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

[1]Martina Restuati, [1]DikySetyaDiningrat

[1]*Biology Department of Mathematics and Natural Sciences Faculty, Medan State University, North Sumatera Indonesia.

corresponding author[U1]

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Abstract

Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine.*Premna pubescens*-Blume (Buasbuas) has been used to increase the body immunity and endurance. *Centella asiatica*(Pegagan) used for medicinal purposes. Native to China, India and Indonesia recently have had interest in herb for its possible health benefits.The aim of the study was to investigate *in vitro*antimicrobial activity of North Sumatera medicinal plants *P. pubescens* and *C. asatica* against the important pathogens. To investigate *in vitro* antimicrobial activity of aerial parts of *P. pubescens* and *C. asatica*, that popularly used as folk medicines. **Materials and Methods:**The organic solvent plant extracts are tested on the various microorganisms including bacteria and fungi using agardiffusion technique.[i-[6]**Results:**The length of the inhibition zone was measured in millimeters from the edge of the well to the inhibition zone. *P. pubescens* showed significant to moderate activity against (14 mm) *Pseudomonas marginalis* and (21 mm) *Streptococcus mutans* with 100 mg/ml DMSO plant drug concentration. The results of (MICs) values are lowest at 66 and highest at 152 mg/ml for *P. pubescens* whereas 0 to 155 mg/ml for *C. asiatica*. **Conclusion:**The extracts were assessed to validate the potential activity of the medicinal plants against microbes. These studies wereconducted to separate the individual componentsof *P. pubescens* and *C. asatica* usingcolumnchromatography to developalternative synthetic agents. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties[U7].

Keywords: [U8]*Premna pubescens*- Blume, *Centella asiatica*, antimicrobial, *Minimum inhibitory concentratio*~~MIC~~, Inhibition zone

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 (Rios & Recio, 2005[i-[9]). Nearly 6-7 thousand species of medicinal plants out of about 17-18 thousand flowering plants are known to be in use in folk and officially recognized systems of medicine in Indonesia, i.e., North Sumatra, Borneo, Celebes and Papua (Roosita et al., 2008).

North Sumatera Indonesia has a rich heritage of knowledge on plant baseddrugs bothforusein preventiveandcurativemedicin[U10]e (Khairiah et al., 2017).Sinceancient civilization, the

variouspartsof different plants were used toeliminate pain,control suffering and counteract disease. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs(Suswardany et al., 2017). It has alsobeenwidelyobservedandacceptedthatthe medicinal value of plants lies in the bioactive phytocomponents present in the plants (Diningrat et al., 2015a; Restuati et al, 2016). Much work has been done on ethnomedicinal plants in North Sumatera Indonesia (Roosita et al., 2008; Khairiah et al., 2017;Diningrat et al., 2015b; Restuati et al, 2016). Medicinal plants represent a rich source of antimicrobial agents (Restuati et al, 2016; Johnson et al., 2010; Koochak et al., 2010). Because[U11] of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines (Perwitasari et al., 2016; Manaf et al., 2017).

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(Leeratiwong et al., 2016). In the traditional North Sumatera Indonesian medicinal system, it has been used toincrease 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 (Khotimah & Muhtadi, 2017; (Wibowo & Harlia, 2016), and also increase appetite (Hidayat, 2015).Compounds derived from the plant have been found to have alkaloid, flavonoid, saponin, steroid, dan fenolik (Diningrat et al., 2015a; Restuati et al, 2016). Antimicrobial activity of *Premna pubescens* Blume was previously screened by (Restuati et al, 2016).

Centella asiatica (Family Mackinlayaceae) common names include Pegagan, Asiatic Pennywort, North Sumatera Indonesian Pennywort, Luei Gong Gen, Takip, kohol, Antanan,

Pegagan, Pegaga, vallaarai Kula kud, Bai Bua Bok and Brahmi. In North Sumatera Indonesia iswell known as "Pegagan" it is used to improve the mental ability (Dogra et al., 2016; Jayaprakash & Nagarajan, 2016). Antibacterial activity of *C.asiatica* was previously screened by (Lin et al., 2017).The aim of the study was to investigate in vitroantimicrobial activity of North Sumatera Indonesian medicinal plants *P. pubescens.* and *C. asiatica* against the important pathogens. In this paper the results of such studies are reported in order to orient future investigations towards the finding of potent, less toxic to human health and safe antimicrobial agents from natural sources.

MATERIALS AND METHODS[i-[12]

Plant materials and extraction: The whole plants of *P. ~~Pubescens~~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 [U13]with 95% (v/v) Methanol[U14] as solvent for 12 h was performed. The resultant extraction was frozen and dried for 24°C/48 h. Yield of Methanol extracts: 30% (Villalobos et al., 2016).

Test microorganisms: Microbial strains of clinical, plant and aquatic origin i.e. *Asperigellus niger*, *Pencilliumexpansum*, *Fusariumoxysporum*,*Xanthomonas compestries*, *Lactobacillus acidophilus*, *Pseudomonasmarginalis*,*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 harvestedby centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 min again and diluted innormal saline to obtain 5 x 10^5 cfu/mL.

Determination of antimicrobial activity: The crude extracts of the different plant parts of different species were subjected to antimicrobial assay using the agar well diffusion method (Balouiri et al., 2016). About 20 ml of nutrient agar was dispensed into sterile universal bottles. Then inoculated with 0.2 ml of cultures mixed gently and poured into sterile petri dishes. After setting a number 3-cup borer (6 mm diameter) was properly sterilized by flaming and used to make three to five 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 50µl of the extract concentrations of 100, 300and 500 mg/ ml DMSO and allow diffusion for 45 minutes. The solvents used to reconstitute the extracts were similarly analyzed. The plates were incubated at 37°C for 24 hours for bacteria. The above procedure is allowed for fungal assays, except the media potato dextrose agar instead of nutrient agar and incubates at 25°C for 48 hours. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in duplicates.

Statistical analysis[i-[15]

RESULTS

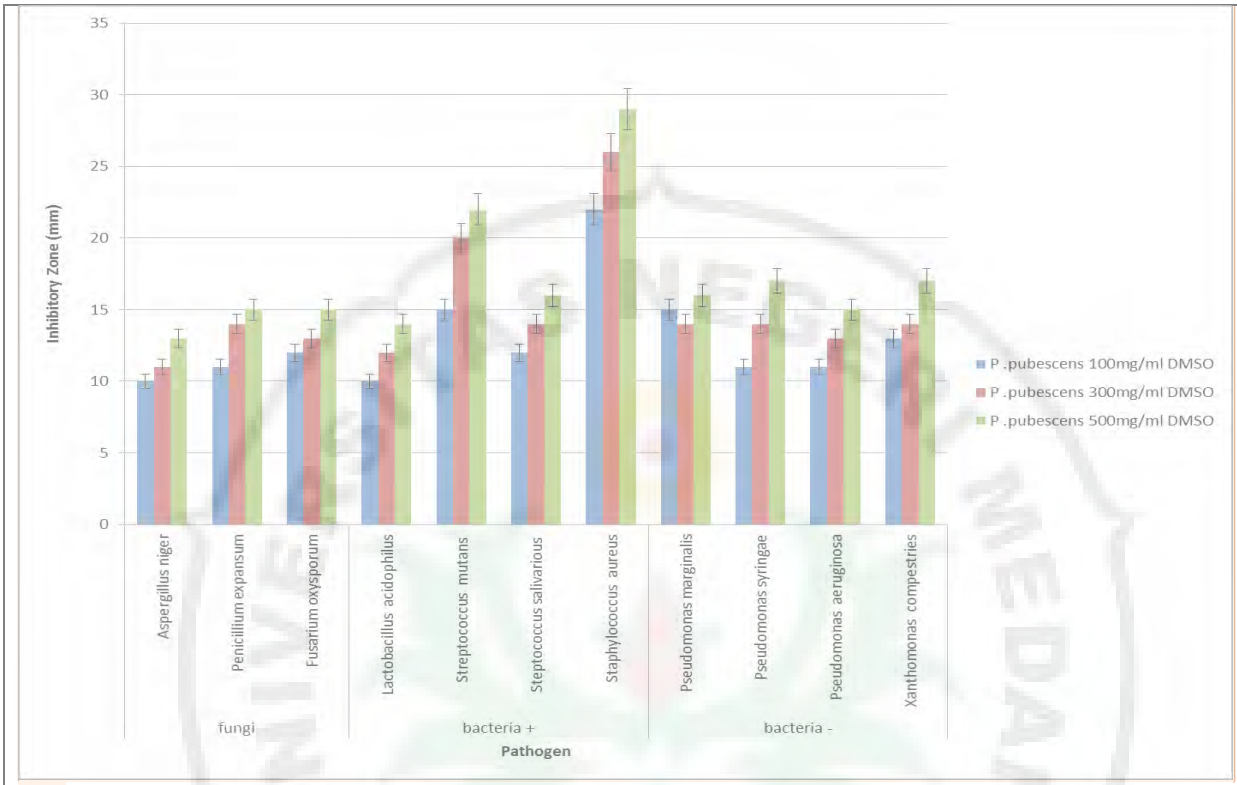
In the study of methanolic extract exhibited different degree of growth inhibition against the tested bacterial and fungal strains. ~~According to Fig. 1a, 2 band Table 1~~, methanolic extracts of *P. pubescens* and *C. asatica* exhibited considerable antimicrobial activity against testedmicrobial strains as shown in table 1 and figure 1a , b. *Premnapubescens* showed significant to moderate activity against *P. Marginalis*and *S. mutans*with 100 mg/ml DMSO

plant drug concentration. *Centellaasiatica*issignificant against *P.syringae* andmoderate against other pathogens *F. oxysporum*, *L. acidophilus*, *S. ~~Salivarious~~salivarious*and *S. ~~Aureus~~aureus*with 100 mg/ml DMSO.

Table 1. Antimicrobial activity ofmethanolic extracts*Premna pubescens*~~.~~ Blume and*Centella asiatica*

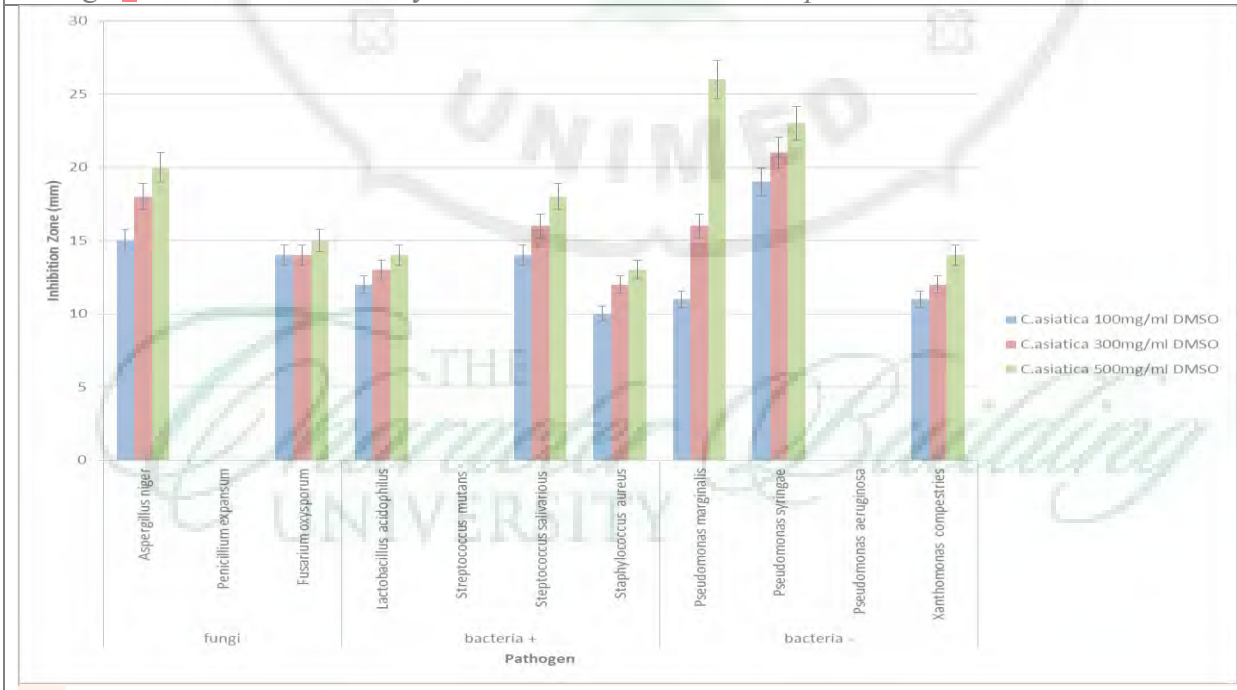
Pathogen		*Premna pubescens*~~.~~ Blume				*Centellaasiatica*			
		A	B	C	MIC	A	B	C	MIC[U16]
Fungi	*Aspergillus niger*	10	11	13	153	15	18	20	66
	Penicillium expansum	11	14	15	101	0	0	0	0
	Fusarium oxysporum	12	13	15	105	14	14	15	96
Bacteria (+)	*Lactobacillus acidophilus*	10	12	14	121	12	13	14	156
	Streptococcus 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 (-)	*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

(0) Value indicates no activity,Volume per well: 50µl, Borer size used: 6mm used Plant Methanolic extract concentrations (A =100, B=300, and C=500 mg/ DMSO ml) MIC-Minimum inhibitory concentration



[U17]

Fig.1a.Antimicrobial activity of methanol extracts*Premna pubescens* Blume



[U18]

Fig. ~~2~~1b. Antimicrobial activity of methanol extracts*C. asiatica*

The results of MICs values are lowest at 66 [U19] andhighest at 1532 mg/ml for *P. pubescens* whereas 0.155 mg/ml for *C.asatica*.The variation of antimicrobial activity of ~~our~~ these 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. asatica* on *P. aeruginosa* and *S. mutans.*

DISCUSSION

These extracts are harmless [U20] and nonphytotoxic; it has been proved that extracts have inhibitory effects on germination and on the viability of fungal spores as well. Both plant extracts showed moderate to good activity against *A. niger*as it is 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 and dried prune. The effectiveness of the active compounds in plant extractscauses the production of growthinhibition zones that appear as clear are as surrounding the wells. However, plant extract was unable to exhibit antibacterial activity against tested bacterial strains. These bacterial strains may have some kinds ofresistance mechanisms e.g.enzymatic inactivation, target sites modificationand decrease of intracellulardrugaccumulation (Santajit& Indrawattana, 2016) or the concentration of thecompoundusedmay notbe sufficient.

The adverse effects of *P. pubescens* consumption are reported to 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(Khairiah et al., 2017).The latex is highly toxic to human eyesandproduces sudden painless dimnessofvisionwithphotophobia (Micheloud et al., 2017). Several phytochemicals are identified in differentparts. *P. pubescens* flowers contain terpenes, multiflorenol, and cyclisadol (Tan et al., 2017). The latex contains caoutchouc, calotropin, calotoxin, calactin,

uscharin, trypsin, voruscharin, uzarigenin, syriogenin and proceroside (Bezerra et al., 2017). Chemical constituents of *P. pubescens* flowersarelupeol, uscharin, proceroside, proceragenin (cardenolide), syriogenin,taraxast-20(30)-en-3-(4-methyl-3-pentenoate),3-thiazolinecardenolide, gigantin, giganteol, isogiganteol, uscharidin, uzarigenin voruscharin a, calotropeol, 3-epimoretenol, a-lactuceryl acetate and alactuceryl isovalerate (Wen et al., 2017). Rootbarkof *P. pubescens* contains triterpenes, A new norditerpenyl ester, namedcalotropterpenyl ester, and twounknown pentacyclictriterpinoids,namely calotropursenylacetateand calotropfriedelenylacetate, akundarolisovalerate, mundarol isovalerate and quercetin, 3-rutinoside (Wen et al., 2017). The principal active medicinals are asclepin and mudarin (Lam et al., 2016).

