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(RESEARCH ARTICLE)

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Antibacterial activity of methanol extract from *Wedelia biflora* (L.) DC. growing in the Can Gio Mangrove Biosphere Reserve, Vietnam

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Abstract

The study investigated antibacterial activity of methanol extract of *Wedelia biflora* (L.) DC. (*Wollastonia biflora* or *Melanthera biflora*) growing in the Can Gio Mangrove Biosphere Reserve, Vietnam by using agar well diffusion method. Antimicrobial activity strength of *Wedelia biflora* (L.) DC. was depended on diameter of antibacterial zone causing by the methanol extract. The findings showed that methanol extract of whole plants, *Wedelia biflora* (L.) DC. exhibited antibacterial activity against Gram (+) bacteria: *Bacillus cereus* and *Staphylococcus aureus* but negative effects on Gram (-) bacteria: *Pseudomonas aeruginosa* and *Escherichia coli*. Its antibacterial activity increased with increase in extract concentration. Only extract with concentration of 1000 mg mL^{-1} had moderately antibacterial strength against *B. cereus* but the extract with other tested concentrations had antibacterial activity at weak level according to Manuanza's rating scale of antibacterial zones.

Keywords: Antibacterial activity; Antibacterial zone; *Wedelia biflora* (L.) DC.; Methanol extract

1. Introduction

The genus Wedelia consisting of approximately 65 – 70 species is widely distributed over the world specifically in tropical and warm temperature regions [1]. Traditionally, its species are used as therapeutic remedies to various diseases. In the Caribbean and Central America, the aerial parts of *Wedelia trilobata* have been used against bronchitis, colds, abdominal pains, dysmenorrhea and fertility enhancer [2]. In the Miskito Indians of eastern Nicaragua, leaves are applied to treat kidney dysfunction, cold, stingray wounds, snakebite, purge and amenorrhea. In the Tamil Nadu, India leaves of *Wedelia biflora* (Linn.) D.C. (*Wollastonia biflora* or *Melanthera biflora*) are employed to cure wounds, ulcers, sore throat, varicose of veins, skin diseases, headache and stomach ache. The root and leave decoction are used to cure stomach aches. The leaves are also credited with diuretic properties [3]. Some cultures, *W. trilobata* are applied to deal with the common cold, hepatitis, infections in Hong Kong; against reproductive problems, amenorrhea, and dysmenorrheal in Trinidad and Tobago [4]. The antimicrobial activity of *Wedelia* species was reported. The essential oils of *Wedelia biflora* showed antibacterial and antifungal property [5]. The essential oil of *Wedelia chinensis* and *Wedelia trilobata* inhibited against tested bacteria and fungi. The oil of *Wedelia trilobata* had been effective against Gram positive and active against all the tested fungi [6]. The growth of test organisms, *B. subtilis* and *C. albicans* was inhibited by ethanol extract of *Wedelia biflora* [7]. Ethanol and hexane extracts of *Wedelia chinensis* leaves inhibited both Gram-positive bacteria: *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus* and Gram-negative bacteria: *Salmonella typhimurium, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli* and fungi: *Aspergillus niger, Aspergillus flavus, Candida albicans, Alternaria alternata* [8].

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In Vietnam, Wedelia are widely dispersed and consist of five species: *Wedelia prostrata*, *Wedelia biflora* (L.) DC., *Wedelia wallichii* Less, *Wedelia urticaefolia* (Blume) DC. ex Wight, and *Wedelia chinensis* (Osbeck) Mer [9]. All five species are used to treat certain diseases/symptoms in traditional medicine, among them, *Wedelia biflora* (L.) DC. is applied as a therapeutic remedy to treat malaria, hematuria, difficulty urinating, hives, boils, ulcers, insect caused bites [4, 9]. However, antibacterial potential of *Wedelia biflora* (L.) DC. have been limitedly screened. Therefore, the present research was conducted to investigate antibacterial activity of methanol extract from *Wedelia biflora* (L.) DC. collected in the Can Gio Mangrove Biosphere Reserve, Vietnam and clarify better its importance in field of medicine.

