



(RESEARCH ARTICLE)



Antibacterial activity of methanol leaf extract and fruit decoction from *Morinda persicifolia* Buch. – Ham

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Abstract

The study investigated antibacterial potentials of *Morinda persicifolia* Buch. – Ham collected from Lang Sen Wetland Reserve, Long An Province in Vietnam. Antimicrobial activity of *M. persicifolia* Buch. – Ham was evaluated based on size of antibacterial zone of methanol leaf extract by agar well diffusion method, values of minimum inhibitory concentration (MIC) by micro-well dilution method, minimum bactericidal concentration (MBC) by spread plate method and MBC/MIC ratios of fruit decoction against five test bacteria. The results showed that the methanol leaf extract produced growth inhibition zones against test bacteria with diameters of smaller than 6 mm (2.41 mm -5.63 mm). The fruit decoction had MIC of 100 mg/mL for *P. aeruginosa* and 200 mg/mL for *B. cereus*, *B. subtilis*, *S. aureus* and *E. coli*; and MBC about 300 mg/mL to *B. cereus* and *E. coli* and 400 mg/mL to *B. cereus*, *B. subtilis*, *S. aureus* and *P. aeruginosa*. MBC/MIC ratios of the fruit decoction against test bacteria were from 1.5 to 4. Thus, the methanol leaf extract was considered antibacterial at weak level and the fruit decoction was considered bactericidal.

Keyword: Antibacterial Activity; Fruit decoction; Growth inhibition zones; MBC/MIC; Methanol leaf extract; *Morinda persicifolia* Buch – Ham

1. Introduction

Genera *Morinda* belonging to Rubiaceae had 65 species distributed many tropical countries [1]. In Vietnam, *Morinda* had 10 species including *M. citrifolia* L., *M. tinctoria* Roxb and *M. persiaefolia* Buch.–Ham. Two first species have been widely used as herbal medicines for long time in many cultures. Traditionally, *M. citrifolia* L was used as a therapeutic remedy to various diseases such as an antibacterial, antitumor, anthelmintic, analgesic, anti-inflammatory, immunostimulant. Noni was found beneficial in many conditions as gastritis, skin diseases, respiratory infections, menstrual and urinary tract disorders and diabetes [2]. Presently, antimicrobial activity of *M. citrifolia* L. was investigated. The studies showed the plant extracts were against to *E. coli* and *S. typhii* and *S. aureus*. The Noni extract inhibited the growth of some fungi such as *A. brasiliences* and *A. flavus* [3]. Different extracts of *M. tinctoria* fruits revealed antimicrobial activity against the pathogens. Ethanol and methanol extract of mature fruits encumbered *S. typhii* and *K. pneumonia* to grow [4]. Antimicrobial activity of different extracts of *M. tinctoria* was because of presence of various secondary metabolites. *M. persiaefolia* Buch. - Ham was mainly found in the Southern Delta and its extract was used to treat hypertension [1]. However, beyond this application, there did not seem to be any medical research, particularly on antibacterial activity on *M. persiaefolia* Buch. - Ham. Therefore, to understand more about its importance in the field of medicine, the study was conducted to investigate antibacterial potentials of methanol leaf extracts and fruit decoction from *M. persiaefolia* Buch. - Ham collected from Lang Sen Wetland Reserve, Long An Province in Vietnam.

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2. Material and methods

2.1. Preparation of *M. persicifolia* Buch. – Ham leaves and fruits

Leaves and fruits of *M. persicifolia* Buch. – Ham grown at Lang Sen Wetland Reserve, Long An Province in Vietnam were collected, washed with water to remove sand and dust, dried at room temperature for one - two days and then dried by oven at 50 °C until their mass were constant [5]. The dried leaves were then grounded into powder. The leaf powder and intact dried fruits were stored separately for further uses.

2.2. Preparation of methanol extract of *M. persicifolia* Buch. – Ham leaves

Fifty grams of the leaf powder of *M. persicifolia* Buch. – Ham were soaked in 500 mL of methanol 96° (1:10 (w/v) for 48 hours. The mixture was then filtered by using Whatman filter paper No. 4. The residue was re-exacted two times in methanol with the same ratio (w/v) [6]. All combined extracts of leaf powder were let to evaporate at room temperature. The filtrates were stored in screw cap bottles at 4 °C for further uses.

