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Research Article

Antimicrobial Profile of *Premna pubescens*. Blume and *Centella asiatica* Extracts Against Bacteria and Fungi Pathogens

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Abstract

Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge on medicinal plants used for preventive and curative medicine *Premna pubescens*. Blume (Buasbuas) has been used to increase the body immunity and endurance. *Centella asiatica* (Pegagan) is used for medicinal purposes. This study is important to find out the antimicrobial capabilities of *Premna pubescens* (*P. pubescens*) and *Centella asiatica* (*C. asiatica*) methanol extracts. This study is expected to provide the scientific foundation for the development of plants that are traditionally believed to be efficacious drug. The aim of the study was to investigate *in vitro* antimicrobial activity of North Sumatera medicinal plants *P. pubescens* and *C. asiatica* against the main pathogens. **Materials and Methods:** The organic solvent plant extracts are tested on the various microorganisms including bacteria and fungi by using agar diffusion technique. The data was analyzed with ANOVA statistics by using SPSS software. **Results:** The length of the inhibition zone was measured in mm from the edge of the well to the inhibition zone. *P. pubescens* showed significant moderate activity against (14 mm) *Pseudomonas marginalis* and (21 mm) *Streptococcus mutans* with 100 mg mL⁻¹ DMSO plant drug concentration. The results of lowest (MICs) values are at 66 and highest ones are at 152 mg mL⁻¹ for *P. pubescens* meanwhile those of (MICs) values are 0-155 mg mL⁻¹ for *C. asiatica*. **Conclusion:** In general, based on the result of this research, it can be said that *P. pubescens* and *C. asiatica* plants can be used as antibacterial and antifungal compounds.

Key words: *Premna pubescens*. Blume, *Centella asiatica*, antibacterial, antifungal, minimal inhibitory concentration

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The number of medicinal plants is nearly 20,000, this data was according to a study conducted by the World Health Organization (WHO) based on publications on pharmacopoeias and medicinal plants in 91 countries. Nearly 6-7 thousand species of medicinal plants from around about 17-18 thousand flowering plants are known as traditional medicine and officially recognized as medicine system in Indonesia, i.e., North Sumatra, Borneo, Celebes and Papua^{1,2}.

North Sumatra is very rich in plants that are believed to have medicinal properties for either prevention or treatment³. Since ancient civilization, the various parts of different plants were used to eliminate pain, control suffering and counteract disease. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs⁴. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytochemicals living in the plants^{5,6}. Much work has been done on ethnomedicinal plants in North Sumatra Indonesia^{3,6,2,7}. Medicinal plants represent a rich source of antimicrobial agents^{6,8,9,10}. Scientists have recently paid more attention to extracts and biologically active compounds isolated from plant species are used in herbal medicines, because they want to avoid from the antibiotics side effect, i.e., pathogenic microbe resistance^{9,11}.

Premna pubescens. Blume (Family Lamiaceae) is a shrub or tree up to 7-10 m height. It is known by various names like bebuas and buasbuas in Indonesia¹². In North Sumatra Indonesian traditionally medicinal system, it has been used to increase the body endurance, treat the unwell or catching a cold, help for blood coagulation and overcome for warm infection in children, help to increase breast milk^{13,14} and also increase appetite^{14,15}. Compounds derived from the plant have been found contain alkaloid, flavonoid, saponin, steroid, dan fenolik^{5,6}. Antimicrobial activity of *Premna pubescens*. Blume was previously screened⁶.

Centella asiatica (Family Mackinlayaceae) commonly names Pegagan, Asiatic Pennywort, North Sumatra Indonesian Pennywort, Luei Gong Gen, Takip, kohol, Antanan, Pegagan, Pegaga, Vallarai Kula kud, Bai Bua Bok and Brahmi. In North Sumatra Indonesia is well-known as "Pegagan" this term is used to improve the mental ability^{6,7}. Antibacterial activity of *C. asiatica* was previously screened⁸. The aim of the study was to investigate *in vitro* antimicrobial activity of North Sumatra Indonesia medicinal plants *P. pubescens* and *C. asiatica* against the main pathogens. In this study, the results of such studies are reported in order to orient future

investigations towards the finding of potent, less toxic to human health hand, safe antimicrobial agents from natural sources.