No inhibition was observed with controls, 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*.*Pseudomonasaeruginosaare* is wide-spread in soil, water and sewage can be considered as an indication oftheirinvolvementinthenaturalprocess of mineralization of organic matter. It has long been a troublesome cause of secondary infections of wound, especially burns, 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 (Miyazaki et al., 2016). *S.aureus* occur harmlessly as a normal flora of the skin and mucous membrane and itisoneofthecommonestbacterialpathogens encountered in the community causing severe food

poisoning or minor skin infections to severe lifethreatening infections (Brooks et al.,2004).*C. asiatica* methanol extracthavingstrong inhibition activity against *P. aeruginosa and S. aureus* was previously reported by (Thangavel Arumugam et al. 2011).

CONCLUSION[U21]S

Premna pubescens and *Centella asiatica* extracts of showed antimicrobial activity against tested pathogens including antibiotic resistant strains. Further studies are being carried out in order to separate the individual components inplantextractsof *P. pubescens* and *C. asiatica* using column chromatographytodevelopbiopesticides as alternativetosynthetic agents. However, it isnecessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic propertie[U22]s.

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REPLY TO REVIEWER'S COMMENTS SHEET

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7	Abstract	Which Statistical method was being used to analyze data? Poorly written future recommendation should not be added in conclusion.	Statistical method used is ANAVZA (analysis of variant) using software of

			SPSS 121 -Soxhiet used is the product of IWAKIOXHET-100 IWAKI soxihiet extractor 100 ML -methanol used is methanol compound P.A.,99% sigma-adrich
8	Introduction	References must be cited in the text in superscript digits at the end of sentence or paragraph before punctuation or full stop[1]. In case of two or more references, separate the superscript digits by comma[1,2,6]. Moreover, If there are more references but in continuous numbers then use dash between superscript digits[2-6].	Will be mentioned
9	Materials and Methods	When the study was carried out? mention the grading of chemich either analytical grade was used or other Please indicate both the manufacturer's name and location (including city, state, and country) for all specialized equipments, kits and reagents used in the experiment Which Statistical method was being used to analyze data?	Will be mentioned
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11	References	References must be in English language References are each must be numbered, ordered sequentially as they appear in the text	Will be repaired

		Some of the references are incomplete or not formatted properly. Such type of references are not acceptable. Go to Http://scialert.net and consult "references" from "guide to author" for having idea of reference categories and formatting	
	figures	Metion the unit in which it was measured Remove this figure as it caused repetition of the data that is represented in table 1	Will be mentioned
	acknowledgement	Funding source with relevant grant number should be mentioned in the Acknowledgement.	Will be mentioned
	conclusion	Poorly written conclude about the findings so that a reader should have a good idea of what you have investigated and discovered. future recommendation should be added separately	Will be added
	result	recheck it as lowest MIC value is 0 according to above table	0 is not the lowest MIC value because 0 has no bioactive compound in killing microbes on the contrast 66 MIC is the lowest one,

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- Other comments those are important to the editor.

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

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corresponding author[U1]

Running title[U2]

Author contribution[U3]

Conflict of interest[U4]

Significance statement [U5]

Abstract

Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine.*Premna pubescens* Blume (Buasbuas) has been used to increase the body immunity and endurance. *Centella asiatica*(Pegagan) used for medicinal purposes. Native to China, India and Indonesia recently have had interest in herb for its possible health benefits.The aim of the study was to investigate *in vitro*antimicrobial activity of North Sumatera medicinal plants *P. pubescens* and *C. asatica* against the important pathogens. To investigate *in vitro* antimicrobial activity of aerial parts of *P. pubescens* and *C. asatica*, that popularly used as folk medicines. **Materials and Methods:**The organic solvent plant extracts are tested on the various microorganisms

including bacteria and fungi using agardiffusion technique.[i-[6]]**Results:**The length of the inhibition zone was measured in millimeters from the edge of the well to the inhibition zone. *P. pubescens* showed significant to moderate activity against (14 mm) *Pseudomonas marginalis* and (21 mm) *Streptococcus mutans* with 100 mg/ml DMSO plant drug concentration. The results of (MICs) values are lowest at 66 and highest at 152 mg/ml for *P. pubescens* whereas 0 to 155 mg/ml for *C. asiatica*. **Conclusion:**The extracts were assessed to validate the potential activity of the medicinal plants against microbes. These studies wereconducted to separate the individual componentsof *P. pubescens* and *C. asatica* usingcolumnchromatography to developalternative synthetic agents. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties[U7].

Keywords: [U8]*Premna pubescens*~~-~~ Blume, *Centella asiatica*, antimicrobial, *Minimum inhibitory concentratio*~~MIC~~, Inhibition zone

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 (Rios & Recio, 2005[i-[9]]). Nearly 6-7 thousand species of medicinal plants out of about 17-18 thousand flowering plants are known to be in use in folk and officially recognized systems of medicine in Indonesia, i.e., North Sumatra, Borneo, Celebes and Papua (Roosita et al., 2008).

North Sumatera Indonesia has a rich heritage of knowledge on plant baseddrugs bothforusein preventiveandcurativemedicin[U10]e (Khairiah et al., 2017).Sinceancient civilization, the variouspartsof different plants were used toeliminate pain,control suffering and counteract disease. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs(Suswardany et al., 2017). It has alsobeenwidelyobservedandacceptedthatthe medicinal value of plants lies in the bioactive phytocomponents present in the plants (Diningrat et al., 2015a; Restuati et al, 2016). Much work has been done on ethnomedicinal plants in North Sumatera Indonesia (Roosita et al., 2008; Khairiah et al., 2017;Diningrat et al., 2015b; Restuati et al, 2016). Medicinal plants

represent a rich source of antimicrobial agents (Restuati et al, 2016; Johnson et al., 2010; Koochak et al., 2010). Because[U11] of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines (Perwitasari et al., 2016; Manaf et al., 2017).

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(Leeratiwong et al., 2016). In the traditional North Sumatera Indonesian medicinal system, it has been used toincrease 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 (Khotimah & Muhtadi, 2017; (Wibowo & Harlia, 2016), and also increase appetite (Hidayat, 2015).Compounds derived from the plant have been found to have alkaloid, flavonoid, saponin, steroid, dan fenolik (Diningrat et al., 2015a; Restuati et al, 2016). Antimicrobial activity of *Premna pubescens* Blume was previously screened by (Restuati et al, 2016).

Centella asiatica (Family Mackinlayaceae) common names include Pegagan, Asiatic Pennywort, North Sumatera Indonesian Pennywort, Luei Gong Gen, Takip, kohol, Antanan, Pegagan, Pegaga, vallaarai Kula kud, Bai Bua Bok and Brahmi. In North Sumatera Indonesia iswell known as "Pegagan" it is used to improve the mental ability (Dogra et al., 2016; Jayaprakash & Nagarajan, 2016). Antibacterial activity of *C.asiatica* was previously screened by (Lin et al., 2017).The aim of the study was to investigate in vitroantimicrobial activity of North Sumatera Indonesian medicinal plants *P. pubescens.* and *C. asiatica* against the important pathogens. In this paper the results of such studies are reported in order to

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

MATERIALS AND METHODS[i-[12]

Plant materials and extraction: The whole plants of *P. ~~Pubescens~~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 [U13]with 95% (v/v) Methanol[U14] as solvent for 12 h was performed. The resultant extraction was frozen and dried for 24°C/48 h. Yield of Methanol extracts: 30% (Villalobos et al., 2016).

Test microorganisms: Microbial strains of clinical, plant and aquatic origin_i.e. *Asperigellus_niger*, *Pencilliumexpansum*, *Fusariumoxysporum*,*Xanthomonas compestries*, *Lactobacillus acidophilus*, *Pseudomonasmarginalis*,*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^{o}C overnight. The cells were harvestedby centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 min again and diluted innormal saline to obtain 5 x 10^5 cfu/mL.

Determination of antimicrobial activity: The crude extracts of the different plant parts of different species were subjected to antimicrobial assay using the agar well diffusion

method (Balouiri et al., 2016). About 20 ml of nutrient agar was dispensed into sterile universal bottles. Then inoculated with 0.2 ml of cultures mixed gently and poured into sterile petri dishes. After setting a number 3-cup borer (6 mm diameter) was properly sterilized by flaming and used to make three to five 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 50µl of the extract concentrations of 100, 300and 500 mg/ ml DMSO and allow diffusion for 45 minutes. The solvents used to reconstitute the extracts were similarly analyzed. The plates were incubated at 37°C for 24 hours for bacteria. The above procedure is allowed for fungal assays, except the media potato dextrose agar instead of nutrient agar and incubates at 25°C for 48 hours. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in duplicates.

Statistical analysis[i-[15]

RESULTS

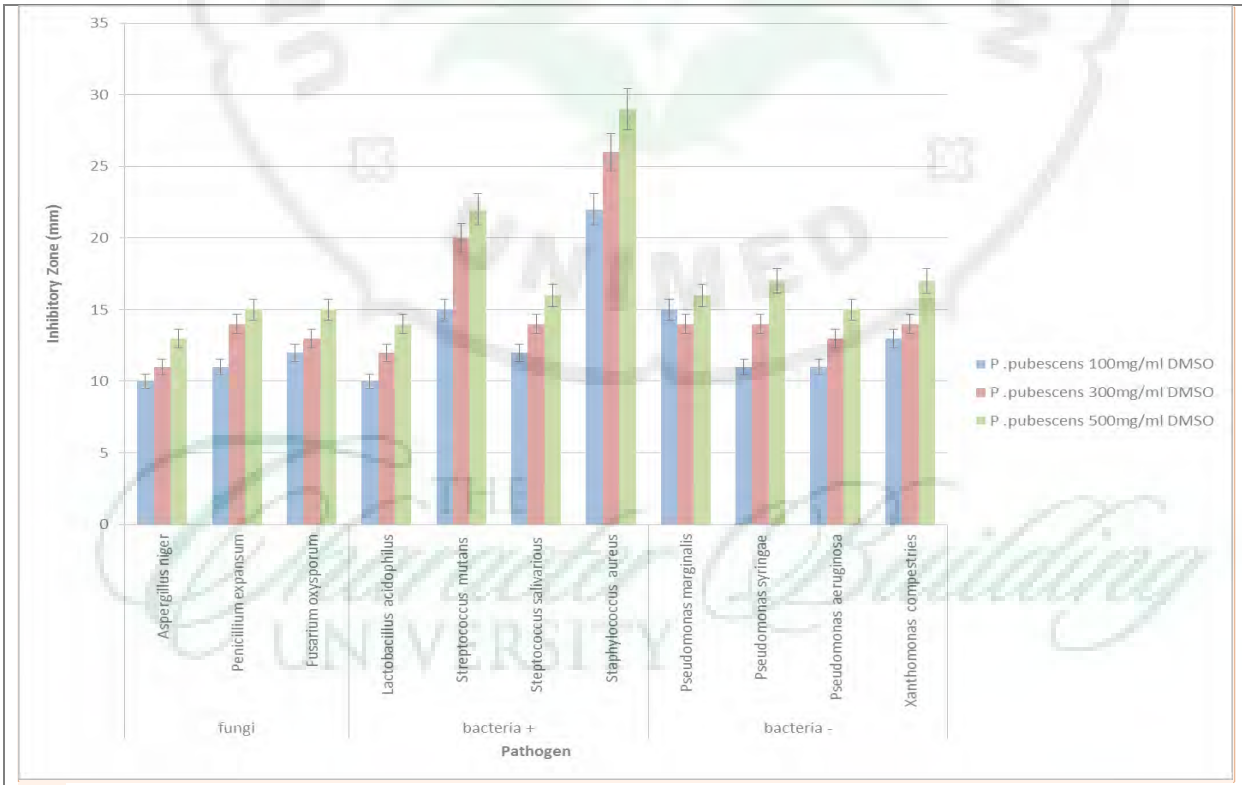
In the study of methanolic extract exhibited different degree of growth inhibition against the tested bacterial and fungal strains. ~~According to Fig. 1a, 2 band Table 1~~, methanolic extracts of *P. pubescens* and *C. asatica* exhibited considerable antimicrobial activity against testedmicrobial strains as shown in table 1 and figure 1a , b. *Premnapubescens* showed significant to moderate activity against *P. Marginalis*and *S. mutans*with 100 mg/ml DMSO plant drug concentration. *Centellaasiatica*issignificant against *P.syringae* andmoderate against other pathogens *F. oxysporum*, *L. acidophilus*, *S. ~~Salivarious~~salivarious*and *S. ~~Aureus~~aureus*with 100 mg/ml DMSO.

Table 1. Antimicrobial activity ofmethanolic extracts*Premna pubescens*~~.~~ Blume and*Centella asiatica*

Pathogen		*Premna pubescens*~~.~~ Blume				*Centellaasiatica*			
		A	B	C	MIC	A	B	C	MIC[U16]
Fungi	*Aspergillus*	10	11	13	153	15	18	20	66

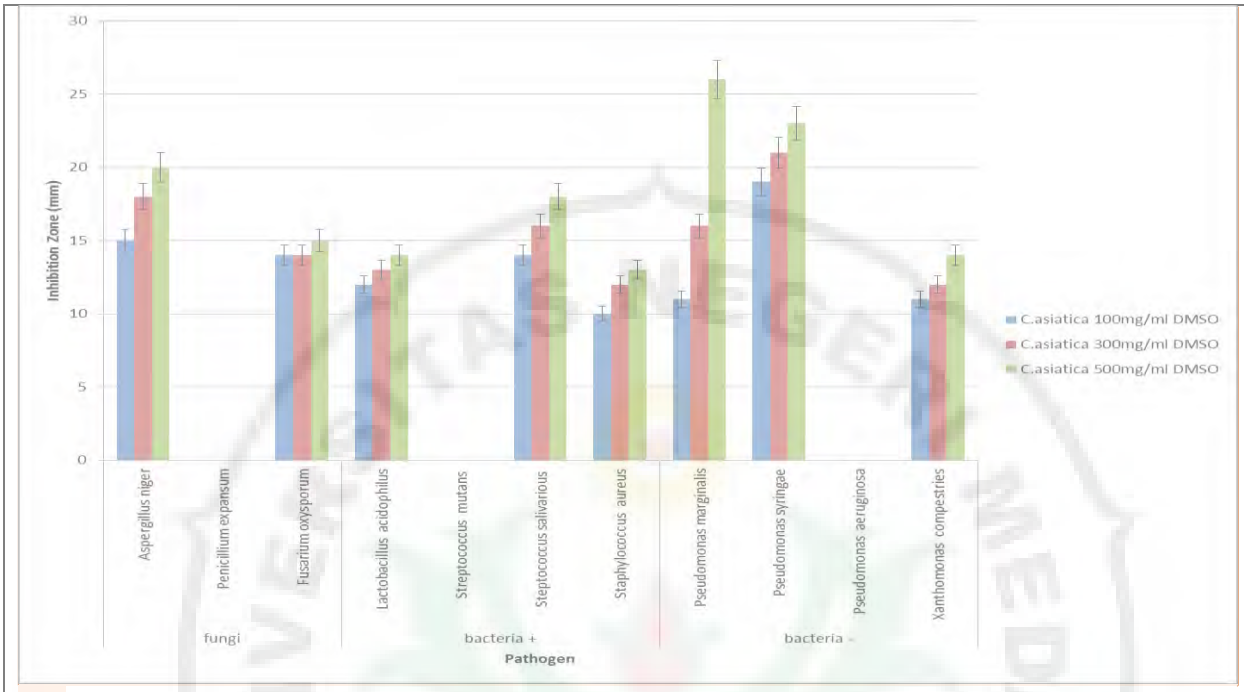
	niger								
	Penicillium expansum	11	14	15	101	0	0	0	0
	Fusarium oxysporum	12	13	15	105	14	14	15	96
Bacteria (+)	*Lactobacillus acidophilus*	10	12	14	121	12	13	14	156
	Streptococcus mutans	15	20	22	101	0	0	0	0
	Steptococcus salivarious	12	14	16	101	14	16	18	128
	Staphylococcus aureus	22	26	29	67	10	12	13	148
Bacteria (-)	*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

(0) Value indicates no activity,Volume per well: 50µl, Borer size used: 6mm used Plant Methanolic extract concentrations (A =100, B=300, and C=500 mg/ DMSO ml) MIC-Minimum inhibitory concentration



[U17]

Fig.1a.Antimicrobial activity of methanol extracts*Premna pubescens*-Blume



[U18]

Fig. ~~2~~1b. Antimicrobial activity of methanol extracts*C. asiatica*

The results of MICs values are lowest at 66 [U19] andhighest at 153~~2~~ mg/ml for *P. pubescens* whereas 0~~.~~.155 mg/ml for *C.asatica*.The variation of antimicrobial activity of ~~our~~ these ~~-~~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. asatica* on *P. aeruginosa* and *S. mutans.*

DISCUSSION

These extracts are harmless [U20] and nonphytotoxic; it has been proved that extracts have inhibitory effects on germination and on the viability of fungal spores as well. Both plant extracts showed moderate to good activity against *A. niger*as it is 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 and dried prune. The effectiveness of the active compounds in plant extractscauses the production of

growthinhibition zones that appear as clear are as surrounding the wells. However, plant extract was unable to exhibit antibacterial activity against tested bacterial strains. These bacterial strains may have some kinds ofresistance mechanisms e.g.enzymatic inactivation, target sites modificationand decrease of intracellulardrugaccumulation (Santajit& Indrawattana, 2016) or the concentration of thecompoundusedmay notbe sufficient.