2.3. Preparation of decoction of *M. persicifolia* Buch. – Ham fruits

100 grams of the dried fruit of *M. persicifolia* Buch. – Ham were placed in a lidded ceramic kettle and boiled slowly in 1000 mL of distilled water at 70 – 80 °C for three hours. The kettle was then incubated in incubator at 50 °C until the volume was reduced to 150 mL and then to 100 mL. The decoction of fruit was stored at 4 °C for later uses.

2.4. Preparation of Bacterial suspension

Test bacteria in the investigation were belonged to two groups: Gram (+) and Gram (-) bacteria. The first group included *Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus*. The second group contained *Pseudomonas aeruginosa* and *Escherichia coli*. The test bacterial were cultured in liquid LB media and incubated on a thermostatic shaker at 37 °C, 120 rpm. After 48 hours of culture, the suspension of each bacterium was collected and measured cell density with a spectrophotometer at 600 nm and adjusted to the McFarland standard 0.5 corresponding to 1.5×10^8 CFU/mL [7]. This standard cell density was applied to all later experiments.

2.5. Antibacterial Activity Screening

2.5.1. Antibacterial Activity of methanol leaf extract

The agar well diffusion method was used to survey antibacterial activity of the leaf extract of *M. persicifolia* Buch. – Ham [8]. 10 mL suspension of each bacterial strain with cell density of 10^8 CFU/mL was added to a 190 mL conical flask containing sterilized warm LB medium (40 - 50 °C) to produce bacterial cell density of 5×10^6 CFU/mL and mixed well. The mixtures were then poured into petri dishes. Four wells on each medium plate were produced with a sterile round glass pipe ($\Phi = 6$ mm). 20 μ L of methanol leaf extract with each concentration of 200 mg/mL, 400 mg/mL, 600 mg/mL, 800 mg/mL and 1000 mg/mL were applied in each medium plate's wells. Negative controls were medium plate's wells containing 20 μ L of methanol 70° and positive controls were medium plate's wells containing 10 μ L of aqueous 1 mg/mL solution of tetracycline or gentamicin. All petri dishes were placed at 5 °C for three hours to allow for pre-diffusion of the extract into the medium and inoculated at 37 °C for 24 – 48 hours.

The antibacterial activity of extract was evaluated based on diameter of zones of growth inhibition against the test bacteria. The larger the diameter of antibacterial zone, the stronger the extract and vice versa. Evaluating the degree of bacterial inhibition of the extract by using the rating scale of Manuanza's antibacterial zone [9].

2.5.2. Antibacterial activity evaluation of fruit decoction

The assays were conducted to determine the lowest tested concentration (MIC) of the extract at which bacteria's growth was inhibited and the minimum bactericidal concentration (MBC) of the extract at which bacteria was killed [10]. MIC of the decoction was determined based on the color change of the resazurin solution from blue to pink because of bacterial growth (micro-well dilution method) [11]. The lowest concentration of the decoction in the tested concentration range that did not change the blue color of the resazurin solution was the MIC value (mg/mL) of the decoction. The decoction was filtered through a bacterial filter. The sterile decoction was then diluted with liquid LB in sterile Eppendorf to obtain a sequential concentration range from 0 mg/mL to 1000 mg/mL with each interval of 100 mg/mL (labeled from E₀ to E₁₀). The standard bacteria suspension was added to these Eppendorf so that bacterial density of the mixture was of 5×10^6 CFU/mL. 200 μ L of each mixture was applied into each well of microtiter plate with 3 replicates. Plates were then incubated at 37 °C for 24 h. After 24 hours of incubation, sterile resazurin 0.1% were

added into all well (20 μ L per well) and further incubated for 20 – 30 minutes for observation of colour change. Sterile resazurin 0.1% was prepared by adding 0.1 g resazurin in 99 mL DPBS (Dulbecco's Phosphate Buffered Saline) solution containing (CaCl₂: 0.1 g/L; KCl: 0.2 g/L; MgCl₂.6H₂O: 0.1 g/L; NaCl: 8 g/L; Na₂HPO₄: 2.16 g/L) [12].

