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Original Article

Antimicrobial activity of *Elatostesma parasiticum*

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ABSTRACT

This research studied antimicrobial activity of aerial parts of *Elatostema parasiticum* (Blume) Blume ex. H. Schroet. The extract and fractions of *Elatostema parasiticum* were tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, *Aspergillus niger* and *Microsporum gypseum* by using micro broth dilution and bioautography. The extract, n-hexane and ethyl acetate fractions of *Elatostema parasiticum* have antimicrobial activities.

Keywords: *Elatostema parasiticum*, antimicrobial, bioautography, extract, fraction

INTRODUCTION

Elatostema parasiticum (urticaceae) is classified as Magnoliophyta division, Magnoliopside class, Hamamelidae subclass, Urticales order, Urticaceae familly, *Elatostema* genus and species of *Elatostema parasiticum* (Blume) Blume ex. H. Schroet. The plant is used by Siwai and Buin communities, Bougainville islands, Papua New Guinea for fever and fobia disorder [1,2]. Several species of urticaceae have antimicrobial activities, i.e *Laportea aestuans* (Gaud.), *Laportea crenulata* Gaudich, *Debregeasia salicifolia* (D. Don) Rendle, *Urtica pilulifer* L. and *Boehmeria cylindrical* (L) Willd. The active substances of the plants above were essential oils, chalcone, isoflavon, alkaloid, fatty acid and triterpenoid [3,4,5,6,7]. The antimicrobial activities from Indonesian urticaceae extracts (*Cypholophus lutescens* (Blume) Wedd., *Dendrocnide stimulans* (L. f) Chew., *Dendrocnide microstigma* (Gaud. ex Wedd.) Kuntze., *Debregeasia longifolia* (Burm. F.) Wedd., *Elatostema repens* (Lour.) Hallier f., *Elatostema sinuatum* (Blume) Hassk., *Elatostema parasiticum* (Blume) Blume ex. H. Schroet., *Elatostema integrifolium* (D. Don.) Wedd., *Myriocarpa longipes* Liebm., *Pilea repens* (Sw.) Liebm., *Pilea melastomoides* (Poir.) Wedd., *Villebrunea scabra* (Blume) Wedd. and *Villebrunea rubescens* (Blume) Blume) has been carried out. The extract of aerial part of *Elatostema parasiticum* showed the best antimicrobial activity among all extracts [8]. Behalf of that, the purpose of this study was to investigate antimicrobial activity of aerial part of *Elatostema parasiticum.*

MATERIALS AND METHODS

Plant Material: The aerial parts of *Elatostema parasiticum* were collected from Bogor botanic garden. Voucher specimens has been determined at the herbarium of Bogoriense, Bogor. The plant materials were washed, dried and grounded to small pieces.

Preparation of Extract and Fractions: About 500 gr of powder of the dried plant material was macerated with ethanol for 24 hours. Maceration process were repeated for 7 times. The ethanol extract was dried using rotary evaporator, it was obtained 75.49 g of extract. That extract was dissolved in hot water, then filtered through filter paper. The filtrate was fractionated by liquid-liquid extracted with n-hexane and ethyl acetate solvents. All fractions were dried using rotary evaporator. The n-hexane, ethyl acetate and water fraction yields were 0.48 g, 2.45 g and 14.82 g, respectively.

Test Microbes: Test microbes were *Staphylococcus aureus* (American Type Culture Collection ATCC (6538)), *Bacillus subtilis* (ATCC

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6636), *Escherichia coli* (ATCC 8939), *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 10231), *Aspergillus niger* (ATCC 16404), and *Microsporum gypseum.* Those were obtained from Microbiology laboratory collection of Bandung Institute of Technology.

Antimicrobial Activity Test

Determination of the Minimal Inhibitory Concentration (MIC): Antimicrobial activities test of extracts were using by micro dilution broth method based on National Committee for Clinical Laboratory Standard (2012). A series of dilutions of extract were prepared in Mueller Hinton broth (MHB) or Sabouraud dextrose broth (SDB) at final concentrations ranging from 1.95 to 1000 µg/mL. The inocula of microorganisms were prepared from 24 hours cultures and suspensions were adjusted to 0.05 x 0.5 McFarland standard suspensions. The tubes were dispensed into 100 µL with different concentrations of extract and 10 µL inoculum. The control tubes contained only MHB or SDB and inoculums suspension. The positive or reference controls were prepared using tetracycline HCl, and ketoconazole. The inoculated tubes of bacteria were incubated at 37^0C for 24 hours, yeasts at 28^0 C for 48 hours and fungi at 28^0C for 120 hours. The MIC was calculated as no visible growth of tested microorganism appeared, which were expressed in µg/ml. The tests were conducted in triplicate. The least concentration of each extract showing a clear of inhibition was taken as the minimal inhibitory concentration (MIC).