2. Material and methods

2.1. Preparation of *Wedelia biflora* (L.) DC.

Wedelia biflora (L.) DC. growing at the Can Gio Mangrove Biosphere Reserve, Vietnam were collected as whole plants containing roots, stems and leaves. The samples were washed with water for sand and dust removal, and dried at room condition for a couple of days. The plants were oven-dried at 50 °C to constant mass. Finally, the dried plants were grounded into powder stored for later uses [10].

2.2. Preparation of methanol extract of *Wedelia biflora* (L.) DC.

Each thirty grams of the powder of *Wedelia biflora* (L.) DC. were soaked in 300 mL of methanol 96° (1:10 (w/v) for 48 hours. The filtrates were collected by filtration with Whatman filter paper No. 4. The residues were furthers oaked twice in methanol with the same ratio of the plant powder to solvent (w/v) [11]. The combining filtrate was left to dry at room condition, then concentrated using a rotary evaporator (Rotary Evaporator RE301, Yamato Scientific), termed as methanol extract from *Wedelia biflora* (L.) DC. and then stored for further uses.

2.3. Preparation of bacterial suspension

Tested bacteria were: two Gram (+) bacteria: *Bacillus cereus, Staphylococcus aureus* and two Gram (-) bacteria*: Pseudomonas aeruginosa*, *Escherichia coli.* The bacteria were grown in liquid nutrient agar media and shook at 37 °C, 120 rpm. After 24 hours of culture, the suspension of each bacterium was used for later experiments [12].

2.4. Study on antibacterial activity of methanol extract of *Wedelia biflora* (L.) DC.

Antibacterial potential of the methanol extract of *Wedelia biflora* (L.) DC. plants was determined by agar well diffusion method [13]. The sterilized solid nutrient agar (NA) medium was used to test antibacterial potential of the methanol extract. Bacterial suspension of each bacterium was spread on surface of each petri dish consisting of 0.2 cm of NA thick. Four wells on each medium plate were created with a sterile round glass pipe (Φ = 5 mm). 20 µL of the methanol extract with concentrations of 200 mg mL^{-1}, 400 mg mL^{-1}, 600 mg mL^{-1}, 800 mg mL^{-1} and 1000 mg mL^{-1}, 20 µL of methanol 70° and 10 µL of aqueous 1.0 mg mL^{-1} solution of tetracycline or gentamicin were applied in wells of each medium plate, randomly. The testing dishes were incubated at 5 °C for a period of three hours and at 37 °C for a period of one - two days [14].

Based on diameter of clear zones inhibiting tested bacteria growth, antibacterial activity strength was determined. The greater the diameter of the clear zone, the higher potential of antibacterial activity of the extract. Degree of bacterial growth inhibition of the extract was evaluated according to rating scale of Manuanza's antibacterial zones [15].

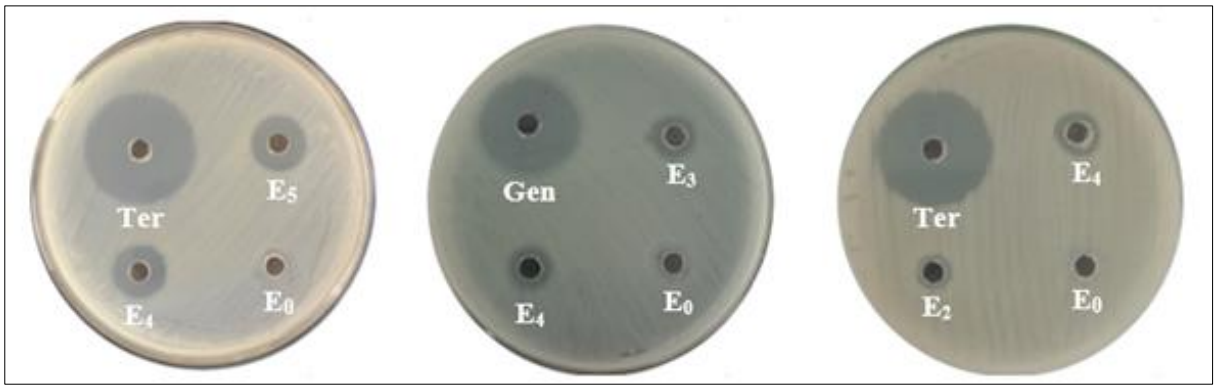
2.5. Experiment design and data analysis

