Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 4, 2014

Original Article

# ANTIMICROBIAL ACTIVITY FROM INDONESIAN URTICACEAE

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***Received: 25 Jan 2014 Revised and Accepted: 13 Feb 2014***

**ABSTRACT**

**Objective**: This research evaluated antimicrobial activities from Indonesian urticaceae extracts (*Cypholophus lutescens, Dendrocnide stimulans, Dendrocnide microstigma, Debregeasia longifolia, Elatostema repens, Elatostema sinuatum, Elatostema parasiticum, Elatostema integrifolium, Myriocarpa longipes, Pilea repens, Pilea melastomoides, Villebrunea scabra* and *Villebrunea rubescens).*

**Methods**: The extracts were evaluated against *Staphylococcus aureus*, *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli*, *Candida albicans*, *Aspergillus niger* and *Microsporum gypseum* by using agar diffusion assay and micro broth dilution.

**Results**: Almost all the extracts inhibitted the growth of *S. aureus*. The highest inhibitory zone was observed in aerial part extract of *Elatostema parasiticum* against *B. subtilis* (12.2 mm), *S. aureus* (10.7 mm), E, *coli* (10.3 mm) and C. *albicans* (8.5 mm). The MIC values of aerial part extract of *Elatostema parasiticum* 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.

**Conclusion:** The aerial part extract of *Elatostema parasiticum* has antimicrobial activity although this plant has not been used for anti-infective in traditional medicine.

**Keywords**: Urticaceae, Infectious, Extract, antimicrobial

## INTRODUCTION

In recent decades, the search for new anti-microbial agents has occupied many research groups in the field of ethnopharmacology. Many focus on determining the antimicrobial activity of plant extracts found essential oils or isolated compounds. Those compounds were isolated or obtained by bio-guided isolation after previously detecting antimicrobial activity on the part of the plant [1].

Urticaceae is widely distributed throughout the world, from tropical to temperate regions. 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 [2,3,4,5,6].

Indonesian urticaceae has been used as a traditional medicine to treat several infectious diseases such as ulcers, carbuncle, dysentery, urinary infectious and itching. According to Backer *et al*., urticaceae on the Java island consist of 22 genera and 76 species, while according to Heyne, useful urticaceae in Indonesia consist of 13 genera [3,7].

Though there is no enough scientific report about antimicrobial activities of Indonesian urticaceae plants, the purpose of this study was to evaluate thirteen urticaceae plants as new potential sources of antimicrobial activities.

## MATERIALS AND METHODS

### Plant Material

Thirteen plants of Indonesian urticaceae were collected from several place in Indonesia. *Cypholophus lutescens* (Blume) Wedd., *Debregeasia longifolia* (Burm. F.) Wedd., *Myriocarpa longipes* Liebm. from Ranca Upas, Ciwidey. *Pilea melastomoides* (Poir.) Wedd. from Manoko garden, Lembang. The others were from Bogor botanic garden. Part of the plant collecting based on empirical usages. Voucher specimens has been deposited and determined at the herbarium of Bandungense the school of life science and technology, Institut Teknologi Bandung. The plant materials were washed, dried and grounded to small pieces.

### Preparation of Extracts

About 100 gr of powder of the dried plant materials were macerated with ethanol for 24 hours. Maceration process were repeated for 7 times. The ethanolic extracts were dried using rotary evaporator.

### Test Microbes

Test microbes were *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6636), *Escherichia coli* (American Type Culture Collection (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 Institut Teknologi Bandung.

