Etnobotany-1

by Diky Diningrat

Submission date: 19-Oct-2018 11:39AM (UTC+0700)
Submission ID: 1022811359
File name: 271-275.pdf (526.5K)
Word count: 3188
Character count: 17829

International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2018.271.275

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

Martina Restuati and Diky Setya Diningrat

Department of Biology, Faculty of Mathematics and Natural Sciences, Medan State University, 20221 Medan, North Sumatera, Indonesia

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

Received: August 09, 2017 Accepted: October 20, 2017 Published: January 15, 2018

Citation: Martina Restuati and Diky Setya Diningrat, 2018. Antimicrobial profile of *Premna pubescens*. Blume and *Centella asiatica* extracts against bacteria and fungi pathogens. Int. J. Pharmacol., 14: 271-275.

Corresponding Author: Diky Setya Diningrat, Department of Biology, Faculty of Mathematics and Natural Sciences, Medan State University, 20221 Medan, North Sumatera, Indonesia Tel: +6181361362400

Competing Interest: The authors have declared that no competing interest exists.

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 treatment[3]. 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 drugs[4]. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents living in the plants[5,6]. Much work has been done on ethnomedicinal plants in North Sumatera 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 Indonesia[12]. In North Sumatera 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 screened[6].

Centella asiatica (Family Mackinlayaceae) commonly names Pegagan, Asiatic Pennywort, North Sumatera Indonesian Pennywort, Luei Gong Gen, Takip, kohol, Antanan, Pegagan, Pegaga, Vallarai Kula kud, Bai Bua Bok and Brahmi. In North Sumatera Indonesia is well-known as "Pegagan" this term is used to improve the mental ability[6,7]. Antibacterial activity of *C. asiatica* was previously screened[8]. The aim of the study was to investigate *in vitro* antimicrobial activity of North Sumatera 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*, *Steptococcus salivarious* 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. salivarious* 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 accumulation[17] 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 cyclisadol[18]. The latex contains caoutchouc, calotropin, calotoxin, calactin, uscharin, trypsin, voruscharin, uzarigenin, syriogenin and proceroside[14]. Chemical

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

	Premna pubescens. Blume				*Centella asiatica*			
Pathogens	A	B	C	MIC (mg mL^{-1})	A	B	C	MIC (mg mL^{-1})
Fungi								
Aspergillus niger	10	11	13	153	15	18	20	66
Penicillium expansum	11	14	15	101	0	0	0	0
Fusarium oxysporum	12	13	15	105	14	14	15	96
Bacteria (Gram +ive)								
Lactobacillus acidophilus	10	12	14	121	12	13	14	156
Streptococcus mutans	15	20	22	101	0	0	0	0
Steptococcus salivarious	12	14	16	101	14	16	18	128
Staphylococcus aureus	22	26	29	67	10	12	13	148
Bacteria (Gram -ive)								
Pseudomonas marginalis	15	14	16	11	11	16	26	145
Pseudomonas syringae	11	14	17	19	19	21	23	81
Pseudomonas aeruginosa	11	13	15	0	0	0	0	0
Xanthomonas compestries	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, proceroside, proceragenin (cardenolide), syriogenin, taraxast-20(30)-en-3-(4-methyl-3-pentenoate), 3-thiazoline cardenolide, gigantin, giganteol, isogiganteol, uscharidin, uzarigenin voruscharin a, calotropeol, 3-epimoretenol, a-lactuceryl acetate and alactuceryl isovalerate[19]. 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-rutinoside[19]. The principal active medicines are asclepin and mudarin[20].

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 solutions[21]. *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 infections[19]. *C. asiatica* methanol extract having strong inhibition activity against *P. aeruginosa* and *S. aureus* was previously reported[22].

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.

ACKNOWLEDGEMENTS

We are thankful for constant encouragement and support from Biology Department, Mathematic and Natural Sciences, BOPTN Grants Funding Medan State University with grant number 178A/UN33.8/KU/2016, Post-doctoral grants from the ministry of research, technology and higher education of Indonesia.

REFERENCES

1. Santajit, S. and N. Indrawattana, 2016. Mechanisms of antimicrobial resistance in ESKAPE pathogens. BioMed Res. Int., Vol. 2016. 10.1155/2016/2475067.
2. Suswardany, D.L., D.W. Sibbritt, S. Supardi, J.F. Pardosi, S. Chang and J. Adams, 2017. A cross-sectional analysis of traditional medicine use for malaria alongside free antimalarial drugs treatment amongst adults in high-risk malaria endemic provinces of Indonesia. PloS One, Vol. 12. 10.1371/journal.pone.0173522.
3. Khairiah, A., Nisyawati and M. Silalahi, 2017. Biodiversity of medicinal plants by Minangkabau ethnic in Guguak Sarai, West Sumatera, Indonesia. AIP Conf. Proc., Vol. 1862. 10.1063/1.4991213.
4. Villalobos, M.D.C., M.J. Serradilla, A. Martin, E. Ordiales, S. Ruiz-Moyano and M.D.G. Cordoba, 2016. Antioxidant and antimicrobial activity of natural phenolic extract from defatted soybean flour by-product for stone fruit postharvest application. J. Sci. Food Agric., 96: 2116-2124.
5. Diningrat, D.S., S.M. Widiyanto, A. Pancoro, A. Iriawati and D. Shim *et al*., 2015. Transcriptome of teak (*Tectona grandis*, L.F) in vegetative to generative stages development. J. Plant Sci., 10: 1-14.
6. Restuati, M., U. Hidayat, A.S.S. Pulungan, N. Pratiwi and D.S. Diningrat, 2016. Antibacterial activity of buasbuas (*Premna pubescens* Blume) leaf extracts against *Bacillus cereus* and *Escherichia coli*. J. Plant Sci., 11: 81-85.
7. Diningrat, D.S., S.M. Widiyanto, A. Pancoro, Iriawati and D. Shim *et al*., 2015. Identification of Terminal Flowering1 (TFL1) genes associated with the teak (*Tectona grandis*) floral development regulation using RNA-seq. Res. J. Bot., 10: 1-13.