Experiments were arranged in RCRD type with three replicates. Data was statistically analyzed by One-way ANOVA. Mean comparisons were based on Duncan's test at 5% level of confidence with IBM SPSS Statistics software version 20.0.

3. Results and discussion

3.1. Antibacterial effects of the methanol extract of *Wedelia biflora* (L.) DC. on *Bacillus cereus*

The results of screening the antibacterial activity of the methanol extract from *Wedelia biflora* (L.) DC. with different concentrations against *Bacillus cereus* were reported in Table 1 and Figure 1. The methanol extract exhibited antibacterial activity at all tested concentrations. The degree of positive effect of the extract increased with increasing extract concentrations and were weak to moderate.



(E0): negative control. (E1): 200 mg mL-1; (E2): 200 mg mL-1; (E3): 600 mg mL-1; (E4): 800 mg mL-1; (E5): 1000 mg mL-1. (E6): 600 mg mL-1; Gen (Gentamicin) and Tet (Tetracycline): positive controls.

Figure 1 Antibacterial effects of the extract of *W. biflora* (L.) DC. on *B. cereus*

As could be seen from Table 1, the methanol extract from concentrations of 200 mg mL^{-1} (E_1) to 800 mg mL^{-1} (E_5) showed inhibitory activity against *B. cereus* with antibacterial ring diameters arranged from 4.23 ± 1.29 mm to 7.11 ± 1.92 mm. The inhibitory effect of the extract with the tested concentrations was weak to moderate and gradually increased with increasing concentrations of the applied extract. Specifically, the applied extract concentration at 1000 mg mL^{-1} (E_5) produced halo rings with average diameter of 9.84 ± 1.84. Thus, the extract with concentration of 1000 mg mL^{-1} approximately reached moderately antibacterial potential (Figure 1).

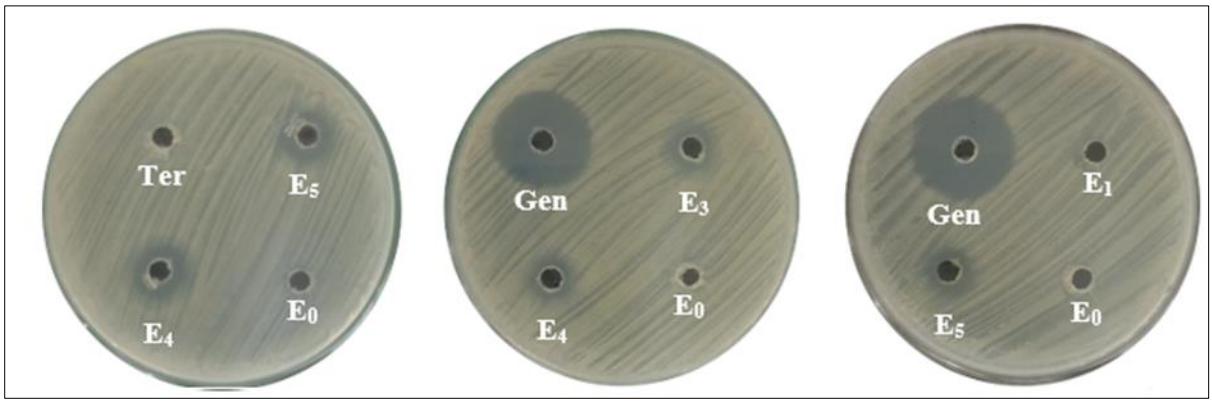
Table 1 Antibacterial effect of the methanol extract of *W. biflora* (L.) DC on *B. cereus*

