacterial strains of *A. hydrophila, E. ictaluri,* and *S. agalactiae* compared to *A. dhakensis*. In particular, the extract showed the strongest resistance on the bacterial strain of *S. agalactiae*, a Gram (+) bacteria. It may be because the composition of the extract contained groups of compounds that act on the cell wall (peptidoglycan) of Gram (+) bacteria. Besides, the resistance to *A. hydrophila* of *Bidens pilosa* L. ethanol extract in this study was lower than that of the methanol extract of *P. amarus* (MIC = 128 µg/mL, according to Britto et al., 2011) and ethanol extract of *Piper betle* leaves (MIC = 25 µg/mL, according to Caruso et al., 2017), but higher than other extracts of *Combretum quadrangulare*, *Kalanchoe pinnata, Premna corymbosa, Wedelia chinensis, Zingiber officinale, Houttuynia cordata and Ageratum conyzoides* (Dao et al., 2020; Tran et al., 2021).

3.5 Antifungal activity against plant pathogens

Similar to antibacterial activity, the essential oil and the extracts of *Bidens pilosa* L. also showed resistance to many human pathogenic fungi such as *C. albicans* (MIC = 315 µg/mL), *C. tropicalis*

Table 5. Minimum Inhibitory Concentrations (MICs) of the ethanol extract from *Bidens pilosa* L.

Microorganisms	*A. dhakensis*	*A. hydrophila*	*E. ictaluri*	*S. agalactiae*
MIC (µg/mL)	1250	625	625	625

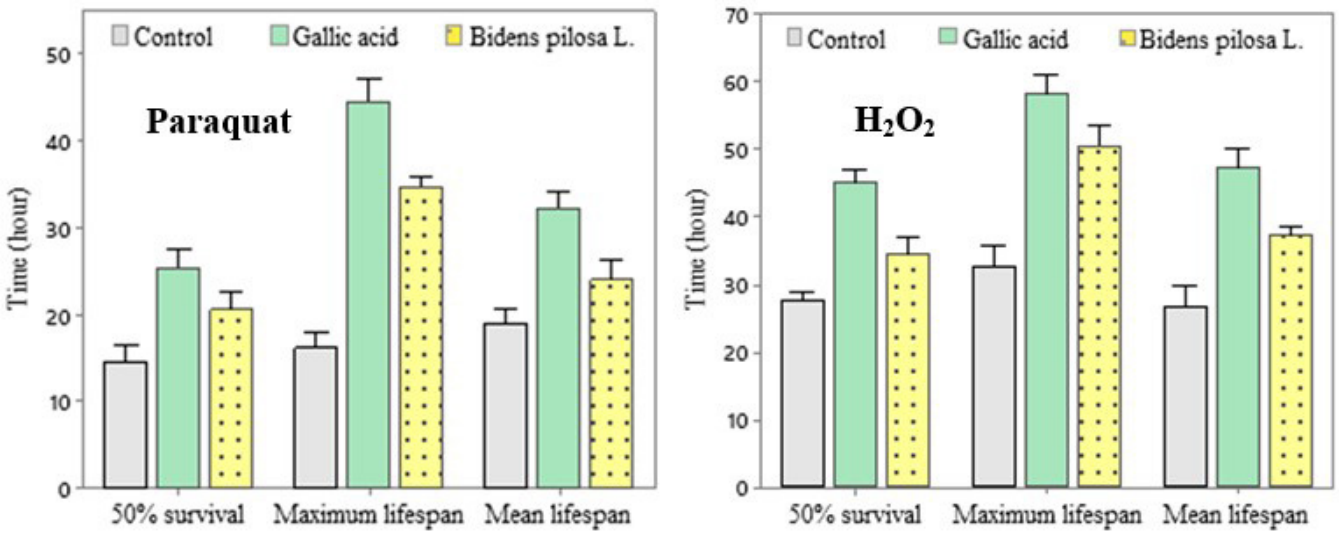

Figure 1. *In vivo* antioxidative effect of *Bidens pilosa* L. extract in the condition of 20 mM Paraquat and H_2O_2 10%.

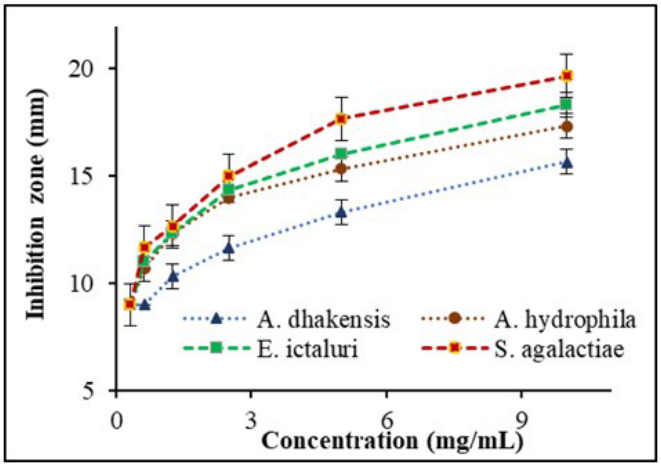

Figure 2. Antibacterial ring diameter of *Bidens pilosa* L. extract.