The adverse effects of *P. pubescens* consumption are reported to 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(Khairiah et al., 2017).The latex is highly toxic to human eyesandproduces sudden painless dimnessofvisionwithphotophobia (Micheloud et al., 2017). Several phytochemicals are identified in differentparts. *P. pubescens* flowers contain terpenes, multiflorenol, and cyclisadol (Tan et al., 2017). The latex contains caoutchouc, calotropin, calotoxin, calactin, uscharin, trypsin, voruscharin, uzarigenin, syriogenin and proceroside (Bezerra et al., 2017). Chemical constituents of *P. pubescens* flowersarelupeol, uscharin, proceroside, proceragenin (cardenolide), syriogenin,taraxast-20(30)-en-3-(4-methyl-3-pentenoate),3-thiazolinecardenolide, gigantin, giganteol, isogiganteol, uscharidin, uzarigenin voruscharin a, calotropeol, 3-epimoretenol, a-lactuceryl acetate and alactuceryl isovalerate (Wen et al., 2017). Rootbarkof *P. pubescens* contains triterpenes, A new norditerpenyl ester, namedcalotropterpenyl ester, and twounknown pentacyclictriterpinoids,namely calotropursenylacetateand calotropfriedelenylacetate, akundarolisovalerate, mundarol isovalerate and quercetin, 3-rutinoside (Wen et al., 2017). The principal active medicinals are asclepin and mudarin (Lam et al., 2016).

No inhibition was observed with controls, 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*.*Pseudomonasaeruginosaare* is wide-spread in soil, water and sewage can be considered as an indication oftheirinvolvementinthenaturalprocess of mineralization of organic matter. It has long been a troublesome cause of secondary infections of wound, especially burns, 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 (Miyazaki et al., 2016). *S.aureus* occur harmlessly as a normal flora of the skin and mucous membrane and itisoneofthecommonestbacterialpathogens encountered in the community causing severe food poisoning or minor skin infections to severe lifethreatening infections (Brooks et al.,2004).*C. asiatica* methanol extracthavingstrong inhibition activity against *P. aeruginosa and S. aureus* was previously reported by (Thangavel Arumugam et al. 2011).

CONCLUSION[U21]S

Premna pubescens and *Centella asiatica* extracts of showed antimicrobial activity against tested pathogens including antibiotic resistant strains. Further studies are being carried out in order to separate the individual components inplantextractsof *P. pubescens* and *C. asiatica* using column chromatographytodevelopbiopesticides as alternativetosynthetic agents. However, it isnecessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic propertie[U22]s.

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REPLY TO REVIEWER'S COMMENTS SHEET

(Article No. 86564)

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7	Abstract	Which Statistical method was being used to analyze data? Poorly written future recommendation should not be added in conclusion.	Statistical method used is ANAVZA (analysis of variant) using software of

			SPSS 121 -Soxhiet used is the product of IWAKIOXHET-100 IWAKI soxihiet extractor 100 ML -methanol used is methanol compound P.A.,99% sigma-adrich
8	Introduction	References must be cited in the text in superscript digits at the end of sentence or paragraph before punctuation or full stop[1]. In case of two or more references, separate the superscript digits by comma[1,2,6]. Moreover, If there are more references but in continuous numbers then use dash between superscript digits[2-6].	Will be mentioned
9	Materials and Methods	When the study was carried out? mention the grading of chemich either analytical grade was used or other Please indicate both the manufacturer's name and location (including city, state, and country) for all specialized equipments, kits and reagents used in the experiment Which Statistical method was being used to analyze data?	Will be mentioned
10	keywords	highlighted keywords are not unique. Provide at least five key words. Key words should be unique and a concise summary of your paper's content and should capture the most important aspects of your paper	Will be provided
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		Some of the references are incomplete or not formatted properly. Such type of references are not acceptable. Go to Http://scialert.net and consult "references" from "guide to author" for having idea of reference categories and formatting	
	figures	Metion the unit in which it was measured Remove this figure as it caused repetition of the data that is represented in table 1	Will be mentioned
	acknowledgement	Funding source with relevant grant number should be mentioned in the Acknowledgement.	Will be mentioned
	conclusion	Poorly written conclude about the findings so that a reader should have a good idea of what you have investigated and discovered. future recommendation should be added separately	Will be added
	result	recheck it as lowest MIC value is 0 according to above table	0 is not the lowest MIC value because 0 has no bioactive compound in killing microbes on the contrast 66 MIC is the lowest one,

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One of the biggest mistakes made by authors is to respond to all the comments, but forget to actually update the paper.
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The comments should include a justification of the change (or lack of change!). In other words, answers to two questions:

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

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corresponding author

Running title

Author contribution

Conflict of interest

Significance statement

Abstract
Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine.*Premna pubescens*-Blume (Buasbuas) has been used to increase the body immunity and endurance. *Centella asiatica*(Pegagan) used for medicinal purposes. Native to China, India and Indonesia recently have had interest in herb for its possible health benefits.The aim of the study was to investigate *in vitro*antimicrobial activity of North Sumatera medicinal plants *P. pubescens* and *C. asatica* against the important pathogens. To investigate *in vitro* antimicrobial activity of aerial parts of *P. pubescens* and *C. asatica*, that popularly used as folk medicines. **Materials and Methods:**The organic solvent plant extracts are tested on the various microorganisms

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Comment [U5]: A statement about the significance of this research work should be included in the manuscript. The significance statement should provide the novelty aspect and significance of this research work with respect to the existing literature and more generally to the society. It should be a short summary which describe what this paper adds to and what was already known. **Start this statement with the following words:** This study discover the ---------- that ...

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including bacteria and fungi using agardiffusion technique.**Results:**The length of the inhibition zone was measured in millimeters from the edge of the well to the inhibition zone. *P. pubescens* showed significant to moderate activity against (14 mm) *Pseudomonas marginalis* and (21 mm) *Streptococcus mutans* with 100 mg/ml DMSO plant drug concentration. The results of (MICs) values are lowest at 66 and highest at 152 mg/ml for *P. pubescens* whereas 0 to 155 mg/ml for *C. asiatica*. **Conclusion:**The extracts were assessed to validate the potential activity of the medicinal plants against microbes. These studies wereconducted to separate the individual componentsof *P. pubescens* and *C. asatica* usingcolumnchromatography to developalternative synthetic agents. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties.

Keywords: *Premna pubescens* Blume, *Centella asiatica*, antimicrobial, *Minimum inhibitory concentration* MIC, Inhibition zone

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 (Rios & Recio, 2005). Nearly 6-7 thousand species of medicinal plants out of about 17-18 thousand flowering plants are known to be in use in folk and officially recognized systems of medicine in Indonesia, i.e., North Sumatra, Borneo, Celebes and Papua (Roosita et al., 2008).

North Sumatera Indonesia has a rich heritage of knowledge on plant baseddrugs bothforusein preventiveandcurativemedicine (Khairiah et al., 2017).Sinceancient civilization, the variouspartsof different plants were used toeliminate pain,control suffering and counteract disease. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs(Suswardany et al., 2017). It has alsobeenwidelyobservedandacceptedthatthe medicinal value of plants lies in the bioactive phytocomponents present in the plants (Diningrat et al., 2015a; Restuati et al, 2016). Much work has been done on ethnomedicinal plants in North Sumatera Indonesia (Roosita et al., 2008; Khairiah et al., 2017;Diningrat et al., 2015b; Restuati et al, 2016). Medicinal plants

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represent a rich source of antimicrobial agents (Restuati et al, 2016; Johnson et al., 2010; Koochak et al., 2010). Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines (Perwitasari et al., 2016; Manaf et al., 2017).

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(Leeratiwong et al., 2016). In the traditional North Sumatera Indonesian medicinal system, it has been used toincrease 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 (Khotimah & Muhtadi, 2017; (Wibowo & Harlia, 2016), and also increase appetite (Hidayat, 2015).Compounds derived from the plant have been found to have alkaloid, flavonoid, saponin, steroid, dan fenolik (Diningrat et al., 2015a; Restuati et al, 2016). Antimicrobial activity of *Premna pubescens*-Blume was previously screened by (Restuati et al, 2016).

Centella asiatica (Family Mackinlayaceae) common names include Pegagan, Asiatic Pennywort, North Sumatera Indonesian Pennywort, Luei Gong Gen, Takip, kohol, Antanan, Pegagan, Pegaga, vallaarai Kula kud, Bai Bua Bok and Brahmi. In North Sumatera Indonesia iswell known as "Pegagan" it is used to improve the mental ability (Dogra et al., 2016; Jayaprakash & Nagarajan, 2016). Antibacterial activity of *C.asiatica* was previously screened by (Lin et al., 2017).The aim of the study was to investigate in vitroantimicrobial activity of North Sumatera Indonesian medicinal plants *P. pubescens.* and *C. asiatica* against the important pathogens. In this paper the results of such studies are reported in order to

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

MATERIALS AND METHODS

Plant materials and extraction: The whole plants of *P. ~~Pubescens~~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 with 95% (v/v) Methanol as solvent for 12 h was performed. The resultant extraction was frozen and dried for 24°C/48 h. Yield of Methanol extracts: 30% (Villalobos et al., 2016).

Test microorganisms: Microbial strains of clinical, plant and aquatic origin_i.e. *Asperigellus_niger*, *Pencilliumexpansum*, *Fusariumoxysporum*,*Xanthomonas compestries*, *Lactobacillus acidophilus*, *Pseudomonasmarginalis*,*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 harvestedby centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 min again and diluted innormal saline to obtain 5 x 10^5 cfu/mL.

Determination of antimicrobial activity: The crude extracts of the different plant parts of different species were subjected to antimicrobial assay using the agar well diffusion

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method (Balouiri et al., 2016). About 20 ml of nutrient agar was dispensed into sterile universal bottles. Then inoculated with 0.2 ml of cultures mixed gently and poured into sterile petri dishes. After setting a number 3-cup borer (6 mm diameter) was properly sterilized by flaming and used to make three to five 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 50µl of the extract concentrations of 100, 300and 500 mg/ ml DMSO and allow diffusion for 45 minutes. The solvents used to reconstitute the extracts were similarly analyzed. The plates were incubated at 37°C for 24 hours for bacteria. The above procedure is allowed for fungal assays, except the media potato dextrose agar instead of nutrient agar and incubates at 25°C for 48 hours. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in duplicates.

Statistical analysis

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RESULTS

In the study of methanolic extract exhibited different degree of growth inhibition against the tested bacterial and fungal strains. ~~According to Fig. 1a, 2 band Table 1~~, methanolic extracts of *P. pubescens* and *C. asatica* exhibited considerable antimicrobial activity against testedmicrobial strains as shown in table 1 and figure 1a , b. *Premnapubescens* showed significant to moderate activity against *P. Marginalis*and *S. mutans*with 100 mg/ml DMSO plant drug concentration. *Centellaasiatica*issignificant against *P.syringae* andmoderate against other pathogens *F. oxysporum*, *L. acidophilus*, *S. ~~Salivarious~~salivarious*and *S. ~~Aureus~~aureus*with 100 mg/ml DMSO.

Table 1. Antimicrobial activity ofmethanolic extracts*Premna pubescens*~~.~~ Blume and*Centella asiatica*

Pathogen		*Premna pubescens*~~.~~ Blume				*Centellaasiatica*			
		A	B	C	MIC	A	B	C	MIC
Fungi	*Aspergillus*	10	11	13	153	15	18	20	66

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	niger								
	Penicillium expansum	11	14	15	101	0	0	0	0
	Fusarium oxysporum	12	13	15	105	14	14	15	96
Bacteria (+)	*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 (-)	*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

(0) Value indicates no activity,Volume per well: 50µl, Borer size used: 6mm used Plant Methanolic extract concentrations (A =100, B=300, and C=500 mg/ DMSO ml) MIC-Minimum inhibitory concentration

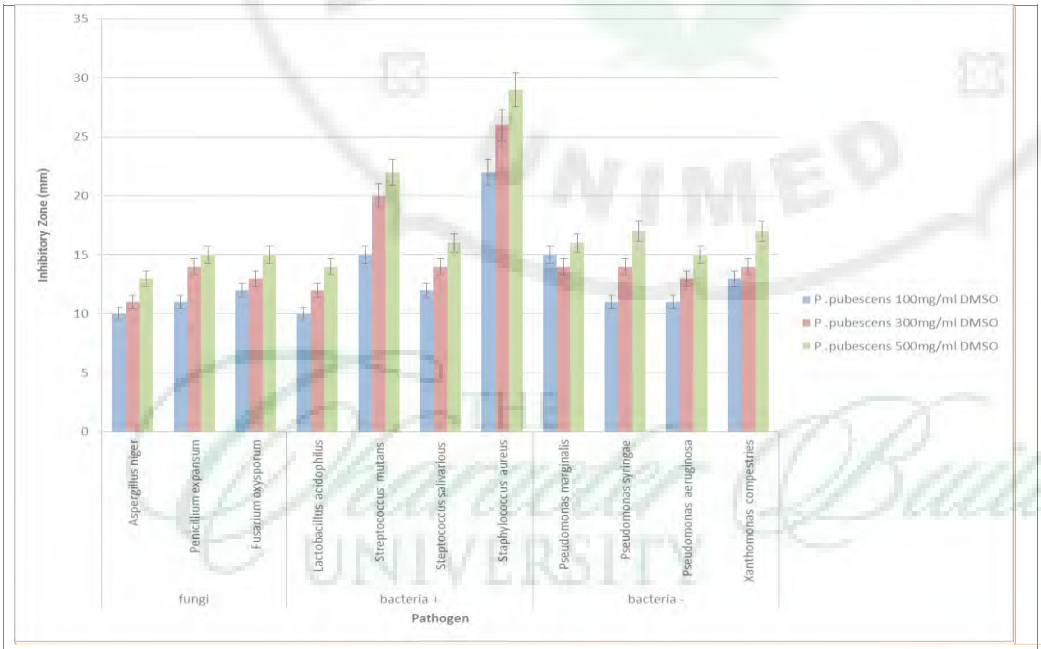

Fig.1a.Antimicrobial activity of methanol extracts*Premna pubescens*-Blume

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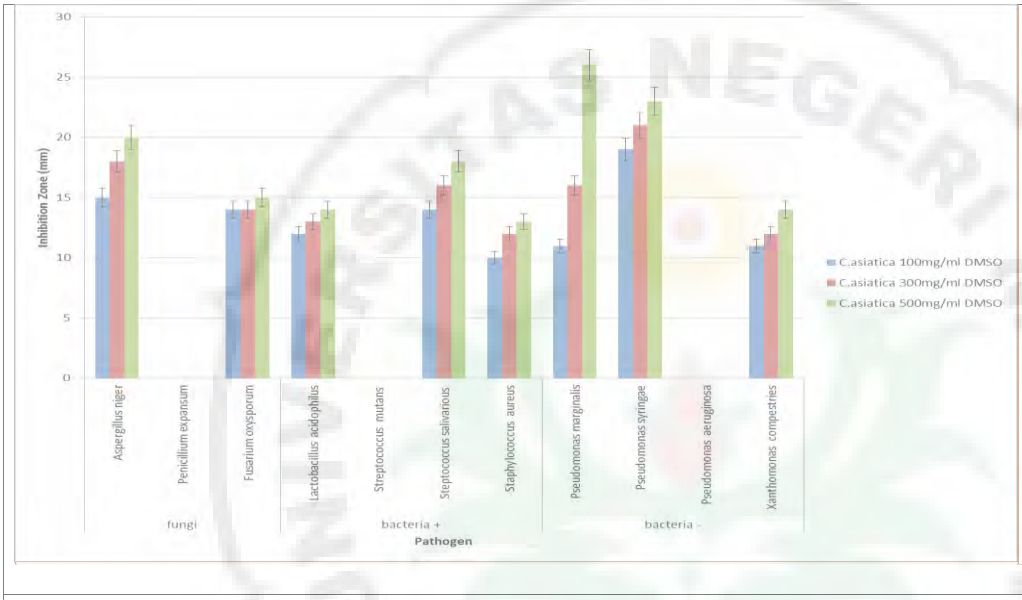