8. Lin, J., H. Jiang and X. Ding, 2017. Synergistic combinations of five single drugs from Centella asiatica for neuronal differentiation. NeuroReport, 28: 23-27.
9. Manaf, S.R., M.D. Hassan, M.M. Noordin, A.A. Razak and R.H. Hayati *et al*., 2017. The effects of dietary supplementation of methanolic extracts of herbal medicine on haematological variable of red hybrid tilapia (*Oreochromis* sp.). Proceedings of the International Seminar on Livestock Production and Veterinary Technology, August 31, 2017, Indonesia, pp: 540-548.
10. Micheloud, J.F., L.A. Colque-Caro, L.R. Comini, J.L. Cabrera, S. Nunez-Montoya, O.G. Martinez and E.J. Gimeno, 2017. Spontaneous photosensitization by *Heterophyllaea pustulata* Hook. f. (Rubiaceae), in sheep from Northwestern Argentina. Trop. Anim. Health Prod., 49: 1553-1556.
11. Perwitasari, D.A., N. Mahdi, M. Muthaharah and I.N. Kertia, 2016. Quality of life of patients prescribed with herbal medicine. J. Pharm. Sci. Commun., 13: 57-60.
12. Leeratiwong, C., P. Chantaranothai and A. Paton, 2016. Taxonomic notes on the genus *Premna* L. (Lamiaceae) in Thailand. Thai Forest Bull. (Botany), 44: 122-124.
13. Khotimah, S.N. and A. Muhtadi, 2017. Plants contain anti-inflammation active compounds. Farmaka, Vol. 14.
14. Bezerra, C.F., E.F. Mota, A.C.M. Silva, A.R. Tome and M.Z.R. Silva *et al*., 2017. Latex proteins from *Calotropis procera*: Toxicity and immunological tolerance revisited. Chem.-Biol. Interact., 274: 138-149.
15. Balouiri, M., M. Sadiki and S.K. Ibnsouda, 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. J. Pharm. Anal., 6: 71-79.
16. Wibowo, M.A. and V.V. Harlia, 2016. Antioxidant activity and toxicity of fruit extracts buas-buas (*Premna serratifolia* Linn). J. Kimia Khatulistiwa, Vol. 5.
17. Tan, Y., D. Li, Y. Chen and B. Li, 2017. *Premna bhamoensis* (Lamiaceae, Premnoideae), a new species from Kachin state, Northeastern Myanmar. PhytoKeys, 83: 93-101.
18. Wen, X., J. Hempel, R.M. Schweiggert, Y. Ni and R. Carle, 2017. Carotenoids and carotenoid esters of red and yellow physalis (*Physalis alkekengi* L. and *P. pubescens* L.) fruits and calyces. J. Agric. Food Chem., 65: 6140-6151.
19. Phillips, C.J., N.A. Wells, M. Martinello, S. Smith, R.J. Woodman and D.L. Gordon, 2016. Optimizing the detection of methicillin-resistant *Staphylococcus aureus* with elevated vancomycin minimum inhibitory concentrations within the susceptible range. Infect. Drug Resistance, 9: 87-92.
20. Lam, K.Y., A.P.K. Ling, R.Y. Koh, Y.P. Wong and Y.H. Say, 2016. A review on medicinal properties of orientin. Adv. Pharmacol. Sci., Vol. 2016.
21. Miyazaki, Y., K. Nakajima, M. Hosaka, N. Ban and T. Takahashi *et al*., 2016. A novel endoscopic catheter for laparoscopy-like irrigation and suction: Its research and development process and clinical evaluation. J. Laparoendoscopic Adv. Surg. Techniques, 26: 943-949.
22. Jayaprakash, S.B. and N. Nagarajan, 2016. Studies on the bioactive compounds and antimicrobial activities of medicinal plant *Centella asiatica* (Linn). J. Med. Plants, 4: 181-185.

Etnobotany-1

ORIGINALITY REPORT

7%	6%	4%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	researchtrend.net Internet Source	1%
2	www.ijprd.com Internet Source	1%
3	www.sciencepub.net Internet Source	1%
4	chushikoku.jsbba.or.jp Internet Source	1%
5	Malayan, Jeevan, Balaji Selvaraj, Aparna Warrier, Sambantham Shanmugam, Manikannan Mathayan, and Thangam Menon. "Anti-mumps virus activity by extracts of Mimosa pudica, a unique Indian medicinal plant", Indian Journal of Virology, 2013. Publication	1%
6	Prabu, Amudha, Prabu Seenivasan, and Vanaja Kumar. "Antimycobacterial activity of certain mangrove plants against multi-drug resistant Mycobacterium tuberculosis", Asian Journal of Medical Sciences, 2014. Publication	1%

7	Eric Van Quaquebeke, Gentiane Simon, Aurélie André, Janique Dewelle et al. " Identification of a Novel Cardenolide (2‘ ‘-Oxovoruscharin) from and the Hemisynthesis of Novel Derivatives Displaying Potent in Vitro Antitumor Activities and High in Vivo Tolerance: Structure−Activity Relationship Analyses ", Journal of Medicinal Chemistry, 2005 Publication	1%
8	www.ijppr.com Internet Source	1%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		

Etnobotany-1

GRADEMARK REPORT

FINAL GRADE

/20

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6