Extract concentration	Diameter (mm) of bacterial inhibition zone
E_0	0.00^{a}
E_1	4.23^{b} ± 1.29
E_2	4.57^{b} ± 0.94
E_3	5.25^{bc} ± 1.03
E_4	7.11^{c} ± 1.92
E_5	9.84^{d} ± 1.84
Gen	20.83^{e} ± 1.98
Tet	25.57^{f} ± 0.97

(E_0): negative control. (E_1): 200 mg mL^{-1}; (E_2): 200 mg mL^{-1}; (E_3): 600 mg mL^{-1}; (E_4): 800 mg mL^{-1}; (E_5): 1000 mg mL^{-1}. (E_6): 600 mg mL^{-1}; Gen (Gentamicin) and Tet (Tetracycline): positive controls; Values in the same vertical columns followed by one or more of the same letters were not significantly different at the 0.05 significance level according to Duncan's test.

3.2. Antibacterial effect of the methanol extract of *Wedelia biflora* (L.) DC. on *Staphylococcus aureus*

The methanol extract of different concentrations from *Wedelia biflora* (L.) DC exhibited antibacterial activity against *Staphylococcus aureus* (Table 2 and Figure 2). The antibacterial potential of the extract expressed at concentration of 200 mg mL^{-1} (E_1) and increased with rise in the tested extract concentrations. The antibacterial activity against *S. aureus* of the extract with different applied concentrations was just at weak degree.



(E_0): negative control. (E_1): 200 mg mL^{-1}; (E_2): 200 mg mL^{-1}; (E_3): 600 mg mL^{-1}; (E_4): 800 mg mL^{-1}; (E_5): 1000 mg mL^{-1}. (E_6): 600 mg mL^{-1}; Gen (Gentamicin) and Tet (Tetracycline): positive controls.

Figure 2 Antibacterial effects of the extract from *W. biflora* (L.) DC on *S. aureus*

From Table 1, the methanol extract from concentrations of 200 mg mL^{-1} (E_1) to 1000 mg mL^{-1} (E_5) exhibited activity against *Staphylococcus aureus* with the antibacterial ring diameters arranged from 4.55 ± 0.74 mm to 6.9 ± 0.71 mm. The inhibitory effect of the extract gradually increased with increasing concentrations but was at weak level.

Table 2 Antibacterial effect of the methanol extract of *W. biflora* (L.) DC on *S. aureus*

Extract concentration	Diameter (mm) of bacterial inhibition zone
E_0	0.00^a
E_1	0.00^a
E_2	4.55^b ± 0.74
E_3	5.31^b ± 0.50
E_4	5.85^{bc} ± 0.51
E_5	6.9^c ± 0.71
Gen	20.34^d ± 2.02
Tet	0.00^a

(E_0): negative control. (E_1): 200 mg mL^{-1}; (E_2): 200 mg mL^{-1}; (E_3): 600 mg mL^{-1}; (E_4): 800 mg mL^{-1}; (E_5): 1000 mg mL^{-1}. (E_6): 600 mg mL^{-1}; Gen (Gentamicin) and Tet (Tetracycline): positive controls; Values in the same vertical columns followed by one or more of the same letters were not significantly different at the 0.05 significance level according to Duncan's test.

3.3. Antibacterial effect of the methanol extract of *Wedelia biflora* (L.) DC. on *Pseudomonas aeruginosa* and *Escherichia coli*

The methanol extract from *Wedelia biflora* (L.) DC. did not express antibacterial potential against *Pseudomonas aeruginosa* and *Escherichia coli* (Table 3 and Figure 3).