Fig. ~~2~~1b. Antimicrobial activity of methanol extracts*C. asiatica*

The results of MICs values are lowest at 66 andhighest at 1532 mg/ml for *P. pubescens* whereas 0~~.~~.155 mg/ml for *C.asatica*.The variation of antimicrobial activity of ~~our~~ these -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. asatica* on *P. aeruginosa* and *S. mutans.*

DISCUSSION

These extracts are harmless and nonphytotoxic; it has been proved that extracts have inhibitory effects on germination and on the viability of fungal spores as well. Both plant extracts showed moderate to good activity against *A. niger*as it is 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 and dried prune. The effectiveness of the active compounds in plant extractscauses the production of

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growthinhibition zones that appear as clear are as surrounding the wells. However, plant extract was unable to exhibit antibacterial activity against tested bacterial strains. These bacterial strains may have some kinds ofresistance mechanisms e.g.enzymatic inactivation, target sites modificationand decrease of intracellulardrugaccumulation (Santajit& Indrawattana, 2016) or the concentration of thecompoundusedmay notbe sufficient.

The adverse effects of *P. pubescens* consumption are reported to 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(Khairiah et al., 2017).The latex is highly toxic to human eyesandproduces sudden painless dimnessofvisionwithphotophobia (Micheloud et al., 2017). Several phytochemicals are identified in differentparts. *P. pubescens* flowers contain terpenes, multiflorenol, and cyclisadol (Tan et al., 2017). The latex contains caoutchouc, calotropin, calotoxin, calactin, uscharin, trypsin, voruscharin, uzarigenin, syriogenin and proceroside (Bezerra et al., 2017). Chemical constituents of *P. pubescens* flowersarelupeol, uscharin, proceroside, proceragenin (cardenolide), syriogenin,taraxast-20(30)-en-3-(4-methyl-3-pentenoate),3-thiazolinecardenolide, gigantin, giganteol, isogiganteol, uscharidin, uzarigenin voruscharin a, calotropeol, 3-epimoretenol, a-lactuceryl acetate and alactuceryl isovalerate (Wen et al., 2017). Rootbarkof *P. pubescens* contains triterpenes, A new norditerpenyl ester, namedcalotropterpenyl ester, and twounknown pentacyclictriterpinoids,namely calotropursenylacetateand calotropfriedelenylacetate, akundarolisovalerate, mundarol isovalerate and quercetin, 3-rutinoside (Wen et al., 2017). The principal active medicinals are asclepin and mudarin (Lam et al., 2016).

No inhibition was observed with controls, 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*.*Pseudomonasaeruginosaare* is wide-spread in soil, water and sewage can be considered as an indication oftheirinvolvementinthenaturalprocess of mineralization of organic matter. It has long been a troublesome cause of secondary infections of wound, especially burns, 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 (Miyazaki et al., 2016). *S.aureus* occur harmlessly as a normal flora of the skin and mucous membrane and itisoneofthecommonestbacterialpathogens encountered in the community causing severe food poisoning or minor skin infections to severe lifethreatening infections (Brooks et al.,2004).*C. asiatica* methanol extracthavingstrong inhibition activity against *P. aeruginosa and S. aureus* was previously reported by (Thangavel Arumugam et al. 2011).

CONCLUSIONS

Premna pubescens and *Centella asiatica* extracts of showed antimicrobial activity against tested pathogens including antibiotic resistant strains. Further studies are being carried out in order to separate the individual components inplantextractsof *P. pubescens* and *C. asiatica* using column chromatographytodevelopbiopesticides as alternativetosynthetic agents. However, it isnecessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties.

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ACKNOWLEDGEMENTS

We are thankful for constant encouragementandsupport from Biology Department, Mathematic and Natural Sciences, BOPTN Grants Funding Medan State University, Post-doctoral grants from the ministry of research, technology and higher education of Indonesia.

Comment [i-[23]: Funding source with relevant grant number should be mentioned in the Acknowledgement.

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Some of the references are incomplete or not formatted properly. Such type of references are not acceptable. Go to Http://scialert.net and consult "references" from "guide to author" for having idea of reference categories and formatting

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7	Abstract	Which Statistical method was being used to analyze data? Poorly written future recommendation should not be added in conclusion.	
8	Introduction	References must be cited in the text in superscript digits at the end of sentence or paragraph before punctuation or full stop[1]. In case of two or more references, separate the superscript digits by comma[1,2,6]. Moreover, If there are more references but in	

		continuous numbers then use dash between superscript digits[2-6].	
9	Materials and Methods	When the study was carried out? mention the grading of chemich either analytical grade was used or other Please indicate both the manufacturer's name and location (including city, state, and country) for all specialized equipments, kits and reagents used in the experiment Which Statistical method was being used to analyze data?	
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11	References	References must be in English language References are each must be numbered, ordered sequentially as they appear in the text Some of the references are incomplete or not formatted properly. Such type of references are not acceptable. Go to Http://scialert.net and consult "references" from "guide to author" for having idea of reference categories and formatting	
	figures	Metion the unit in which it was measured Remove this figure as it caused repetition of the data that is represented in table 1	
	acknowledgement	Funding source with relevant grant number should be mentioned in the Acknowledgement.	
	conclusion	Poorly written conclude about the findings so that a reader should have a good idea of what you have investigated and discovered. future recommendation should be added separately	
	result	recheck it as lowest MIC value is 0 according to above table	

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One of the biggest mistakes made by authors is to respond to all the comments, but forget to actually update the paper.
Don't be that person: one way to make sure you remember is to always include line numbers in your changes. That way, you actually have to make the change first before including the line numbers in your response.

How to prepare the revised paper?

1. Before you start editing the document, make sure Tracking changes is enabled! In MS Word go to Review menu and enable 'Track changes'.
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3. Mark your every change by adding a comment, excluding typos or similar minor editorial errors.

The comments should include a justification of the change (or lack of change!). In other words, answers to two questions:

- Why I made this change?
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If you decide to explain or justify why you believe your change (or lack of it) adds to the paper, always make sure to clearly indicate parts of paper you refer to (e.g. I mentioned this fact already in the Introduction, p. 1 paragraph 2. I believe bringing up this information there clarifies the issue from the very beginning).
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Summary: Track changes + put a comment with justification on every change you make

b. Cover letter

It is a good academic practice to attach a cover letter to submissions of new and revised papers to journals. Cover letter sent with the paper after major review usually includes:

- Courteous thanks to the editor, etc.
- General confirmation on carrying out all minor editorial corrections (typos etc.)
- Confirmation of implementing the requested changes. Explanations on general character of changes. You may mention 1-3 fundamental changes you made and explain how you dealt with it.
- A list of tasks given by the reviewer with precise indication where in the document you included the changes.
- Other comments those are important to the editor.

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

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corresponding author

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Running title

Author contribution

Conflict of interest

Significance statement

Abstract

Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine.*Premna pubescens*-Blume (Buasbuas) has been used to increase the body immunity and endurance. *Centella asiatica*(Pegagan) used for medicinal purposes. Native to China, India and Indonesia recently have had interest in herb for its possible health benefits.The aim of the study was to investigate *in vitro*antimicrobial activity of North Sumatera medicinal plants *P. pubescens* and *C. asatica* against the important pathogens. To investigate *in vitro* antimicrobial activity of aerial parts of *P. pubescens* and *C. asatica*, that popularly used as folk medicines. **Materials and Methods:**The organic solvent plant extracts are tested on the various microorganisms including bacteria and fungi using agardiffusion technique.**Results:**The length of the inhibition zone was measured in millimeters from the edge of the well to the inhibition zone. *P. pubescens* showed significant to moderate activity against (14 mm) *Pseudomonas marginalis* and (21 mm) *Streptococcus mutans* with 100 mg/ml DMSO plant drug concentration. The results of (MICs) values are lowest at 66 and highest at 152 mg/ml for *P. pubescens* whereas 0 to 155 mg/ml for *C. asiatica*. **Conclusion:**The extracts were assessed to validate the potential activity of the medicinal plants against microbes. These studies wereconducted to separate the individual componentsof *P. pubescens* and *C. asatica* usingcolumnchromatography to developalternative synthetic agents. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties.

Keywords: *Premna pubescens*- Blume, *Centella asiatica*, antimicrobial, *Minimum inhibitory concentratio*~~MIC~~, Inhibition zone

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 (Rios & Recio, 2005). Nearly 6-7 thousand species of medicinal plants out of about 17-18 thousand flowering plants are known to be in use in folk and officially recognized systems of medicine in Indonesia, i.e., North Sumatra, Borneo, Celebes and Papua (Roosita et al., 2008).

Comment [U2]: Provide the running title of the article as it is necessary according to the format of the journal.

Comment [U3]: There is a series of questions that will enable you to state the contributions of each author. Each author listed on the manuscript should have a real and concrete contribution to the submission. Every single person who contributed to the manuscript should be listed. More information about authorship can be collected from *Editorial Policies*

Comment [U4]: A statement on conflicts of interest should be included in the manuscript. Either mention: 'none declared', or specify the authors' financial or other interests which should be known to the readers.

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North Sumatera Indonesia has a rich heritage of knowledge on plant baseddrugs bothforusein preventiveandcurativemedicine (Khairiah et al., 2017).Sinceancient civilization, the variouspartsof different plants were used toeliminate pain,control suffering and counteract disease. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs(Suswardany et al., 2017). It has alsobeenwidelyobservedandacceptedthatthe medicinal value of plants lies in the bioactive phytocomponents present in the plants (Diningrat et al., 2015a; Restuati et al, 2016). Much work has been done on ethnomedicinal plants in North Sumatera Indonesia (Roosita et al., 2008; Khairiah et al., 2017;Diningrat et al., 2015b; Restuati et al, 2016). Medicinal plants represent a rich source of antimicrobial agents (Restuati et al, 2016; Johnson et al., 2010; Koochak et al., 2010). Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines (Perwitasari et al., 2016; Manaf et al., 2017).

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(Leeratiwong et al., 2016). In the traditional North Sumatera Indonesian medicinal system, it has been used toincrease 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 (Khotimah & Muhtadi, 2017; (Wibowo & Harlia, 2016), and also increase appetite (Hidayat, 2015).Compounds derived from the plant have been found to have alkaloid, flavonoid, saponin, steroid, dan fenolik (Diningrat et al., 2015a; Restuati et al, 2016). Antimicrobial activity of *Premna pubescens* Blume was previously screened by (Restuati et al, 2016).

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Centella asiatica (Family Mackinlayaceae) common names include Pegagan, Asiatic Pennywort, North Sumatera Indonesian Pennywort, Luei Gong Gen, Takip, kohol, Antanan, Pegagan, Pegaga, vallaarai Kula kud, Bai Bua Bok and Brahmi. In North Sumatera Indonesia iswell known as "Pegagan" it is used to improve the mental ability (Dogra et al., 2016; Jayaprakash & Nagarajan, 2016). Antibacterial activity of *C.asiatica* was previously screened by (Lin et al., 2017).The aim of the study was to investigate in vitroantimicrobial activity of North Sumatera Indonesian medicinal plants *P. pubescens.* and *C. asiatica* against the important pathogens. In this paper the results of such studies are reported in order to orient future investigations towards the finding of potent, less toxic to human health and safe antimicrobial agents from natural sources.

MATERIALS AND METHODS

Plant materials and extraction: The whole plants of *P. ~~Pubescens~~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 with 95% (v/v) Methanol as solvent for 12 h was performed. The resultant extraction was frozen and dried for 24°C/48 h. Yield of Methanol extracts: 30% (Villalobos et al., 2016).

Test microorganisms: Microbial strains of clinical, plant and aquatic origin i.e. *Asperigellus niger*, *Pencilliumexpansum*, *Fusariumoxysporum*,*Xanthomonas compestries*, *Lactobacillus acidophilus*, *Pseudomonasmarginalis*,*Pseudomonas syringae*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Steptococcus salivarious* and *Staphylococcus aureus*

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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 harvestedby centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 min again and diluted innormal saline to obtain 5 x 10^5 cfu/mL.

Determination of antimicrobial activity: The crude extracts of the different plant parts of different species were subjected to antimicrobial assay using the agar well diffusion method (Balouiri et al., 2016). About 20 ml of nutrient agar was dispensed into sterile universal bottles. Then inoculated with 0.2 ml of cultures mixed gently and poured into sterile petri dishes. After setting a number 3-cup borer (6 mm diameter) was properly sterilized by flaming and used to make three to five 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 50µl of the extract concentrations of 100, 300and 500 mg/ ml DMSO and allow diffusion for 45 minutes. The solvents used to reconstitute the extracts were similarly analyzed. The plates were incubated at 37°C for 24 hours for bacteria. The above procedure is allowed for fungal assays, except the media potato dextrose agar instead of nutrient agar and incubates at 25°C for 48 hours. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in duplicates.

Statistical analysis

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RESULTS

In the study of methanolic extract exhibited different degree of growth inhibition against the tested bacterial and fungal strains. ~~According to Fig. 1a, 2 band Table 1~~, methanolic extracts of *P. pubescens* and *C. asatica* exhibited considerable antimicrobial activity against

testedmicrobial strains as shown in table 1 and figure 1a , b. *Premnapubescens* showed significant to moderate activity against *P. Marginalis*and *S. mutans*with 100 mg/ml DMSO plant drug concentration. *Centellaasiatica*issignificant against *P.syringae* andmoderate against other pathogens *F. oxysporum*, *L. acidophilus*, *S. ~~Salivarious~~salivarious*and *S. ~~Aureus~~aureus*with 100 mg/ml DMSO.