MBC of the extract was determined by spread plate method [13]. 10 μ L of test solution of wells that did not change colour of resazurin solution was spread on Nutrient agar media (extract yeast: 3 g/L; peptone: 5 g/L; agar: 15 g/L). All plates were incubated at 37 °C for 24 hours. The MBC value was the lowest concentration in the concentration range of the extract that could kill all bacteria in the well (colonies did not appear on the culturing medium).

2.6. Experiment design and data analysis

The experiments were arranged in RCRD type with three replicates. Data for quantitative experiments was analyzed by using One-way ANOVA and comparisons of means were carried out based on Duncan's test at 5% level of confidence with the support of IBM SPSS Statistics software version 20.0.

3. Results and discussion

3.1. Antibacterial activity of methanol leaf extract

Effects of antibacterial activity of methanol extract of *M. persicifolia* Buch. – Ham leave on four test bacteria were represented in Table 1 and Figure 1. At concentration of 200 mg/mL, the methanol extract showed inhibition of tested bacteria's growth except *S. aureus*. For Gram (+) bacteria, the lowest concentration of the extract affected growth of *S. aureus* with diameter of bacterial inhibition zone about 2.41 mm. Although at higher concentrations, the extract produced larger rings of bacterial inhibition up to 3.63 mm in diameter at extract 1000 mg/mL, the results were not statistically different. Similar trends occurred to *B. cereus* and *B. subtilis*, greater concentration of the extract caused increases in sizes of antibacterial zones. However, those rises in antibacterial effects of the extract were not significantly different at the 0.05 significance level. For Gram (-) bacteria, *P. saeruginosa*, extract concentration of 200 mg/mL created an antibacterial ring with diameter of 2.8 mm. When applied extract concentration were increased, diameters of antibacterial rings became larger. At extract concentration of 1000 mg/mL, size of antibacterial zone was 1.79 times greater than that at extract concentration of 200 mg/mL. Effects of bacteria's growth inhibition were increased together with increases in applied extract concentration.

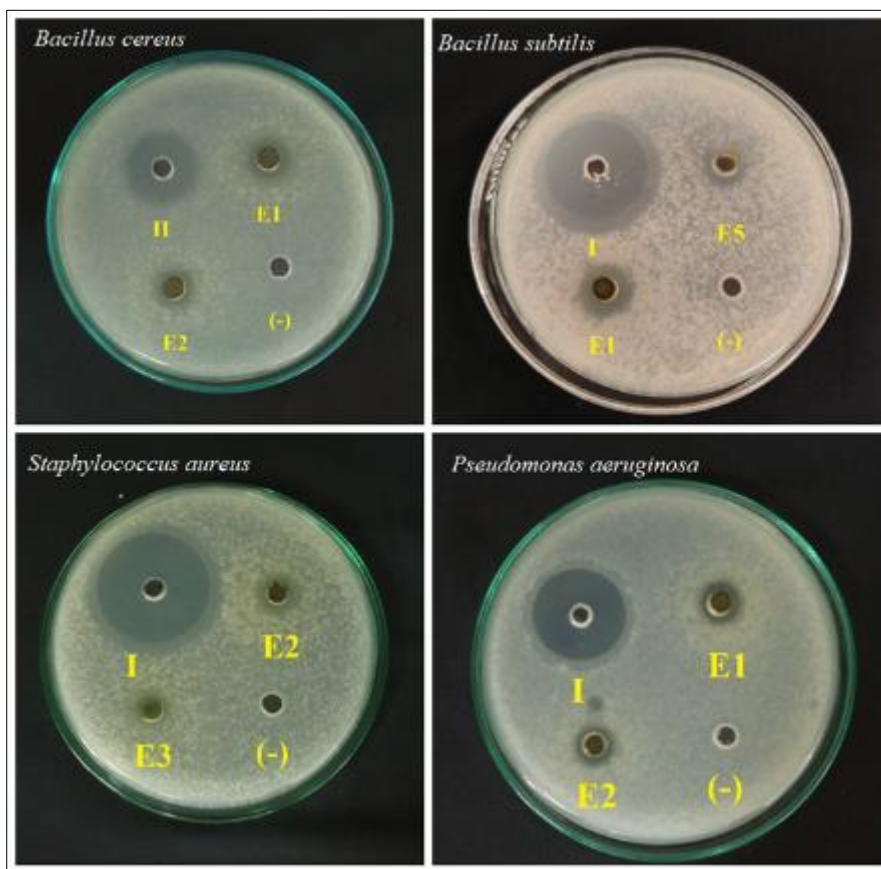
Table 1 Antibacterial effects of methanol leaf extract on tested bacteria