Table 6. Percentage of inhibition of fungal growth after 7 days.

Concentrations of extract (µg/mL)	Percentage of mycellal growth inhibitor (%)	
	Colletotrichum sp.	*Fusarium oxysporum*
5000	100 ± 0	100 ± 0
2500	100 ± 0	100 ± 0
1250	100 ± 0	79.39 ± 0.76
625	81.92 ± 0.98	62.72 ± 2.74
312.5	71.19 ± 1.69	34.65 ± 1.52
156.25	49.72 ± 0.98	27.19 ± 1.52

(MIC = 198 µg/mL) mL), *C. parapsilosis* (MIC < 31 µg/mL), *T. rubrum* (MIC < 31 µg/mL), *T. mentagrofites* (MIC < 31 µg/mL), *Corticum rolfsii* (MIC < 31 µg/mL), *P. notatum* (MIC = 100 µg/mL) (Ashafa & Afolayan, 2009; Angelini et al., 2021). However, to find and apply native plant species to prevent and treat plant-damaging fungal strains, two fungi of *Colletotrichum* sp. and *Fusarium oxysporum* were selected to test the antifungal activity through the environmental toxicity assay. The study results showed that the percentage inhibition of fungal growth was proportional to the high concentration of *Bidens pilosa* L. extract in the concentration range from 156.25 to 5000 µg/mL. Specifically, at concentration of > 1250 µg/mL, *Bidens pilosa* L. completely inhibited 100% of the growth of *Colletotrichum* sp (Table 6).

Similarly, *Fusarium oxysporum* was also 100% inhibited at concentration of > 2500 µg/mL. Notably, the study results showed that the extract had a better ability to resist *Colletotrichum* sp. than *Fusarium oxysporum* with MIC values of 1250 and 2500 µg/mL, respectively. The results also indicated that the ethanol extract *Bidens pilosa* L. had stronger activity against *Colletotrichum* sp. than the methanol extract from *M. calabura* root (MIC = 10000 µg/mL according to Ramasamy et al., 2017), equivalent to *Chloranthus japonicus* root extract (Park et al., 2017) and the resistance activity to *Fusarium oxysporum* was stronger than the methanol extract from *Thymus vulgaris* (MIC = 8000 µg/mL), as well as *Zingiber officinale* (MIC = 16000 µg/mL according to Al-Rahmah et al., 2013), but 2,5 times less than that of *Piper hispidum* essential oil (MIC = 500 µg/mL) (Tangarife-Castaño et al., 2014).

4 Conclusions

The research results concluded that *Bidens pilosa* L. extract expressed its *in vitro* antioxidant capacity on 4 test methods. TAC had the strongest antioxidant activity with $Abs_{0,5}$ value of 139.14 ± 4.34 µg/mL. Fruit flies reared in the diet supplemented with 0.5 g/mL of *Bidens pilosa* extract could prolong the average lifespan under oxidative stress induced by 20 mM Paraquat and H_2O_2 10%. In addition, the ethanol extract from *Bidens pilosa* L. contained almost all groups of active substances such as polyphenols, flavonoids, alkaloids, tannins, and glycosides, but did not have saponins. Total polyphenols and total flavonoids were also determined to be 107.49 ± 4.04 mg GAE/g and 165.63 ± 2.90 mg QE/g, respectively. Regarding the antimicrobial activity, the extract showed the ability to inhibit *A. hydrophila*, *A. dhakensis*, *E. ictaluri* and well inhibit *S. agalactiae*; resistant to *Colletotrichum* sp. better than *Fusarium oxysporum*. The result has provided more scientific basis and medicinal value of *Bidens pilosa* L. and the potential application of this plant in the development of functional foods with antioxidant effects; or using *Bidens pilosa* L. extract as an alternative antibiotic therapy in the prevention and treatment of diseases in aquatic animals and plants caused by microorganisms.

Conflict of interest

The authors declare that they have no conflicts of interest.

Author contributions

Conceptualization, TTM and NTT; methodology, TTM and NHS; formal analysis, TTM and NTT; investigation, TTM, NHS and NTT; resources, TTM and NTT; supervision, TTM and NTT; writing, review and editing, TTM, NHS, and NTT. All authors have read and agreed to the published version of the manuscript.

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