Table 1. Antimicrobial activity ofmethanolic extracts*Premna pubescens~~.~~* Blume and*Centella asiatica*

Pathogen		*Premna pubescens~~.~~* Blume				*Centellaasiatica*			
		A	B	C	MIC	A	B	C	MIC
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 (+)	*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 (-)	*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

(0) Value indicates no activity,Volume per well: 50µl, Borer size used: 6mm used Plant Methanolic extract concentrations (A =100, B=300, and C=500 mg/ DMSO ml) MIC-Minimum inhibitory concentration

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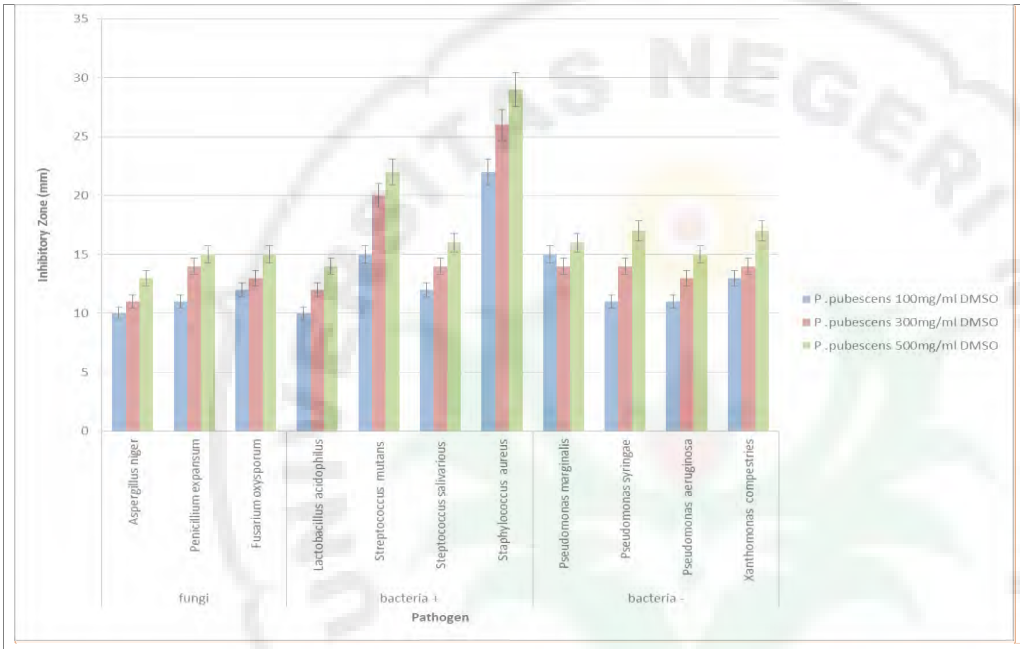

Fig.1a.Antimicrobial activity of methanol extracts*Premna pubescens*-Blume

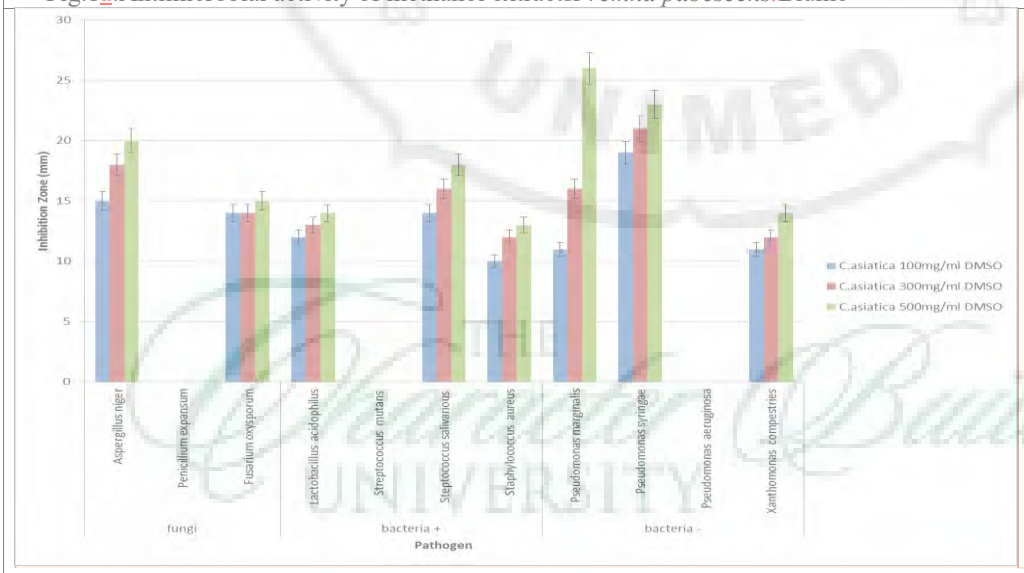

Fig. ~~2~~1b. Antimicrobial activity of methanol extracts*C. asiatica*

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The results of MICs values are lowest at 66 andhighest at 1532 mg/ml for *P. pubescens* whereas 0.155 mg/ml for *C.asatica*.The variation of antimicrobial activity of ~~our~~ these -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. asatica* on *P. aeruginosa* and *S. mutans.*

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DISCUSSION

These extracts are harmless and nonphytotoxic; it has been proved that extracts have inhibitory effects on germination and on the viability of fungal spores as well. Both plant extracts showed moderate to good activity against *A. niger*as it is 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 and dried prune. The effectiveness of the active compounds in plant extractscauses the production of growthinhibition zones that appear as clear are as surrounding the wells. However, plant extract was unable to exhibit antibacterial activity against tested bacterial strains. These bacterial strains may have some kinds ofresistance mechanisms e.g.enzymatic inactivation, target sites modificationand decrease of intracellulardrugaccumulation (Santajit& Indrawattana, 2016) or the concentration of thecompoundusedmay notbe sufficient.

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The adverse effects of *P. pubescens* consumption are reported to 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(Khairiah et al., 2017).The latex is highly toxic to human eyesandproduces sudden painless dimnessofvisionwithphotophobia (Micheloud et al., 2017). Several phytochemicals are identified in differentparts. *P. pubescens* flowers contain terpenes, multiflorenol, and cyclisadol (Tan et al., 2017). The latex contains caoutchouc, calotropin, calotoxin, calactin,

uscharin, trypsin, voruscharin, uzarigenin, syriogenin and proceroside (Bezerra et al., 2017). Chemical constituents of *P. pubescens* flowersarelupeol, uscharin, proceroside, proceragenin (cardenolide), syriogenin,taraxast-20(30)-en-3-(4-methyl-3-pentenoate),3-thiazolinecardenolide, gigantin, giganteol, isogiganteol, uscharidin, uzarigenin voruscharin a, calotropeol, 3-epimoretenol, a-lactuceryl acetate and alactuceryl isovalerate (Wen et al., 2017). Rootbarkof *P. pubescens* contains triterpenes, A new norditerpenyl ester, namedcalotropterpenyl ester, and twounknown pentacyclictriterpinoids,namely calotropursenylacetateand calotropfriedelenylacetate, akundarolisovalerate, mundarol isovalerate and quercetin, 3-rutinoside (Wen et al., 2017). The principal active medicinals are asclepin and mudarin (Lam et al., 2016).

No inhibition was observed with controls, 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*.*Pseudomonasaeruginosaare* is wide-spread in soil, water and sewage can be considered as an indication oftheirinvolvementinthenaturalprocess of mineralization of organic matter. It has long been a troublesome cause of secondary infections of wound, especially burns, 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 (Miyazaki et al., 2016). *S.aureus* occur harmlessly as a normal flora of the skin and mucous membrane and itisoneofthecommonestbacterialpathogens encountered in the community causing severe food

poisoning or minor skin infections to severe lifethreatening infections (Brooks et al.,2004).*C. asiatica* methanol extracthavingstrong inhibition activity against *P. aeruginosa and S. aureus* was previously reported by (Thangavel Arumugam et al. 2011).

CONCLUSIONS

Premna pubescens and *Centella asiatica* extracts of showed antimicrobial activity against tested pathogens including antibiotic resistant strains. Further studies are being carried out in order to separate the individual components inplantextractsof *P. pubescens* and *C. asiatica* using column chromatographytodevelopbiopesticides as alternativetosynthetic agents. However, it isnecessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties.

ACKNOWLEDGEMENTS

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4	Conflict of interest	A statement on conflicts of interest should be included in the manuscript. Either mention: 'none declared', or specify the authors' financial or other interests which should be known to the readers.	I will submit in cover letter
5	Author's contribution	There is a series of questions that will enable you to state the contributions of each author. Each author listed on the manuscript should have a real and concrete contribution to the submission. Every single person who contributed to the manuscript should be listed. More information about authorship can be collected from *Editorial Policies*	I will submit in cover letter
6	Significance statement	A statement about the significance of this research work should be included in the manuscript. The significance statement should provide the novelty aspect and significance of this research work with respect to the existing literature and more generally to the society. It should be a short summary which describe what this paper adds to and what was already known. **Start this statement with the following words:** This study discover the ---------- that can be beneficial for ..... **And the last sentence of this statement could be such as:** This study will help the researcher to uncover the critical areas of ---------- that many researchers were not able to explore. Thus a new theory on ---------- may be arrived at. **A Model Significance Statement:** This study discovers the possible synergistic effect of vitamin E, calcium, and vitamin D combination that can be beneficial for osteoporosis-induced ovariectomized rats. This study will help the researcher to uncover the critical area of postmenopausal bone loss that many researchers were not able to explore. Thus, a new theory on these micronutrients combination, and possibly other combinations, may be arrived at.	I will submit in cover letter
7	Abstract	Which Statistical method was being used to analyze data? Poorly written future recommendation should not be added in conclusion.	Statistical method used is ANAVA (analysis of variant) using software of

			SPSS 121 -Soxhlet used is the product of IWAKI SOXHLET-100 IWAKI soxhlet extractor 100 ML -methanol used is methanol compound P.A. 99,9% sigma-adrich
8	Introduction	References must be cited in the text in superscript digits at the end of sentence or paragraph before punctuation or full stop[1]. In case of two or more references, separate the superscript digits by comma[1,2,6]. Moreover, If there are more references but in continuous numbers then use dash between superscript digits[2-6].	Will be mentioned
9	Materials and Methods	When the study was carried out? mention the grading of chemich either analytical grade was used or other Please indicate both the manufacturer's name and location (including city, state, and country) for all specialized equipments, kits and reagents used in the experiment Which Statistical method was being used to analyze data?	Will be mentioned
10	keywords	highlighted keywords are not unique. Provide at least five key words. Key words should be unique and a concise summary of your paper's content and should capture the most important aspects of your paper	Will be provided
11	References	References must be in English language References are each must be numbered, ordered sequentially as they appear in the text	Will be repaired

		Some of the references are incomplete or not formatted properly. Such type of references are not acceptable. Go to Http://scialert.net and consult "references" from "guide to author" for having idea of reference categories and formatting	
	figures	Metion the unit in which it was measured Remove this figure as it caused repetition of the data that is represented in table 1	Will be mentioned
	acknowledgement	Funding source with relevant grant number should be mentioned in the Acknowledgement.	Will be mentioned
	conclusion	Poorly written conclude about the findings so that a reader should have a good idea of what you have investigated and discovered. future recommendation should be added separately	Will be added
	result	recheck it as lowest MIC value is 0 according to above table	0 is not the lowest MIC value because 0 has no bioactive compound activity in killing microbes on the contrast 66 MIC is the lowest one

Don't forget to make the changes

One of the biggest mistakes made by authors is to respond to all the comments, but forget to actually update the paper.
Don't be that person: one way to make sure you remember is to always include line numbers in your changes. That way, you actually have to make the change first before including the line numbers in your response.

How to prepare the revised paper?

1. Before you start editing the document, make sure Tracking changes is enabled! In MS Word go to Review menu and enable 'Track changes'.
2. You have to address every comment from both reviewers.
3. Mark your every change by adding a comment, excluding typos or similar minor editorial errors.

The comments should include a justification of the change (or lack of change!). In other words, answers to two questions:

- Why I made this change?
- What is the result?

If you decide to explain or justify why you believe your change (or lack of it) adds to the paper, always make sure to clearly indicate parts of paper you refer to (e.g. I mentioned this fact already in the Introduction, p. 1 paragraph 2. I believe bringing up this information there clarifies the issue from the very beginning).
Don't add comments with questions. Bear in mind that:

- Our editors won't answer them

- There will be only two stages of corrections – after major and minor review. There is no space for discussions with the reviewers.

If you remove something, ~~don't mark it like this~~. Instead, mark the whole paragraph by adding a comment with explanations of what you did and how it helps to improve the paper.
Summary: Track changes + put a comment with justification on every change you make

b. Cover letter
It is a good academic practice to attach a cover letter to submissions of new and revised papers to journals. Cover letter sent with the paper after major review usually includes:

- Courteous thanks to the editor, etc.
- General confirmation on carrying out all minor editorial corrections (typos etc.)
- Confirmation of implementing the requested changes. Explanations on general character of changes. You may mention 1-3 fundamental changes you made and explain how you dealt with it.
- A list of tasks given by the reviewer with precise indication where in the document you included the changes.
- Other comments those are important to the editor.

List of change after reviewed

1. Title '
   Before: Antimicrobial Profile of *Premna pubescens* Blume and *Centella asiatica* Extracts Against Bacteria and Fungi Pathogens
   After: Antimicrobial Profile of *Premna pubescens*. Blume and *Centella asiatica* Extracts Against Bacteria and Fungi Pathogens
2. Abstract:
   Before:
   Abstract
   Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine. *Premna pubescens*.Blume (Buasbuas) has been used to increase the body immunity and endurance. *Centella asiatica* (Pegagan) used for medicinal purposes. Native to China, India and Indonesia recently have had interest in herb for its possible health benefits.The aim of the study was to investigate in vitro antimicrobial activity of North Sumatera medicinal plants *P. pubescens* and *C. asatica* against the important pathogens. To investigate in vitro antimicrobial activity of aerial parts of *P. pubescens* and *C. asatica*, that popularly used as folk medicines. **Materials and Methods:** The organic solvent plant extracts are tested on the various microorganisms including bacteria and fungi using agardiffusion technique. **Results:** The length of the inhibition zone was measured in millimeters from the edge of the well to the inhibition zone. *P. pubescens* showed significant to moderate activity against (14 mm) *Pseudomonas marginalis* and (21 mm) *Streptococcus mutans* with 100 mg/ml DMSO plant drug concentration. The results of (MICs) values are lowest at 66 and highest at 152 mg/ml for *P. pubescens* whereas 0 to 155 mg/ml for *C. asiatica*. **Conclusion:** The extracts were assessed to validate the potential activity of the medicinal plants against microbes. These studies wereconducted to separate the individual componentsof *P. pubescens* and *C. asatica* using column chromatography to develop alternative synthetic agents. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties.
   After:

Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine. *Premna pubescens.* Blume (Buasbuas) has been used to increase the body immunity and endurance. *Centella asiatica* (Pegagan) used for medicinal purposes. This research is important performed to find out the antimicrobial capabilities of *P. pubescens* and *C. asiatica* methanol extracts. This research 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. asatica* against the important pathogens. **Materials and Methods:** The organic solvent plant extracts are tested on the various microorganisms including bacteria and fungi using agar diffusion technique. The data were analyzed with Anova statistics using SPSS software. **Results**: The length of the inhibition zone was measured in millimeters from the edge of the well to the inhibition zone. *P. pubescens* showed significant to moderate activity against (14 mm) *Pseudomonas marginalis* and (21 mm) *Streptococcus mutans* with 100 mg/ml DMSO plant drug concentration. The results of (MICs) values are lowest at 66 and highest at 152 mg/ml for *P. pubescens* whereas 0 to 155 mg/ml for *C. asiatica*. **Conclusion:** In general, based on the result of this research, it can be said that *P. pubescens* and *C. asatica* plants can be used as antibacterial and antifungal compounds.

3. Keyword:

   Before: *Premna pubescens. Blume, Centella asiatica*, antimicrobial, MIC, Inhibition zone

   After: *Premna pubescens.* Blume*, Centella asiatica*, antibacterial, antifungal, Minimal inhibitory concentration

4. Running title

   Before: -

   After:

Corresponding Author: Diky Setya Diningrat, Biology Department of Mathematics and Natural Sciences Faculty, Medan State University, Jl. Willem Iskandar Pasar V Medan, North Sumatera, Indonesia, Tel: +6181361362400 email: dikysd@unimed.ac.id

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

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

5. Citation

   Before: countries (Rios & Recio, 2005).

   After: countries[1].

6. Before: North Sumatera Indonesia has a rich heritage of knowledge on plant baseddrugs both for use in preventive and curative medicine (Khairiah et al., 2017).

   After: North Sumatra is very rich in plants that are believed to have medicinal properties for either prevention or treatment[7].

7. Before: Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines (Perwitasari et al., 2016; Manaf et al., 2017).

   After: Many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines, because of the side effects and the resistance that pathogenic microorganisms build against antibiotics[12,16].

8. Before: **Plant materials and extraction:** The whole plants of *P. Pubescenspubescens* 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 with 95% (v/v) Methanol as solvent for 12 h was performed. The resultant extraction was frozen and dried for 24°C/48 h. Yield of Methanol extracts: 30% (Villalobos et al., 2016).