Determination of the Minimal Bactericidal/n Fungicidal Concentration (MBC/MFC): The minimal bactericidal/fungicidal concentration of the plant extract on the clinical bacterial isolates was done according to the method highlighted in National Committee for Clinical Laboratory Standard (2000). Briefly 5µL that was pipetted from the microbe mixture obtained in the determination of MIC stage was streaked out on the nutrient agar/ Sabouraud dextrose agar at 37^0C for 24 hours, yeasts at 28^0C for 48 hours and fungi at 28^0C for 120 hours. The least concentration of the extract with no visible growth was taken as the minimal bactericidal/fungicidal concentration.

Bioautography: 50 µL of extract or fraction (5mg/mL) were applied to pre-coated silica gel F_{254} thin layer chromatography (TLC) plate which developed with toluene : ethyl acetate (7:3 v/v) and dried for complete removal of solvents and placed on inoculated agar surface (0.5 McFarland standard suspensions) for 30 minutes to allow diffusion. The plate was removed and the agar layer was incubated at 37^0C for 24 hours, yeasts at 28^0C for 48 hours and fungi at 28^0C for 120 hours The zones of inhibition growth appear in the places, where the antimicrobial compounds were in contact with the agar layer.

RESULTS

The result of the antimicrobial test are shown in table 1, 2 and 3. Table 1 shows the MIC values of extract, fractions and isolated compound of all tested microbes, table 2 shows the MBC/MFC values of them, while table 3 shows retention factor values of zones of inhibition of bioautography**.**

Table 1: Minimal inhibitory concentration of *Elatostema parasiticum* in microbroth dilution method

No	Sample	Minimal inhibitory concentration (µg/mL) against						
		Sa	Bs	Ec	Pa	Ca	An	Mg
1	Ethanol extract	125	250	125	-	1000	1000	1000
2	N-hexane fraction	62.50	62.50	500	500	1000	-	-
3	Ethyl acetate fraction	500	1000	-	-	-	-	-
4	Water fraction	-	-	-	-	-	-	-
	Tetracycline HCL	0.39	0.09	1.56	12.5			
	Ketoconazole					12.5	0.78	1.56

Sa: ***Staphylococcus aureus*****; Bs:** ***Bacillus subtilis*****; Ec:** ***Escherichia coli*****; Pa:** ***Pseudomonas aeruginosa*****; Ca:** ***Candida albicans*****; An:** ***Aspergillus niger*****; Mg:** ***Microsporum gypseum***

Table 2: Minimal bactericidal/fungicidal concentration of *Elatostema parasiticum*

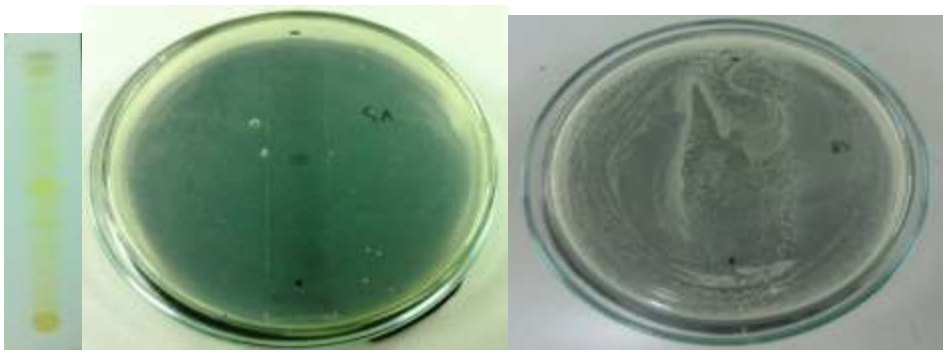
No	Sample	Minimal bactericidal/fungicidal concentration (ppm) against						
		Sa	Bs	Ec	Pa	Ca	An	Mg
1	Ethanol extract	1000	500	>1000	-	>1000	>1000	>1000
2	N-hexane fraction	1000	500	>1000	>1000	>1000	-	-
3	Ethyl acetate fraction	>1000	>1000	-	-	-	-	-
4	Water fraction	-	-	-	-	-	-	-
	Tetracycline HCL	0.39	0.78	1.56	12.5			
	Ketoconazole					12.50	1.56	1.56

Sa: *Staphylococcus aureus*; Bs: *Bacillus subtilis*; Ec: *Escherichia coli*; Pa: *Pseudomonas aeruginosa*; Ca: *Candida albicans*; An: *Aspergillus niger*; Mg: *Microsporum gypseum*