### Antimicrobial Activity Test

Determination of antimicrobial activity was done using Disc Diffusion following the method described by National Committee for Clinical Laboratory Standard (2002). The test microbes was transfered aseptically with an inoculating loop to a test tube containing 5 mL of nutrient broth or Sabouraud dextrose broth. Sufficient inocula were added until the turbidity equal to 0.5 McFarland ($10^8$ cfu/mL) standards The test tube suspension (1 mL) was added to 15–20 mL of nutrient agar or Sabouraud dextrose agar before setting aside the seeded agar plate (9 cm in diameter) to solidify for 15 minutes. Disks of Whatman's No.1 filter paper, 6 mm in diameter, were used to screen the antimicrobial activity. Each sterile disk was impregnated with 20 $\mu$L of extract (corresponding to 200 $\mu$g crude extract/mL), tetracycline HCl, ketoconazol (20 $\mu$g/mL, as positive control for bacteria, yeast and fungi, respectively), or 5 % DMSO (v/v) (as negative control), before it was placed on the surface of the seeded plates. The plates of bacteria were incubated at $37^{o}$ C for 24 hours, yeasts at $28^{0}$ C for 48 hours and fungi at $28^{0}$ C for 120 hours then examined for zones of growth inhibition. The tests were conducted in triplicate.

### 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 0.5 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 ketoconazol. The inoculated tubes of bacteria were incubated at 37° C for 24 hours, yeasts at 28° C for 48 hours and fungi at 28° C 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 MIC.

**Determination of the Minimal Bactericidal/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° C for 24 hours, yeasts at 28° C for 48 hours and fungi at 28° C for 120 hours. The least concentration of the extract with no visible growth was taken as the minimal bactericidal/fungicidal concentration.

**RESULTS AND DISCUSSION**

The result of the antimicrobial test are shown in table 1, 2 and 3. Table 1 shows results of inhibition zones in the agar diffusion assay. Table 2 shows the MIC values of plant extracts of all tested microbes, while table 3 shows the MBC/MFC values of them. Almost all the extracts except *Dendrocnide microstigma, Debregeasia longifolia, Myriocarpa longipes, Pilea repens* and *Pilea melastomoides* showed zone of inhibition against *S. aureus*. The highest inhibitory zone was observed in aerial part extract of *Elatostema parasiticum* against *B. subtilis* (12.2 mm), *S. aureus* (10.7 mm), E, *coli* (10.3 mm) and C. *albicans* (8.5 mm). From the table 1, none of extracts inhibited the growth of. *P. aeruginosa, M. gypseum* and *A. niger* by using agar diffusion assay, but some inhibited the growth of them by using micro dilution broth (table 2). The extract of leaves and bark of *Villebrunea rubescens* inhibited the growth of *P. aeruginosa* (MIC: 1000 and 500 µg/ml). The extract of *Elatostema repens, Elatostema sinuatum, Elatostema parasiticum, Elatostema integrifolium,* and *Villebrunea rubescens* (leaves and bark) inhibited the growth of *A. niger* (with the same MIC of 1000 µg/ml) and *M. gypseum* (1000 µg/ml and 250 µg/ml for *V. rubescens* leaves). All the extract except *Myriocarpa longipes* inhibited the growth of *S. aureus* by using micro broth dilution, but the extract have not inhibitted the growth of that bacteria by using agar diffusion assay (table 1).

**Table 1: Results of the antimicrobial tests of the investigated plants in agar diffusion assay**

| No | Sample | Inhibitory zone (mm) against | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Sa | Bs | Ec | Pa | Ca | An | Mg |
| 1 | *Cypholophus lutescens* (l) | 9.7 | - | - | - | - | - | - |
| 2 | *Dendrocnide stimulans*. (l) | 8.8 | - | - | - | - | - | - |
| 3 | *Dendrocnide microstigma* (l) | - | - | - | - | - | - | - |
| 4 | *Debregeasia longifolia* (l) | - | - | - | - | - | - | - |
| 5 | *Elatostema repens* (ap) | 8.4 | - | - | - | - | - | - |
| 6 | *Elatostema sinuatum* (ap) | 8.8 | - | - | - | - | - | - |
| 7 | *Elatostema parasiticum* (ap) | 10.7 | 12.2 | 10.3 | - | 8.5 | - | - |
| 8 | *Elatostema integrifolium* (ap) | 8.5 | - | - | - | - | - | - |
| 9 | *Myriocarpa longipes* Liebm. (l) | - | - | - | - | - | - | - |
| 10 | *Pilea repens* (ap) | - | - | - | - | - | - | - |
| 11 | *Pilea melastomoides* ( (ap) | - | - | - | - | - | - | - |
| 12 | *Villebrunea scabra* (l) | 8.8 | - | - | - | - | - | - |
| 13 | *Villebrunea scabra* (b) | 8.7 | - | - | - | - | - | - |
| 14 | *Villebrunea rubescens* (l) | 8.7 | - | - | - | - | - | - |
| 15 | *Villebrunea rubescens* (b) | 8.5 | - | - | - | - | - | - |
| | Tetrasiklin HCL | 21.6 | 18.1 | 17.6 | 10.2 | 15.5 | 15.9 | 17.6 |
| | Ketokonazol | | | | | | | |