MATERIALS AND METHODS

Plant materials and extraction: This research project was conducted from July 2016 to November 2016 in Cell and Molecular Biology Laboratory of Medan State University. The whole plants of *P. pubescens* and *C. asiatica* were collected from Medan State University campus plant collections aged 5-7 years for *P. pubescens* and 5-7 weeks for *C. asiatica* in Medan, North Sumatra, Indonesia. The botanical identification of the collected materials was conducted in herbarium of Bogor botanical garden. The samples were separated and oven dried at 28°C room for 1 week. The samples were grounded into powder form using the grinder. Extraction using Soxhlet apparatus (IWAKI SOXHLET-100 IWAKI soxhlet extractor 100 ML) with 95% (v/v) Methanol PA (Sigma-Aldrich) as solvent for 12 h was performed. The resultant extraction was frozen and dried for 24°C/48 h. Yield of Methanol extracts: 30%^{16,4}.

Test microorganisms: Microbial strains of clinical, plant and aquatic origin i.e. *Aspergillus niger*, *Penicillium expansum*, *Fusarium oxysporum*, *Xanthomonas compestries*, *Lactobacillus acidophilus*, *Pseudomonas marginalis*, *Pseudomonas syringae*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Streptococcus salivarius* and *Staphylococcus aureus* including both fungi and bacteria were procured from Microbial Type Culture Collection (MTCC) Biology Department Medan State University. Active cultures were generated by inoculating a loopful of culture in separate 100 mL nutrient/potato dextrose broths and incubating on a shaker at 37°C overnight. The cells were harvested by centrifuging at 4000 rpm for 5 min, washed with normal saline, spin at 4000 rpm for 5 min again and diluted in normal saline to obtain 5×10^5 cfu mL⁻¹.

Determination of antimicrobial activity: The antimicrobial assay of both plant crude extracts was conducted by using the agar well-diffusion method 20 mL of nutrient agar was dispensed into sterile universal bottles. Then they are inoculated with 0.2 mL of cultures and mixed gently as well as poured into sterile petri dishes. After setting a number 3 cup borer (6 mm diameter) was properly sterilized by flaming and used to make 3-5 uniform cups/wells in each petri dish. A drop of molten nutrient agar was used to seal the base

of each cup. The cups/wells were filled with 50 μL of the extract concentrations of 100, 300 and 500 mg mL^{-1} DMSO and allow diffusion for 45 min. The solvents used to reconstitute the extracts were similarly analyzed. The plates were incubated at 37°C for 24 h for bacteria. The procedure above is also allowed for fungal assays, however media used is not nutrient agar but potato dextrose agar and incubated at 25°C for 48 h. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in duplicates.

Statistical analysis: All data were statistically analyzed with SPSS software (version 16). One-way analysis of variance (ANOVA) was used to study significant difference between means and significance level at $p = 0.05^{16,4}$.

RESULTS

In the study of methanolic extract exhibited different degree of growth inhibition against the tested bacterial and fungal strains. Methanolic extracts of *P. pubescens* and *C. asiatica* exhibited considerable antimicrobial activity against tested microbial strains (Table 1). *Premna pubescens* showed significant moderate activity against *P. marginalis* and *S. mutans* with 100 mg mL^{-1} DMSO medicinal plant concentration. *Centella asiatica* is significant against *P. syringae* and moderate against other pathogens *F. oxysporum*, *L. acidophilus*, *S. salivarius* and *S. aureus* with 100 mg mL^{-1} DMSO.

The results of lowest MICs value are at 66 and highest at 153 mg mL^{-1} for *P. pubescens* meanwhile those of highest ones are at 0,155 mg mL^{-1} for *C. asiatica*. The variation of antimicrobial activity of our extracts might be due to the

distribution of antimicrobial substances which is varied from fraction to fraction of the crude extract. No inhibitions were observed with *P. pubescens* on *P. expansum* and *C. asiatica* on *P. aeruginosa* as well as *S. mutans*.

DISCUSSION

These extracts has proved that it has inhibitory effects on germination and on the viability of fungal spores as well. Both plant extracts showed moderate good activity against *A. niger* as a saprophyte in soil causing black mould of onion, garlic and shallot, stem root of *Dracaena*, root stalk rot of *Sansevieria* and boll rot of cotton, spoilage of cashew kernels, dates, figs, vanilla pods as well as dried prune. The effectiveness of the active compounds in plant extracts causes the production of growth inhibition zones that appear as clear as around the wells. However, plant extract was unable to exhibit antibacterial activity against tested bacterial strains. These bacterial strains may have some kinds of resistance mechanisms e.g. enzymatic inactivation, target sites modification and decrease of intracellular drug accumulation¹⁷ or the concentration of the compound used may not be sufficient.