   After: **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-adrich) as solvent for 12 h was performed. The resultant extraction was frozen and dried for 24°C/48 h. Yield of Methanol extracts: 30%[23].
9. Before: No mentioned of statistical analysis

   After: **Statistical analysis**: All data were statistically analyzed with SPSS software (version 16). One-way analysis of variance (ANOVA) was used to study significant difference among means with significance level at p=0.05[23].
10. Before:

RESULTS

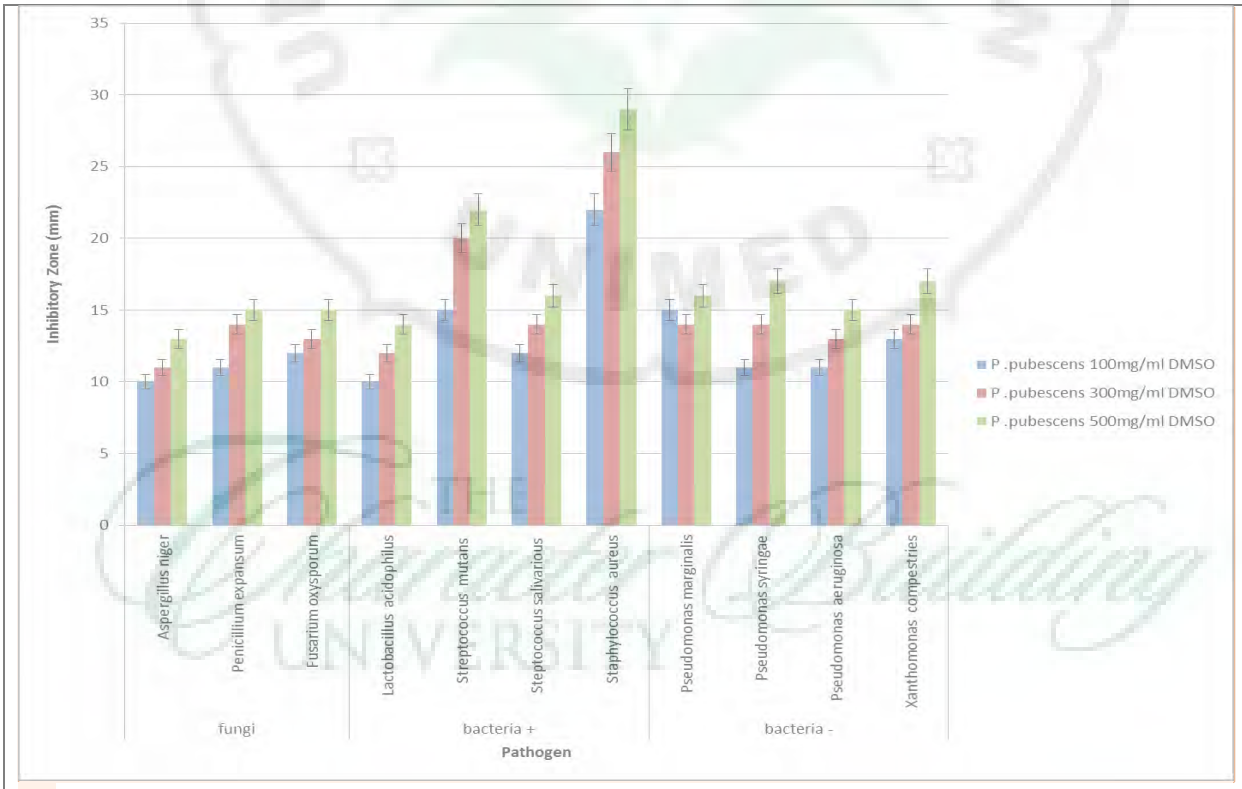
In the study of methanolic extract exhibited different degree of growth inhibition against the tested bacterial and fungal strains. ~~According to Fig. 1a, 2 band Table 1~~, methanolic extracts of *P. pubescens* and *C. asatica* exhibited considerable antimicrobial activity against testedmicrobial strains as shown in table 1 and figure 1a , b. *Premnapubescens* showed significant to moderate activity against *P. Marginalis*and *S. mutans*with 100 mg/ml DMSO plant drug concentration. *Centellaasiatica*issignificant against *P.syringae* andmoderate against other pathogens *F. oxysporum*, *L. acidophilus*, *S. ~~Salivarious~~salivarious*and *S. ~~Aureus~~aureus*with 100 mg/ml DMSO.

Table 1. Antimicrobial activity ofmethanolic extracts*Premna pubescens*~~.~~ Blume and*Centella asiatica*

Pathogen		*Premna pubescens*~~.~~ Blume				*Centellaasiatica*			
		A	B	C	MIC	A	B	C	MIC[U1]
Fungi	*Aspergillus*	10	11	13	153	15	18	20	66

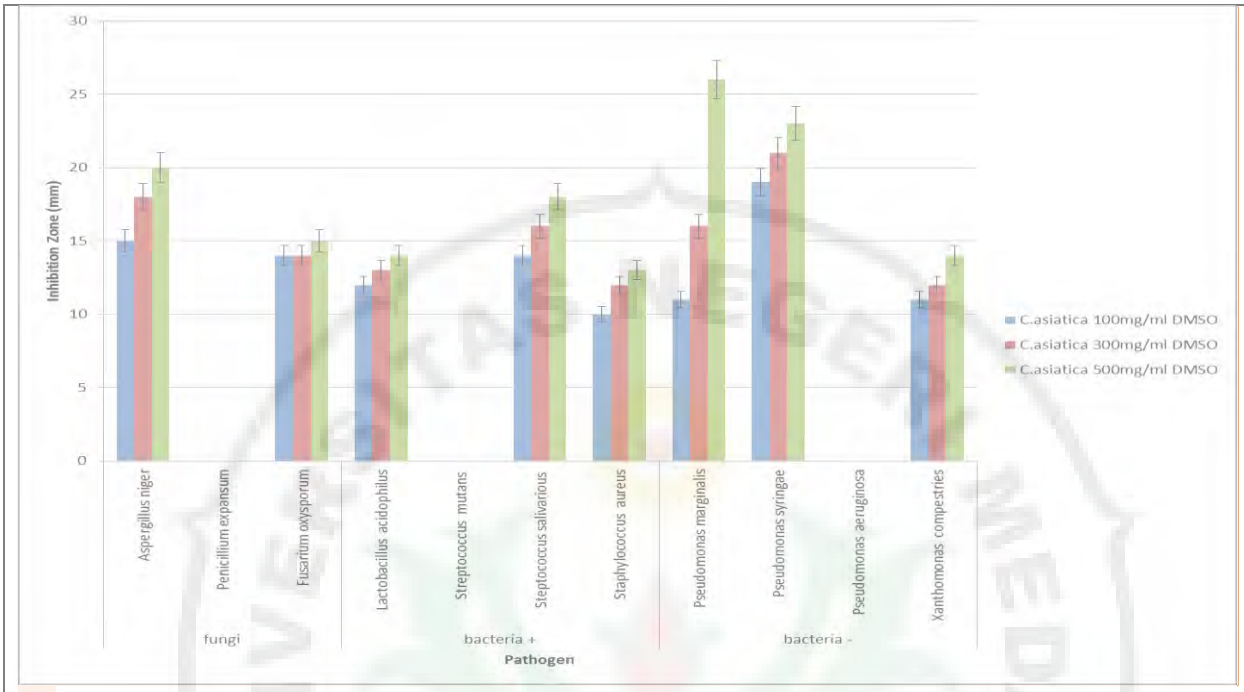
	niger								
	Penicillium expansum	11	14	15	101	0	0	0	0
	Fusarium oxysporum	12	13	15	105	14	14	15	96
Bacteria (+)	*Lactobacillus acidophilus*	10	12	14	121	12	13	14	156
	Streptococcus mutans	15	20	22	101	0	0	0	0
	Steptococcus salivarious	12	14	16	101	14	16	18	128
	Staphylococcus aureus	22	26	29	67	10	12	13	148
Bacteria (-)	*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

(0) Value indicates no activity,Volume per well: 50µl, Borer size used: 6mm used Plant Methanolic extract concentrations (A =100, B=300, and C=500 mg/ DMSO ml) MIC-Minimum inhibitory concentration



[U2]

Fig.1a.Antimicrobial activity of methanol extracts*Premna pubescens*-Blume



[U3]

Fig. ~~2~~1b. Antimicrobial activity of methanol extracts*C. asiatica*

After:

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. asatica* exhibited considerable antimicrobial activity against tested microbial strains. *Premna pubescens* showed significant to moderate activity against *P. marginalis* and *S. mutans* with 100 mg/ml DMSO plant drug 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 DMSO.

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

Pathogen		*Premna pubescens*. Blume				*Centella asiatica*			
		A	B	C	MIC (mg/ml)	A	B	C	MIC (mg/ml)
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 (+)	*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 (-)	*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

(0) Value indicates no activity,Volume per well: 50µl, Borer size used: 6mm used Plant Methanolic extract concentrations (A =100, B=300, and C=500 mg/ DMSO ml) MIC-Minimum inhibitory concentration

11. Before:

DISCUSSION

These extracts are harmless [U4] and nonphytotoxic; it has been proved that extracts have inhibitory effects on germination and on the viability of fungal spores as well.

After:

DISCUSSION

These extracts has been proved that extracts have inhibitory effects on germination and on the viability of fungal spores as well.

12. Before:

CONCLUSIONS

Premna pubescens and Centella asiatica extracts of showed antimicrobial activity against tested pathogens including antibiotic resistant strains. Further studies are being carried out in order to separate the individual components inplantextractsof P. pubescens and C. asiatica using column chromatographytodevelopbiopesticides as alternativetosynthetic agents. However, it isnecessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties.

After:

.

CONCLUSION

Premna pubescens and *Centella asiatica* extracts of 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 pharmaco-kinetic properties.

13. Before:
    ACKNOWLEDGEMENTS

    We are thankful for constant encouragementandsupport from Biology Department, Mathematic and Natural Sciences, BOPTN Grants Funding Medan State University, Post-doctoral grants from the ministry of research, technology and higher education of Indonesia.
    After:
    ACKNOWLEDGEMENTS

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14. Reference format:
    Before:

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After:

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COVER LETTER FOR SUBMISSION OF NEW MANUSCRIPTS

Martina Restuati (author)
Diky Setya Diningrat (corresponding author)

Subject: **SUBMISSION OF NEW MANUSCRIPT FOR EVALUATION**

I am enclosing herewith a manuscript entitled "[**Antimicrobial Profile of Premna pubescens. Blume and Centella asiatica Extracts Against Bacteria and Fungi Pathogens**]" submitted to "**[International Journal of Pharmacology]**" for possible evaluation.

With the submission of this manuscript I would like to undertake that the above mentioned manuscript has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere; and that my Institute's **[Medan State University]** representative is fully aware of this submission.

Select Type of Submitted manuscript:

- Research Article

This research project was conducted from July 2016 to November 2017
Starting date Ending date

My Research Project was partially or fully sponsored by BOPTN Downstream Articles Research Grant with grant number 178A/UN33.8/KU/2016, and another project was partially sponsored by Post-Doctoral Research Grant from Ministry of Research, Technology and Higher education Republic Indonesia. The team leader of these research are Dr. Diky Setya Diningrat and Dr. Martina Restuati is a member.

Detail of the each author with his/her contribution in this paper is as under:

Name of the author and e-mail ID	Types of contribution
Dr. Diky Setya Diningrat	Dr. Diky Setya Diningrat researched extract test on microbe
Dr. Martina Restuati	Dr. Martina Restuati researched extract of bioactive compound, blume and centella

I would also like to share the following information with Editor-in-Chief
For quick understanding about the importance of the project following are the significant findings of my submitted article?

This research identified extract activity of Premna and Centella on microbe however it is not new

research, this covers previous research published by the same author, but the latest and different one is the following:

1. kinds of microbe are equated
2. Conducting comparison activity of bioactive compound between Premna and Centella on the same microbe.

Therefore, The findings can be used as reference and development of bioactive compound on pharmacy industry

How findings of this research work are unique in their nature?

It compares anti-microbial compound from 2 species of plant commonly believed to be traditional efficacious drug in north Sumatra. Microbe is used as completely as representing ordinary pathogen which functions in human life and it comes from group of positive and negative Gram bacteria as well as fungus group

A paragraph explaining why your manuscript is appropriate for the selected journal

Because at the objective of research indicates this research compares 2 species of plant which function as anti-microbe and the conclusion states that the findings of research can be used as reference on development of both species of plants in pharmacy industry.

Revised Paper:

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

Martina Restuati and Diky Setya Diningrat
Biology Department of Mathematics and Natural Sciences Faculty, Medan State University, North Sumatera, Indonesia

Abstract

Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine. *Premna pubescens.* Blume (Buasbuas) has been used to increase the body immunity and endurance. *Centella asiatica* (Pegagan) used for medicinal purposes. This research is important performed to find out the antimicrobial capabilities of *P. pubescens* and *C. asiatica* methanol extracts. This research 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. asatica* against the important pathogens. **Materials and Methods:** The organic solvent plant extracts are tested on the various microorganisms including bacteria and fungi using agar diffusion technique. The data were analyzed with Anova statistics using SPSS software. **Results:** The length of the inhibition zone was measured in millimeters from the edge of the well to the inhibition zone. *P. pubescens* showed significant to moderate activity against (14 mm) *Pseudomonas marginalis* and (21 mm) *Streptococcus mutans* with 100 mg/ml DMSO plant drug concentration. The results of (MICs) values are lowest at 66 and highest at 152 mg/ml for *P. pubescens* whereas 0 to 155 mg/ml for *C. asiatica*. **Conclusion:** In general, based on the result of this research, it can be said that *P. pubescens* and *C. asatica* plants can be used as antibacterial and antifungal compounds.

Keywords: *Premna pubescens*. Blume, *Centella asiatica*, antibacterial, antifungal, Minimal inhibitory concentration

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		possibly other combinations, may be arrived at.	
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	figures	Metion the unit in which it was measured Remove this figure as it caused repetition of the data that is represented in table 1	

	acknowledgement	Funding source with relevant grant number should be mentioned in the Acknowledgement.	
	conclusion	Poorly written conclude about the findings so that a reader should have a good idea of what you have investigated and discovered. future recommendation should be added separately	
	result	recheck it as lowest MIC value is 0 according to above table	

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One of the biggest mistakes made by authors is to respond to all the comments, but forget to actually update the paper.
Don't be that person: one way to make sure you remember is to always include line numbers in your changes. That way, you actually have to make the change first before including the line numbers in your response.

How to prepare the revised paper?

1. Before you start editing the document, make sure Tracking changes is enabled! In MS Word go to Review menu and enable 'Track changes'.
2. You have to address every comment from both reviewers.
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- Other comments those are important to the editor.

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

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corresponding author[U1]

Running title[U2]

Author contribution[U3]

Conflict of interest[U4]

Significance statement [U5]

Abstract

Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine.*Premna pubescens* Blume (Buasbuas) has been used to increase the body immunity and endurance. *Centella asiatica*(Pegagan) used for medicinal purposes. Native to China, India and Indonesia recently have had interest in herb for its possible health benefits.The aim of the study was to investigate *in vitro*antimicrobial activity of North Sumatera medicinal plants *P. pubescens* and *C. asatica* against the important pathogens. To investigate *in vitro* antimicrobial activity of aerial parts of *P. pubescens* and *C. asatica*, that popularly used as folk medicines. **Materials and Methods:**The organic solvent plant extracts are tested on the various microorganisms including bacteria and fungi using agardiffusion technique.[i-[6]**Results:**The length of the inhibition zone was measured in millimeters from the edge of the well to the inhibition zone. *P. pubescens* showed significant to moderate activity against (14 mm) *Pseudomonas marginalis* and (21 mm) *Streptococcus mutans* with 100 mg/ml DMSO plant drug concentration. The results of (MICs) values are lowest at 66 and highest at 152 mg/ml for *P. pubescens* whereas 0 to 155 mg/ml for *C. asiatica*. **Conclusion:**The extracts were assessed to validate the potential activity of the medicinal plants against microbes. These studies wereconducted to separate the individual componentsof *P. pubescens* and *C. asatica* usingcolumnchromatography to developalternative synthetic agents. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties[U7].