(l): leaves; (ae): aerial part; (b): bark; Sa: *Staphylococcus aureus*; Bs: *Bacillus subtilis*; Ec: *Escherichia coli*; Pa: *Pseudomonas aeruginosa*; Ca: *Candida albicans*; An: *Aspergillus niger*; Mg: *Microsporum gypseum*

**Table 2: Minimal inhibitory concentration of the investigated plants in microbroth dilution method**

| No | Sample | Minimal inhibitory concentration (µg/mL) against | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Sa | Bs | Ec | Pa | Ca | An | Mg |
| 1 | *Cypholophus lutescens* (l) | 125 | - | - | - | - | - | - |
| 2 | *Dendrocnide stimulans* (l) | 125 | - | - | - | - | - | - |
| 3 | *Dendrocnide microstigma* (l) | 125 | - | - | - | - | - | - |
| 4 | *Debregeasia longifolia* (l) | 125 | - | - | - | - | - | - |
| 5 | *Elatostema repens* (ap) | 250 | 500 | - | - | - | 1000 | 1000 |
| 6 | *Elatostema sinuatum* (ap) | 250 | - | - | - | - | 1000 | 1000 |
| 7 | *Elatostema parasiticum* (ap) | 125 | 250 | 125 | - | 1000 | 1000 | 1000 |
| 8 | *Elatostema integrifolium* (ap) | 250 | - | - | - | - | - | - |
| 9 | *Myriocarpa longipes* (l) | - | - | - | - | - | 1000 | 1000 |
| 10 | *Pilea repens* (ap) | 1000 | - | - | - | - | - | - |
| 11 | *Pilea melastomoides* (ap) | 500 | - | - | - | - | - | - |
| 12 | *Villebrunea scabra* (l) | 125 | - | - | - | - | - | - |
| 13 | *Villebrunea scabra* (b) | 125 | - | - | - | - | - | - |
| 14 | *Villebrunea rubescens* (l) | 125 | - | - | 500 | - | - | - |
| 15 | *Villebrunea rubescens* (b) | 125 | - | - | 1000 | - | 1000 | 250 |
| | Tetrasiklin HCL | 0.4 | 0.1 | 1.6 | 12.5 | - | 1000 | 1000 |
| | Ketokonazol | | | | | 12.5 | 0.8 | 1.6 |

(l): leaves; (ae): aerial part; (b): bark; Sa: *Staphylococcus aureus*; Bs: *Bacillus subtilis*; Ec : *Escherichia coli*; Pa: *Pseudomonas aeruginosa*; Ca: *Candida albicans*; An: *Aspergillus niger*; Mg: *Microsporum gypseum*