Methanol extract/Antibiotics	Diameter of antibacterial zones (mm)			
	Gram (+) bacteria			Gram (-) bacterium
	<i>B. cereus</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>P. saeruginosa</i>
E1	5.63 ^a ± 0.54	5.25 ^b ± 1.76	3.63 ^a ± 0.96	5.02 ^c ± 0.25
E2	5.01 ^a ± 0.43	3.81 ^{ab} ± 0.21	2.93 ^a ± 0.63	4.13 ^{bc} ± 0.68
E3	4.95 ^a ± 0.42	3.74 ^{ab} ± 0.37	2.68 ^a ± 0.79	3.78 ^{ab} ± 0.25
E4	4.64 ^a ± 0.69	3.70 ^{ab} ± 0.46	2.41 ^a ± 0.51	3.65 ^{ab} ± 0.40
E5	4.16 ^a ± 0.48	2.74 ^a ± 0.31	-	2.80 ^a ± 0.70
E0	-	-	-	-
(I) Gentamicin	18.20 ^c ± 1.41	23.45 ^d ± 0.75	26.48 ^c ± 1.11	18.60 ^e ± 1.08
(II) Tetracycline	15.52 ^b ± 2.18	15.46 ^c ± 1.37	10.72 ^b ± 1.96	15.41 ^d ± 0.88

(E0): negative control, (E1): 1000 mg/mL; (E2): 800 mg/mL; (E3): 600 mg/mL; (E4): 400 mg/mL; (E5): 200 mg/mL, gentamicin and tetracycline: positive control; Values in the same vertical column followed by one or more of the same letters were not significantly different at the 0.05 significance level according to Duncan's test.

According to Manuanza's rating scale of s antibacterial zone, the degree of bacterial inhibition of the extract were evaluated as strong activity with diameter of antibacterial zone equal or greater than 15 mm; moderate activity with diameter of antibacterial zone between 10 mm and 14 mm; and weak activity with diameter of antibacterial zone equal or smaller than 9 mm [9]. Thus, bacterial inhibition activity of the extract was at weak level and was not clear difference

from Gram (+) and (-) bacteria because diameters of antibacterial zones caused by all tested extract concentration were smaller than 6 mm.



(E1): 1000 mg/mL; (E2): 800 mg/mL; (E3): 600 mg/mL; (E5): 200 mg/mL. (-): negative control (I)-Gentamicin and (II)-Tetracycline.

Figure 1 Some results on the halo zones of methanol extracts of *M. persicifolia* Buch. – Ham

Presently, there were some studies on antibacterial activity of *Morinda* methanol leaf extract. When conveyed antibacterial activities of leaf methanol extract of *Morinda elliptica*, Wakawa et al., [3] found that at 500 µg/ml, the extract was moderately active. Mean values of the zone of growth inhibition observed against *E. coli* and *Staphylococcus aureus* was of 10.667 mm and 10.333 mm, respectively. The similar result also reported when Sunder et al., investigated antibacterial activity in solvent extract of different parts of *Morinda citrifolia*. The zone of inhibition produced by leaf methanol extract against *E. coli* and *Staphylococcus aureus* were of 10.31 mm, 11.6 mm, respectively [14]. Moreover, in evaluation of antibacterial activity of *Morinda citrifolia*, *Vitex trifolia* and *Chromolaena odorata*, the methanol, ethanol and ethyl acetate extracts had good inhibitory activity on almost all the test microbes. Among the three solvents tested, methanol extracts showed maximum inhibitory potential against all the tested microorganisms than the others. However, the methanol leaf extract of *Morinda citrifolia* showed zones of growth inhibition against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were just about 8 mm, 7 mm and 9 mm, respectively [15]. Thus, antibacterial activity of some *Morinda* leaf methanol extract was from weak to moderate level and seemed to have no difference from Gram (+) and (-) bacteria.