Table 3: Zones of inhibition growth of *Elatostema parasiticum* bioautography

No	Sample	Zones of inhibition growth (retention factor) against						
		Sa	Bs	Ec	Pa	Ca	An	Mg
1	Ethanol extract	0.49	0.49	-	-	-	-	-
2	N-hexane fraction	0.49	0.49	-	-	-	-	-
3	Ethyl acetate fraction	0.49; 0.25	0.49; 0.25	-	-	-	-	-

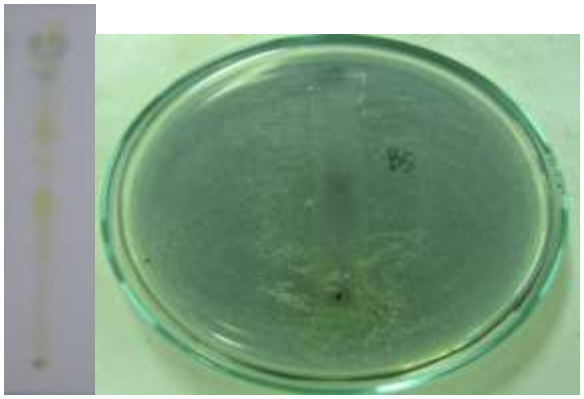
Sa: *Staphylococcus aureus*; Bs: *Bacillus subtilis*; Ec: *Escherichia coli*; Pa: *Pseudomonas aeruginosa*; Ca: *Candida albicans*; An: *Aspergillus niger*; Mg: *Microsporum gypseum*



(a) (b) (c)

Figure 1: The result of bioautography of extract

Explanation: (a) The result of TLC of Elatostema *parasiticum* extract using toluene : ethyl acetate (7:3 v/v) as developer solvent; (b) The result of bioautography of *Elatostema parasiticum* extract againts *Staphylococcus aureus;* (c) The result of bioautography of *Elatostema parasiticum* extract againts *Bacillus subtilis*



(a) (b)

Figure 2: The result of bioautography of ethyl acetate fraction

Explanation: (a) The result of TLC of ethyl acetate fraction of *Elatostema parasiticum* using toluene : ethyl acetate (7:3 v/v) as developer solvent; (b) The result of ethyl acetate fraction of *Elatostema parasiticum* againts *Bacillus subtilis*

DISCUSSION AND CONCLUSION

Ethanol extract inhibited the growth almost all test microbes (except *Pseudomonas aeruginosa*). It contained complex compound, because ethanol is a universal solvent. The MIC values of ethanol extract against *S. aureus* (125 µg/ml), *B. subtilis* (250 µg/ml), *Escherichia coli* (125 µg/ml), *C. albicans* (1000 µg/ml), *A. niger* (1000 µg/ml), and *M. gypseum* (1000 µg/ml) respectively (table 1). Only, the n-hexane fraction inhibited the growth of P. *aeruginosa* (MIC of 500 µg/ml) but it did not inhibit the growth of *A. niger* and *M. gypseum.* The ethyl acetate fraction inhibited the growth of the same bacteria, *S. aureus* and B. *subtilis.* The lowest MIC value was observed in n-hexane fraction. The MIC values of n-hexane fraction of *Elatostema parasiticum* against *S. aureus* and B. *subtilis*, 62.5 µg/ml, each, respectively. Ethyl acetate solvent is more polar than n-hexane. MIC value of ethyl acetate fraction against *S. aureus* dan *B. subtillis* had lower activity than n-hexane fraction, so it assumed that mixed of bioactive compounds decrease the activity. Almost all sample show MBC/MFC value of >1000 µg/ml, except n-hexane and ethyl acetate fractions against *Bacillus subtillis*. All the samples except water fraction inhibited the growth of *S. aureus* and B. *subtilis* by using micro broth dilution method, so the bioautography of water fraction has not been carried out. The results of bioautography showed that extract, n-hexane and ethyl acetate fraction contained the same zones of inhibition growth (0.49 Rf with toluene : ethyl acetate (7:3 v/v) as developer solvent). Another zone of inhibition growth in ethyl acetate fraction had lower value of 0.49 retention factor (Rf), it showed that another bioactive compound in ethyl acetate was more polar than compound with 0.49 Rf. So, the ethanol of extract, n-hexane and ethyl acetate fractions of *Elatostema parasiticum* have antibacterial activity. The compound with 0.49 Rf (with toluene : ethyl acetate (7:3 v/v) as developer solvent) in the n-hexane extract was one of the agent responsible to the antibacterial activity of this plant.

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