Almost all the extracts showed MBC/MFC value of >1000 µg/ml against all the microbes. The extract of *Dendrocnide stimulans, Dendrocnide microstigma, Elatostema parasiticum* and leaves of *Villebrunea rubescens* showed the same MBC value of 1000 µg/ml against *S. aureus.* The extract of *Elatostema parasiticum* showed the lowest MBC value of 500 µg/ml against *B. subtilis.*The extract of the aerial part of *Elatostema parasiticum* showed the best antimicrobial activity among all extracts, by using diffusion assay and micro broth dilution. The MIC values of aerial part extract of *Elatostema parasiticum* 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. Only the extract of *Elatostema parasiticum* inhibited the growth of *B. subtilis, E. coli*, and *C. albicans* by using agar diffusion assay. Only that extract inhibited the growth of *Escherichia coli* and *Candida albicans* by using micro broth dilution

From 16 samples, there were 3 samples (*Dendrocnide stimulans, Elatostema repens*, *Villebrunea rubescens*) that were used in traditional medicines for infectious diseases, but those extract only have antimicrobial activity againts *Staphylococcus aureus* by using both methods. While, the extract of the aerial part of *Elatostema parasiticum* (Blume) Blume ex. H. Schroet was the best antimicrobial activity among all extracts.The extract of *Elatostema parasiticum* (Blume) Blume ex. H. Schroet was promising to be developed for antibacterial compounds, especially in clinical bacterial (i.e *Staphylococcus aureus* and *Escherichia coli*) and there is no scientific report about antimicrobial activity of that plant.

**Table 3: Minimal bactericidal/fungicidal concentration of the investigated plants**

| No | Sample | Minimal bactericidal/fungicidal concentration (ppm) against | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Sa | Bs | Ec | Pa | Ca | An | Mg |
| 1 | *Cypholophus lutescens* (l) | >1000 | - | - | - | - | - | - |
| 2 | *Dendrocnide stimulans* (l) | 1000 | - | - | - | - | - | - |
| 3 | *Dendrocnide microstigma* (l) | 1000 | - | - | - | - | - | - |
| 4 | *Debregeasia longifolia* (l) | >1000 | - | - | - | - | - | - |
| 5 | *Elatostema repens* (ap) | >1000 | >1000 | - | - | - | >1000 | >1000 |
| 6 | *Elatostema sinuatum* (ap) | >1000 | - | - | - | - | >1000 | >1000 |
| 7 | *Elatostema parasiticum* (ap) | 1000 | 500 | >1000 | - | >1000 | >1000 | 1000 |
| 8 | *Elatostema integrifolium* (l) | >1000 | - | - | - | - | >1000 | >1000 |
| 9 | *Myriocarpa longipes* (l) | - | - | - | - | - | - | - |
| 10 | *Pilea repens* (ap) | >1000 | - | - | - | - | - | - |
| 11 | *Pilea melastomoides* (ap) | >1000 | - | - | - | - | - | - |
| 13 | *Villebrunea scabra* (l) | >1000 | - | - | - | - | - | - |
| 14 | *Villebrunea scabra* (b) | >1000 | - | - | - | - | - | - |
| 15 | *Villebrunea rubescens* (l) | 1000 | - | - | >1000 | - | >1000 | >1000 |
| 16 | *Villebrunea rubescens* (b) | >1000 | - | - | >1000 | - | >1000 | >1000 |
| | Tetrasiklin HCL | 0.4 | 0.1 | 1.6 | 12.5 | 12.5 | 0.8 | 1.6 |
| | Ketokonazol | | | | | | | |

(l): leaves; (ae): aerial part; (b): bark; Sa: *Staphylococcus aureus*; Bs: *Bacillus subtilis*; Ec : *Escherichia coli*; Pa: *Pseudomonas aeruginosa*; Ca: *Candida albicans*; An: *Aspergillus niger*; Mg: *Microsporum gypseum*

## CONCLUSION

The ethanolic extract of the aerial part of *Elatostema parasiticum* has antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli*, *Candida albicans*, *Aspergillus niger*, and *Microsporum gypseum*, although this plant has not been used for anti-infective in traditional medicine.

## ACKNOWLEDGEMENT

The reference control of this study (ketoconazol and tetracycline HCL) was supported by Sanbe Farma Industry, Cimahi, Indonesia.

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