3.2. Antibacterial activity of fruit decoction

The results of the antibacterial activity test of the fruit decoction were shown in Table 2. The fruit decoction showed antibacterial activity against *B. cereus*, *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*. It had a minimum inhibitory concentration (MIC) of 100 mg/mL for *P. aeruginosa* and 200 mg/mL for *B. cereus*, *B. subtilis*, *S. aureus* and *E. coli*. Thus, antibacterial ability of the fruit decoction on *P. aeruginosa* was higher than that of other bacteria. Minimum bactericidal concentrations (MBCs) of the extract to *B. cereus* and *E. coli* were about 300 mg/mL and to *B. cereus*, *B. subtilis*, *S. aureus* and *P. aeruginosa* were of 400 mg/mL. The MBC/MIC ratio was used to appreciate antibacterial activity. A MBC/MIC ratio was equal or smaller than four, antibacterial effect of the extract was considered as bactericidal, while a MBC/MIC ratio was greater than four, antibacterial effect of the extract was defined as bacteriostatic [16]. From Table 2, the *in vitro* ratios of MBC to MIC of fruit extract to test bacterial were from 1.5 – 2 to *B. cereus*, *B. subtilis*, *S. aureus* and *E. coli*

and four to *P. saeruginosa*. Thus, antibacterial activity of the fruit decoction was considered as bactericidal and was not clear difference from Gram (+) and (-) bacteria.

Table 2 MIC and MBC values and MBC/MIC ratios of fruit decoction of *M. persicifolia* Huch. – Ham on test bacteria

Tested bacteria	MIC values (mg/ml)	MBC values (mg/ml)	MBC/MIC ratios
<i>B. cereus</i>	200	300	1.5
<i>B. subtilis</i>	200	400	2
<i>S. aureus</i>	200	400	2
<i>E. coli</i>	200	300	1.5
<i>P. saeruginosa</i>	100	400	4

MIC and MBC of *Morinda persicifolia* Huch. – Ham extracts have been not studied or less studied but there were researches of MIC and MBC of *Morinda*'s species. MIC values of methanol seed extracts of *Morinda citrifolia* fruits against *S. aureus* 0198, *S. haemolyticus* 562B, *S. haemolyticus* 731B and *S. aureus* 0198 were reported about 16 mg/mL in average [17]. The MIC values of *Morinda citrifolia* Linn against *Staphylococcus aureus* were 35.34 mg/mL for noni fruit ethanol, 117.40 mg/mL for noni fruit methanol, respectively [18]. In a study of Haziz Sina et al. [19] on Minimum inhibitory (MIC) and bactericidal (MBC) concentration of *M. Citrifolia* juices, MIC values were from 2.5 to 5 mg /mL, while MBC values ranged from 10 to 20 mg/mL. Based on MBC/MIC ratios, the reports showed that fresh juices had bacteriostatic effects on *Staphylococcus epidermidis* and *Escherichia coli*.

4. Conclusion

The methanol leaf extract and fruit decoction of *M. persicifolia* Buch. – Ham showed antimicrobial activity against the test bacteria. The extract produced growth inhibition zones against the test bacteria with diameters of 2.41 mm to 5.63 mm. The fruit decoction of *M. persicifolia* Huch. – Ham had MICs of 100 mg/mL for *P. aeruginosa* and 200 mg/mL for *B. cereus*, *B. subtilis*, *S. aureus* and *E. coli*; and MBCs about 300 mg/mL to *B. cereus* and *E. coli* and 400 mg/mL to *B. cereus*, *B. subtilis*, *S. aureus* and *P. aeruginosa*. Its MBC/MIC ratios were from 1.5 – 4. The methanol leaf extract had weak level of antibacterial activity and the fruit decoction was considered bactericidal.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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