Keywords: [U8]*Premna pubescens* Blume, *Centella asiatica*, antimicrobial, *Minimum inhibitory concentratio*MIC, Inhibition zone

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 (Rios & Recio, 2005[i-[9]). Nearly 6-7 thousand species of medicinal plants out of about 17-18 thousand flowering plants are known to be in use in folk and officially recognized systems of medicine in Indonesia, i.e., North Sumatra, Borneo, Celebes and Papua (Roosita et al., 2008).

North Sumatera Indonesia has a rich heritage of knowledge on plant baseddrugs bothforusein preventiveandcurativemedicin[U10]e (Khairiah et al., 2017).Sinceancient civilization, the variouspartsof different plants were used toeliminate pain,control suffering and counteract disease. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs(Suswardany et al., 2017). It has alsobeenwidelyobservedandacceptedthatthe medicinal value of plants lies in the bioactive phytocomponents present in the plants (Diningrat et al., 2015a; Restuati et al, 2016). Much work has been done on ethnomedicinal plants in North Sumatera Indonesia (Roosita et al., 2008; Khairiah et al., 2017;Diningrat et al., 2015b; Restuati et al, 2016). Medicinal plants represent a rich source of antimicrobial agents (Restuati et al, 2016; Johnson et al., 2010; Koochak et al., 2010). Because[U11] of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines (Perwitasari et al., 2016; Manaf et al., 2017).

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(Leeratiwong et al., 2016). In the

traditional North Sumatera Indonesian medicinal system, it has been used toincrease 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 (Khotimah & Muhtadi, 2017; (Wibowo & Harlia, 2016), and also increase appetite (Hidayat, 2015).Compounds derived from the plant have been found to have alkaloid, flavonoid, saponin, steroid, dan fenolik (Diningrat et al., 2015a; Restuati et al, 2016). Antimicrobial activity of *Premna pubescens*-Blume was previously screened by (Restuati et al, 2016).

Centella asiatica (Family Mackinlayaceae) common names include Pegagan, Asiatic Pennywort, North Sumatera Indonesian Pennywort, Luei Gong Gen, Takip, kohol, Antanan, Pegagan, Pegaga, vallaarai Kula kud, Bai Bua Bok and Brahmi. In North Sumatera Indonesia iswell known as "Pegagan" it is used to improve the mental ability (Dogra et al., 2016; Jayaprakash & Nagarajan, 2016). Antibacterial activity of *C.asiatica* was previously screened by (Lin et al., 2017).The aim of the study was to investigate in vitroantimicrobial activity of North Sumatera Indonesian medicinal plants *P. pubescens.* and *C. asiatica* against the important pathogens. In this paper the results of such studies are reported in order to orient future investigations towards the finding of potent, less toxic to human health and safe antimicrobial agents from natural sources.

MATERIALS AND METHODS[i-[12]

Plant materials and extraction: The whole plants of *P. ~~Pubescens~~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 [U13]with 95% (v/v) Methanol[U14] as solvent for 12 h was performed. The resultant extraction was frozen and dried for 24°C/48 h. Yield of Methanol extracts: 30% (Villalobos et al., 2016).

Test microorganisms: Microbial strains of clinical, plant and aquatic origin i.e. *Asperigellus niger*, *Pencilliumexpansum*, *Fusariumoxysporum*,*Xanthomonas compestries*, *Lactobacillus acidophilus*, *Pseudomonasmarginalis*,*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^{o}C overnight. The cells were harvestedby centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 min again and diluted innormal saline to obtain 5 x 10^{5} cfu/mL.

Determination of antimicrobial activity: The crude extracts of the different plant parts of different species were subjected to antimicrobial assay using the agar well diffusion method (Balouiri et al., 2016). About 20 ml of nutrient agar was dispensed into sterile universal bottles. Then inoculated with 0.2 ml of cultures mixed gently and poured into sterile petri dishes. After setting a number 3-cup borer (6 mm diameter) was properly sterilized by flaming and used to make three to five 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 50µl of the extract concentrations of 100, 300and 500 mg/ ml DMSO and allow diffusion for 45 minutes. The solvents used to reconstitute the extracts were similarly analyzed. The plates were incubated at 37°C for 24 hours for bacteria. The above procedure is allowed for

fungal assays, except the media potato dextrose agar instead of nutrient agar and incubates at 25°C for 48 hours. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in duplicates.

Statistical analysis[i-[15]

RESULTS

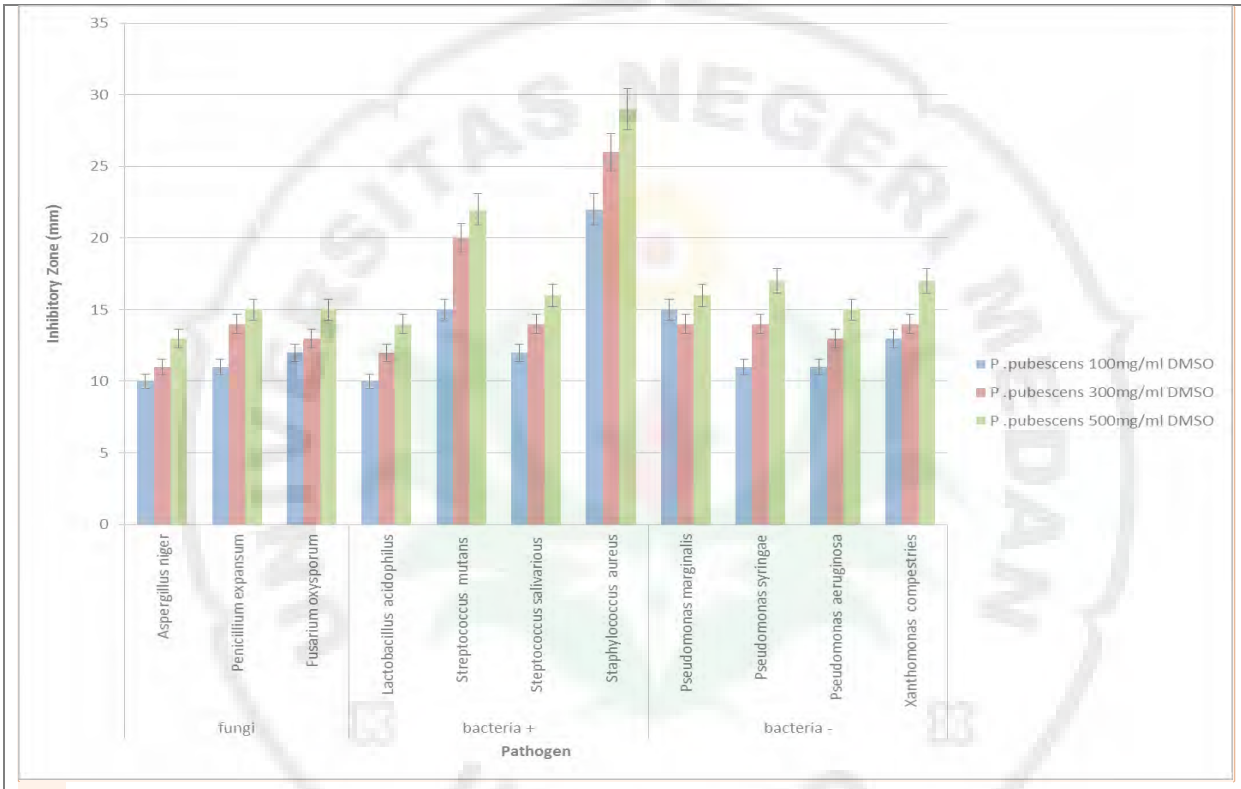
In the study of methanolic extract exhibited different degree of growth inhibition against the tested bacterial and fungal strains. ~~According to Fig. 1a, 2 band Table 1~~, methanolic extracts of *P. pubescens* and *C. asatica* exhibited considerable antimicrobial activity against testedmicrobial strains as shown in table 1 and figure 1a , b. *Premnapubescens* showed significant to moderate activity against *P. Marginalis*and *S. mutans*with 100 mg/ml DMSO plant drug concentration. *Centellaasiatica*issignificant against *P.syringae* andmoderate against other pathogens *F. oxysporum*, *L. acidophilus*, *S. ~~Salivarious~~salivarious*and *S. ~~Aureus~~aureus*with 100 mg/ml DMSO.

Table 1. Antimicrobial activity ofmethanolic extracts*Premna pubescens*~~.~~ Blume and*Centella asiatica*

Pathogen		*Premna pubescens*~~.~~ Blume				*Centellaasiatica*			
		A	B	C	MIC	A	B	C	MIC[U16]
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 (+)	*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 (-)	*Pseudomonas marginalis*	15	14	16	11	11	16	26	145
	Pseudomonas syringae	11	14	17	19	19	21	23	81
	Pseudomonas	11	13	15	0	0	0	0	0

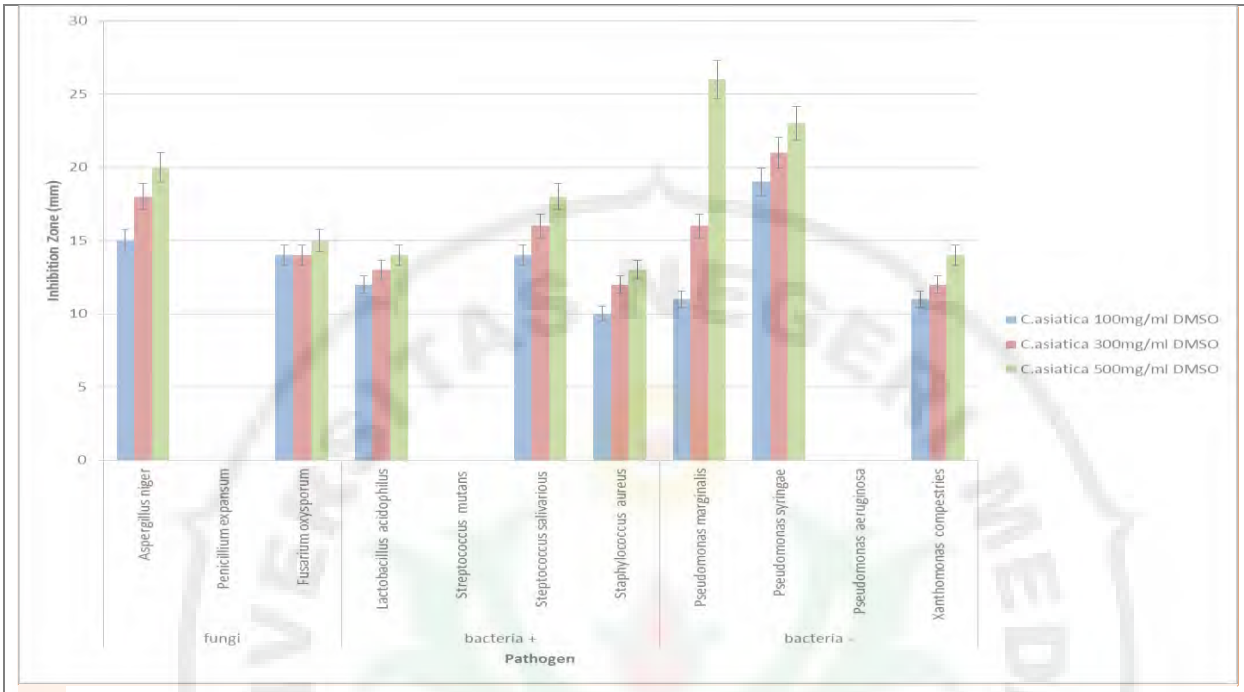
	aeruginosa								
	Xanthomonas compestries	13	14	17	11	11	12	14	153

(0) Value indicates no activity,Volume per well: 50µl, Borer size used: 6mm used Plant Methanolic extract concentrations (A =100, B=300, and C=500 mg/ DMSO ml) MIC-Minimum inhibitory concentration



[U17]

Fig.1a.Antimicrobial activity of methanol extracts*Premna pubescens*-Blume



[U18]

Fig. ~~2~~1b. Antimicrobial activity of methanol extracts*C. asiatica*

The results of MICs values are lowest at 66 [U19] andhighest at 153~~2~~ mg/ml for *P. pubescens* whereas 0~~.~~.155 mg/ml for *C.asatica*.The variation of antimicrobial activity of ~~our~~ these ~~-~~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. asatica* on *P. aeruginosa* and *S. mutans.*

DISCUSSION

These extracts are harmless [U20] and nonphytotoxic; it has been proved that extracts have inhibitory effects on germination and on the viability of fungal spores as well. Both plant extracts showed moderate to good activity against *A. niger*as it is 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 and dried prune. The effectiveness of the active compounds in plant extractscauses the production of

growthinhibition zones that appear as clear are as surrounding the wells. However, plant extract was unable to exhibit antibacterial activity against tested bacterial strains. These bacterial strains may have some kinds ofresistance mechanisms e.g.enzymatic inactivation, target sites modificationand decrease of intracellulardrugaccumulation (Santajit& Indrawattana, 2016) or the concentration of thecompoundusedmay notbe sufficient.

The adverse effects of *P. pubescens* consumption are reported to 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(Khairiah et al., 2017).The latex is highly toxic to human eyesandproduces sudden painless dimnessofvisionwithphotophobia (Micheloud et al., 2017). Several phytochemicals are identified in differentparts. *P. pubescens* flowers contain terpenes, multiflorenol, and cyclisadol (Tan et al., 2017). The latex contains caoutchouc, calotropin, calotoxin, calactin, uscharin, trypsin, voruscharin, uzarigenin, syriogenin and proceroside (Bezerra et al., 2017). Chemical constituents of *P. pubescens* flowersarelupeol, uscharin, proceroside, proceragenin (cardenolide), syriogenin,taraxast-20(30)-en-3-(4-methyl-3-pentenoate),3-thiazolinecardenolide, gigantin, giganteol, isogiganteol, uscharidin, uzarigenin voruscharin a, calotropeol, 3-epimoretenol, a-lactuceryl acetate and alactuceryl isovalerate (Wen et al., 2017). Rootbarkof *P. pubescens* contains triterpenes, A new norditerpenyl ester, namedcalotropterpenyl ester, and twounknown pentacyclictriterpinoids,namely calotropursenylacetateand calotropfriedelenylacetate, akundarolisovalerate, mundarol isovalerate and quercetin, 3-rutinoside (Wen et al., 2017). The principal active medicinals are asclepin and mudarin (Lam et al., 2016).

No inhibition was observed with controls, 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*.*Pseudomonasaeruginosaare* is wide-spread in soil, water and sewage can be considered as an indication oftheirinvolvementinthenaturalprocess of mineralization of organic matter. It has long been a troublesome cause of secondary infections of wound, especially burns, 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 (Miyazaki et al., 2016). *S.aureus* occur harmlessly as a normal flora of the skin and mucous membrane and itisoneofthecommonestbacterialpathogens encountered in the community causing severe food poisoning or minor skin infections to severe lifethreatening infections (Brooks et al.,2004).*C. asiatica* methanol extracthavingstrong inhibition activity against *P. aeruginosa and S. aureus* was previously reported by (Thangavel Arumugam et al. 2011).

CONCLUSION[U21]S

Premna pubescens and *Centella asiatica* extracts of showed antimicrobial activity against tested pathogens including antibiotic resistant strains. Further studies are being carried out in order to separate the individual components inplantextractsof *P. pubescens* and *C. asiatica* using column chromatographytodevelopbiopesticides as alternativetosynthetic agents. However, it isnecessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic propertie[U22]s.