The adverse effects of *P. pubescens* consumption are reported can cause blisters, lesions and eruptions when taken by patients for the treatment of joint pains and gastrointestinal problems. Due to its toxicity, the latex extracted from the stem has traditionally been used to make poison arrows^{3,10}. Several phytochemicals are identified in different parts. *P. pubescens* flowers contain terpenes, multiflorenol and cyclisadol¹⁸. The latex contains caoutchouc, calotropin, calotoxin, calactin, uscharin, trypsin, voruscharin, uzarigenin, syriogenin and proceroiside¹⁴. Chemical

Table 1: Antimicrobial activity of methanolic extracts *Premna pubescens*. Blume and *Centella asiatica*

Pathogens	<i>Premna pubescens</i> . Blume				<i>Centella asiatica</i>			
	A	B	C	MIC (mg mL^{-1})	A	B	C	MIC (mg mL^{-1})
Fungi								
<i>Aspergillus niger</i>	10	11	13	153	15	18	20	66
<i>Penicillium expansum</i>	11	14	15	101	0	0	0	0
<i>Fusarium oxysporum</i>	12	13	15	105	14	14	15	96
Bacteria (Gram +ive)								
<i>Lactobacillus acidophilus</i>	10	12	14	121	12	13	14	156
<i>Streptococcus mutans</i>	15	20	22	101	0	0	0	0
<i>Streptococcus salivarius</i>	12	14	16	101	14	16	18	128
<i>Staphylococcus aureus</i>	22	26	29	67	10	12	13	148
Bacteria (Gram -ive)								
<i>Pseudomonas marginalis</i>	15	14	16	11	11	16	26	145
<i>Pseudomonas syringae</i>	11	14	17	19	19	21	23	81
<i>Pseudomonas aeruginosa</i>	11	13	15	0	0	0	0	0
<i>Xanthomonas campestris</i>	13	14	17	11	11	12	14	153

Value indicates no activity, Volume per well: 50 μL , Borer size used: 6 mm used plant methanolic extract concentrations (A = 100, B = 300 and C = 500 mg/DMSO mL)
MIC: Minimum inhibitory concentration

constituents of *P. pubescens* flowers are lupeol, uscharin, proceroiside, proceragenin (cardenolide), syriogenin, taraxast-20(30)-en-3-(4-methyl-3-pentenoate), 3-thiazoline cardenolide, gigantol, giganteol, isogiganteol, uscharidin, uzarigenin voruscharin a, calotropeol, 3-epimoretenol, a-lactuceryl acetate and alactuceryl isovalerate¹⁹. Root bark of *P. pubescens* contains triterpenes, a new norditerpenyl ester named as calotropterpenyl ester and two unknown pentacyclic triterpenoids named as calotropursenyl acetate and calotropfriedelenyl acetate, akundarol isovalerate, mundarol isovalerate and quercetin, 3-rutinoside¹⁹. The principal active medicines are asclepin and mudarin²⁰.

No inhibition was observed with control which proves that solvents could not act as antimicrobial agents. In almost all tests, crude methanolic extracts showed better inhibition against all the tested bacterial and fungal strains indicating that active ingredients in plant materials could be extracted into methanol. However, the highest antibacterial activity of *P. pubescens* was observed due to the presence of secondary metabolites such as alkaloids, flavonoids and steroids against *S. aureus*. *Pseudomonas aeruginosa* are which is wide-spread in soil, water and sewage can be considered as an indication of their involvement in the natural process of mineralization of organic matter. It has long been a troublesome cause of secondary infections of wound, especially burns and giving rise to blue green pus. It produces meningitis, when introduced by lumber puncture and urinary tract infection when introduced by catheters and instruments or irrigating solutions²¹. *S. aureus* occur harmlessly as a normal flora of the skin and mucous membrane and it is one of the commonest bacterial pathogens encountered in the community causing severe food poisoning or minor skin infections to severe life threatening infections¹⁹. *C. asiatica* methanol extract having strong inhibition activity against *P. aeruginosa* and *S. aureus* was previously reported²².

CONCLUSION

Premna pubescens and *Centella asiatica* extracts showed antimicrobial activity as anti-bacterial and anti-fungal against tested pathogens including antibiotic resistant strains. Future recommendation: It is necessary to determine the toxicity of the active constituents, their side effects and pharmacokinetic properties.

SIGNIFICANCE STATEMENTS

This research compared the antibacterial and antifungal effects of the ethanol extracts of *Premna pubescens* and

Centella asiatica. *Centella asiatica* ethanol extract is more effective as an antifungal than *P. pubescens* where *C. asiatica* effective inhibits *Aspergillus* and *fusarium* growth but *P. pubescens* is more effective in inhibiting *penicillium* growth. *Premna pubescens* is more effective as an antibacterial than *C. asiatica*. Either Gram-positive or Gram-negative bacteria, *Premna's* efficacy as an antibacterial is much better than *C. asiatica*. As such, it help us in determining the development of bioactive compounds from *C. asiatica* as antifungal and *P. pubescens* as antibacterial.

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