Table 3 Antibacterial effect of the methanol extract of *W. biflora* (L.) DC on *P. aeruginosa* and *E. coli*

Extract concentration	Diameter (mm) of bacterial inhibition zone	
	P. aeruginosa.	***E. coli***
E_0	-	-
E_1	-	-
E_2	-	-
E_3	-	-
E_4	-	-

E_5	-	-
Gen	$14.33^{c} \pm 0.86$	$17.79^{b} \pm 1.05$
Tet	$8.37^{b} \pm 1.14$	$21.85^{c} \pm 1.04$

(E_0): negative control. (E_1): 200 mg mL^{-1}; (E_2): 200 mg mL^{-1}; (E_3): 600 mg mL^{-1}; (E_4): 800 mg mL^{-1}; (E_5): 1000 mg mL^{-1}. (E_6): 600 mg mL^{-1}; Gen (Gentamicin) and Tet (Tetracycline): positive controls; Values in the same vertical columns followed by one or more of the same letters were not significantly different at the 0.05 significance level according to Duncan's test.

(E_0): negative control. (E_1): 200 mg mL^{-1}; (E_2): 200 mg mL^{-1}; (E_3): 600 mg mL^{-1}; (E_4): 800 mg mL^{-1}; (E_5): 1000 mg mL^{-1}. (E_6): 600 mg mL^{-1}; Gen (Gentamicin) and Tet (Tetracycline): positive controls;

Figure 3 Antibacterial effects of some methanol extracts of *W. biflora* (L.) DC. (A) on *P. aeruginosa* and *E. coli* (B)

The results of the present study showed that *W. biflora* (L.) DC. extract had positive effects on Gram (+) bacteria but negative effects on Gram (-) bacteria. For Gram (+) bacteria, the extracts' effects at the same concentration on different bacteria were different. *Bacillus cereus* was more sensitive to the extract than *Staphylococcus aureus*. Besides moderate effect on *B. cereus* at concentration of 1000mg mL^{-1}, the extract at other tested concentrations had weak effects on *B. cereus* and *S. aureus.* A research of Gowri et al., 2014 on antimicrobial activity of the leaf extract of *Wedelia biflora* with different solvents found that chloroform and methanol extracts had greater potential against the growth of the tested bacteria compared to that of hexane, aqueous, and ethyl acetate extracts. *Staphylococcus aureus* was more susceptible than *Bacillus subtilis* [16]. Another investigation on antibacterial activity of methanolic extract of *W. trilobata* flower against *Bacillus cereus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Shigella flexneri* showed that flower extracts exhibited a moderate inhibitory activity against all tested bacterial species [17]. When screened effects of methanol extract of *Wedelia chinensis* Osbeck against Gram (+) (*Bacillus cereus, B. subtilis and Stapylococcus aureus*) and Gram (-) bacteria (*Escherichia coli*, *Proteus rettgeri* and *Pseudomonas aeruginosa*), Darah et al., 2013 found that the leaf extract had higher antibacterial activity against Gram (+) bacteria than Gram (+) bacteria and moderate effects on *Bacillus cereus, B. subtilis* but strong effects on *Stapylococcus aureus* [18].

Antibacterial potential of *W. biflora* methanol extract were related to the present of various antibacterial compounds belonging to terpenes, flavones, flavonols, alkaloids and phenylpropanoids. Interaction of polyphenols with bacterial plasma membranes causes a myriad of effects, perturb plasma membrane functionality and contribute to their antibacterial capacity [19]. The different sensitiveness in antibiotics between Gram (+) and Gram (-) bacteria is because

of different structure in their cell wall. The outer phospholipid membrane of Gram (-) bacteria has lipopolysaccharide components protecting Gram (-) bacteria from antibiotics while Gram-positive bacteria do not have this structure. The Gram (+) bacteria are lack of an outer membrane lipopolysaccharide and therefore, more sensitive to the drugs [20].

4. Conclusion

The methanol extract of *Wedelia biflora (L.)* DC. growing in the Can Gio Mangrove Biosphere Reserve, Vietnam exhibited antimicrobial activity against tested bacteria. 1000 mg mL^{-1} concentrated extract had moderately inhibitory strength on *Bacillus cereus* but the extract with other tested concentrations had weak effects on both *Bacillus cereus* and *Stapylococcus aureus* according to Manuanza's rating scale of antibacterial zones. The extract had negative effects on *Escherichia coli* and *Pseudomonas aeruginosa*.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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