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

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corresponding author[U1]

Running title[U2]

Author contribution[U3]

Conflict of interest[U4]

Significance statement [U5]

Abstract

Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine.*Premna pubescens* Blume (Buasbuas) has been used to increase the body immunity and endurance. *Centella asiatica*(Pegagan) used for medicinal purposes. Native to China, India and Indonesia recently have had interest in herb for its possible health benefits.The aim of the study was to investigate *in vitro*antimicrobial activity of North Sumatera medicinal plants *P. pubescens* and *C. asatica* against the important pathogens. To investigate *in vitro* antimicrobial activity of aerial parts of *P. pubescens* and *C. asatica*, that popularly used as folk medicines. **Materials and Methods:**The organic solvent plant extracts are tested on the various microorganisms including bacteria and fungi using agardiffusion technique.[i-[6]**Results:**The length of the inhibition zone was measured in millimeters from the edge of the well to the inhibition zone. *P. pubescens* showed significant to moderate activity against (14 mm) *Pseudomonas marginalis* and (21 mm) *Streptococcus mutans* with 100 mg/ml DMSO plant drug concentration. The results of (MICs) values are lowest at 66 and highest at 152 mg/ml for *P. pubescens* whereas 0 to 155 mg/ml for *C. asiatica*. **Conclusion:**The extracts were assessed to validate the potential activity of the medicinal plants against microbes. These studies wereconducted to separate the individual componentsof *P. pubescens* and *C. asatica* usingcolumnchromatography to developalternative synthetic agents. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties[U7].

Keywords: [U8]*Premna pubescens* Blume, *Centella asiatica*, antimicrobial, *Minimum inhibitory concentratio*MIC, Inhibition zone

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 (Rios & Recio, 2005[i-[9]). Nearly 6-7 thousand species of medicinal plants out of about 17-18 thousand flowering plants are known to be in use in folk and officially recognized systems of medicine in Indonesia, i.e., North Sumatra, Borneo, Celebes and Papua (Roosita et al., 2008).

North Sumatera Indonesia has a rich heritage of knowledge on plant baseddrugs bothforusein preventiveandcurativemedicin[U10]e (Khairiah et al., 2017).Sinceancient civilization, the variouspartsof different plants were used toeliminate pain,control suffering and counteract disease. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs(Suswardany et al., 2017). It has alsobeenwidelyobservedandacceptedthatthe medicinal value of plants lies in the bioactive phytocomponents present in the plants (Diningrat et al., 2015a; Restuati et al, 2016). Much work has been done on ethnomedicinal plants in North Sumatera Indonesia (Roosita et al., 2008; Khairiah et al., 2017;Diningrat et al., 2015b; Restuati et al, 2016). Medicinal plants represent a rich source of antimicrobial agents (Restuati et al, 2016; Johnson et al., 2010; Koochak et al., 2010). Because[U11] of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines (Perwitasari et al., 2016; Manaf et al., 2017).

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(Leeratiwong et al., 2016). In the

traditional North Sumatera Indonesian medicinal system, it has been used toincrease 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 (Khotimah & Muhtadi, 2017; (Wibowo & Harlia, 2016), and also increase appetite (Hidayat, 2015).Compounds derived from the plant have been found to have alkaloid, flavonoid, saponin, steroid, dan fenolik (Diningrat et al., 2015a; Restuati et al, 2016). Antimicrobial activity of *Premna pubescens*-Blume was previously screened by (Restuati et al, 2016).

Centella asiatica (Family Mackinlayaceae) common names include Pegagan, Asiatic Pennywort, North Sumatera Indonesian Pennywort, Luei Gong Gen, Takip, kohol, Antanan, Pegagan, Pegaga, vallaarai Kula kud, Bai Bua Bok and Brahmi. In North Sumatera Indonesia iswell known as "Pegagan" it is used to improve the mental ability (Dogra et al., 2016; Jayaprakash & Nagarajan, 2016). Antibacterial activity of *C.asiatica* was previously screened by (Lin et al., 2017).The aim of the study was to investigate in vitroantimicrobial activity of North Sumatera Indonesian medicinal plants *P. pubescens.* and *C. asiatica* against the important pathogens. In this paper the results of such studies are reported in order to orient future investigations towards the finding of potent, less toxic to human health and safe antimicrobial agents from natural sources.

MATERIALS AND METHODS[i-[12]

Plant materials and extraction: The whole plants of *P. ~~Pubescens~~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 [U13]with 95% (v/v) Methanol[U14] as solvent for 12 h was performed. The resultant extraction was frozen and dried for 24°C/48 h. Yield of Methanol extracts: 30% (Villalobos et al., 2016).

Test microorganisms: Microbial strains of clinical, plant and aquatic origin i.e. *Asperigellus niger*, *Pencilliumexpansum*, *Fusariumoxysporum*,*Xanthomonas compestries*, *Lactobacillus acidophilus*, *Pseudomonasmarginalis*,*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^{o}C overnight. The cells were harvestedby centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 min again and diluted innormal saline to obtain 5 x 10^{5} cfu/mL.

Determination of antimicrobial activity: The crude extracts of the different plant parts of different species were subjected to antimicrobial assay using the agar well diffusion method (Balouiri et al., 2016). About 20 ml of nutrient agar was dispensed into sterile universal bottles. Then inoculated with 0.2 ml of cultures mixed gently and poured into sterile petri dishes. After setting a number 3-cup borer (6 mm diameter) was properly sterilized by flaming and used to make three to five 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 50µl of the extract concentrations of 100, 300and 500 mg/ ml DMSO and allow diffusion for 45 minutes. The solvents used to reconstitute the extracts were similarly analyzed. The plates were incubated at 37°C for 24 hours for bacteria. The above procedure is allowed for

fungal assays, except the media potato dextrose agar instead of nutrient agar and incubates at 25°C for 48 hours. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in duplicates.

Statistical analysis[i-[15]

RESULTS

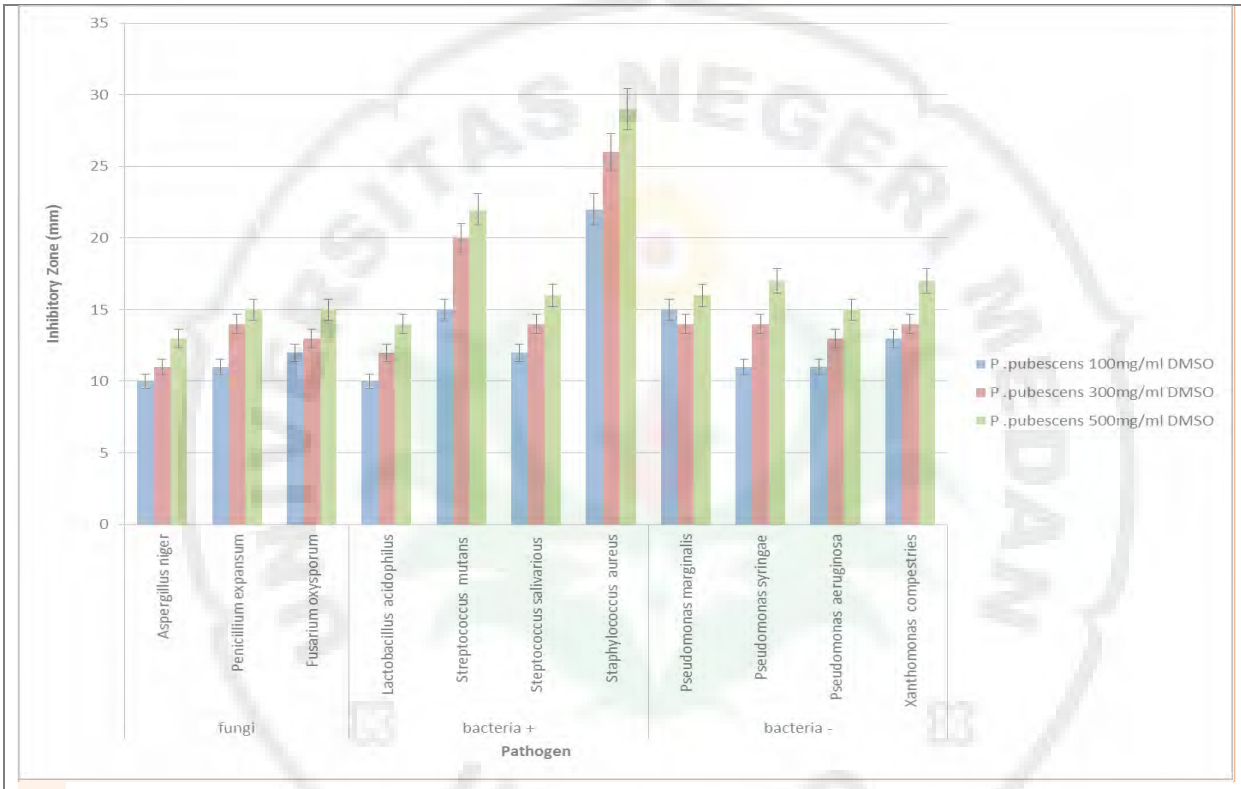
In the study of methanolic extract exhibited different degree of growth inhibition against the tested bacterial and fungal strains. ~~According to Fig. 1a, 2 band Table 1~~, methanolic extracts of *P. pubescens* and *C. asatica* exhibited considerable antimicrobial activity against testedmicrobial strains as shown in table 1 and figure 1a , b. *Premnapubescens* showed significant to moderate activity against *P. Marginalis*and *S. mutans*with 100 mg/ml DMSO plant drug concentration. *Centellaasiatica*issignificant against *P.syringae* andmoderate against other pathogens *F. oxysporum*, *L. acidophilus*, *S. ~~Salivarious~~salivarious*and *S. ~~Aureus~~aureus*with 100 mg/ml DMSO.

Table 1. Antimicrobial activity ofmethanolic extracts*Premna pubescens*~~.~~ Blume and*Centella asiatica*

Pathogen		*Premna pubescens*~~.~~ Blume				*Centellaasiatica*			
		A	B	C	MIC	A	B	C	MIC[U16]
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 (+)	*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 (-)	*Pseudomonas marginalis*	15	14	16	11	11	16	26	145
	Pseudomonas syringae	11	14	17	19	19	21	23	81
	Pseudomonas	11	13	15	0	0	0	0	0

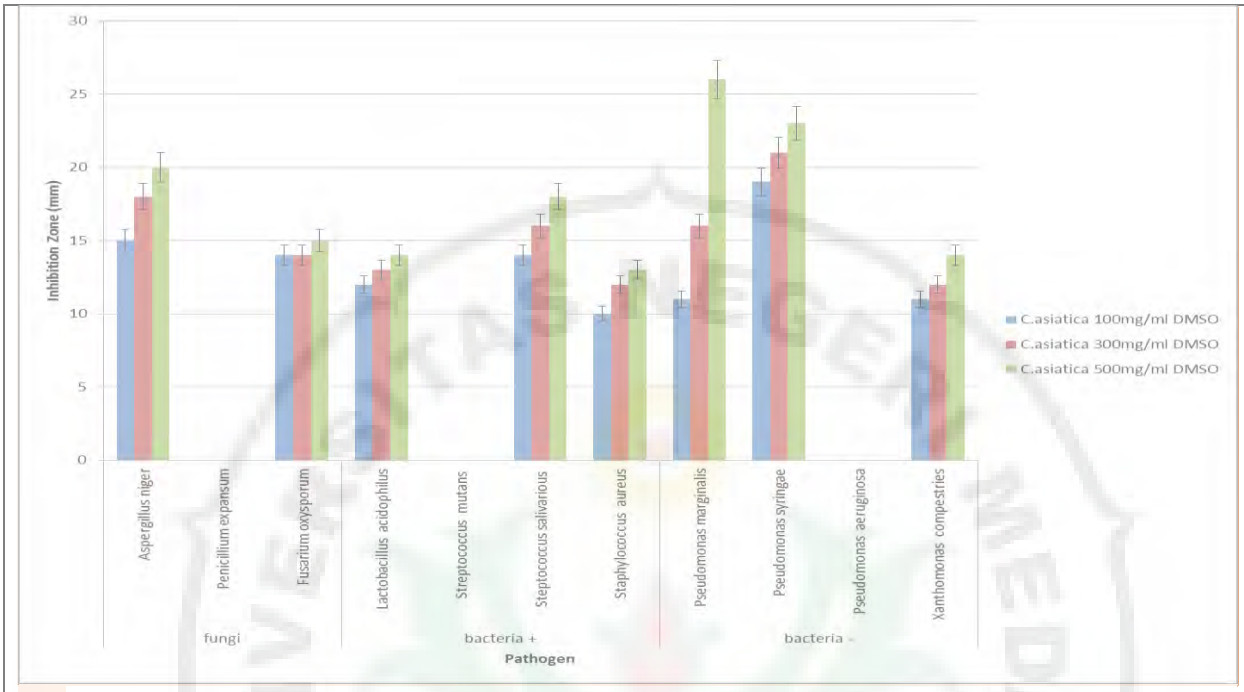
	aeruginosa								
	Xanthomonas compestries	13	14	17	11	11	12	14	153

(0) Value indicates no activity,Volume per well: 50µl, Borer size used: 6mm used Plant Methanolic extract concentrations (A =100, B=300, and C=500 mg/ DMSO ml) MIC-Minimum inhibitory concentration



[U17]

Fig.1a.Antimicrobial activity of methanol extracts*Premna pubescens*-Blume



[U18]

Fig. ~~2~~1b. Antimicrobial activity of methanol extracts*C. asiatica*

The results of MICs values are lowest at 66 [U19] andhighest at 153~~2~~ mg/ml for *P. pubescens* whereas 0~~.~~.155 mg/ml for *C.asatica*.The variation of antimicrobial activity of ~~our~~ these ~~-~~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. asatica* on *P. aeruginosa* and *S. mutans.*

DISCUSSION

These extracts are harmless [U20] and nonphytotoxic; it has been proved that extracts have inhibitory effects on germination and on the viability of fungal spores as well. Both plant extracts showed moderate to good activity against *A. niger*as it is 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 and dried prune. The effectiveness of the active compounds in plant extractscauses the production of

growthinhibition zones that appear as clear are as surrounding the wells. However, plant extract was unable to exhibit antibacterial activity against tested bacterial strains. These bacterial strains may have some kinds ofresistance mechanisms e.g.enzymatic inactivation, target sites modificationand decrease of intracellulardrugaccumulation (Santajit& Indrawattana, 2016) or the concentration of thecompoundusedmay notbe sufficient.

The adverse effects of *P. pubescens* consumption are reported to 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(Khairiah et al., 2017).The latex is highly toxic to human eyesandproduces sudden painless dimnessofvisionwithphotophobia (Micheloud et al., 2017). Several phytochemicals are identified in differentparts. *P. pubescens* flowers contain terpenes, multiflorenol, and cyclisadol (Tan et al., 2017). The latex contains caoutchouc, calotropin, calotoxin, calactin, uscharin, trypsin, voruscharin, uzarigenin, syriogenin and proceroside (Bezerra et al., 2017). Chemical constituents of *P. pubescens* flowersarelupeol, uscharin, proceroside, proceragenin (cardenolide), syriogenin,taraxast-20(30)-en-3-(4-methyl-3-pentenoate),3-thiazolinecardenolide, gigantin, giganteol, isogiganteol, uscharidin, uzarigenin voruscharin a, calotropeol, 3-epimoretenol, a-lactuceryl acetate and alactuceryl isovalerate (Wen et al., 2017). Rootbarkof *P. pubescens* contains triterpenes, A new norditerpenyl ester, namedcalotropterpenyl ester, and twounknown pentacyclictriterpinoids,namely calotropursenylacetateand calotropfriedelenylacetate, akundarolisovalerate, mundarol isovalerate and quercetin, 3-rutinoside (Wen et al., 2017). The principal active medicinals are asclepin and mudarin (Lam et al., 2016).

No inhibition was observed with controls, 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*.*Pseudomonasaeruginosaare* is wide-spread in soil, water and sewage can be considered as an indication oftheirinvolvementinthenaturalprocess of mineralization of organic matter. It has long been a troublesome cause of secondary infections of wound, especially burns, 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 (Miyazaki et al., 2016). *S.aureus* occur harmlessly as a normal flora of the skin and mucous membrane and itisoneofthecommonestbacterialpathogens encountered in the community causing severe food poisoning or minor skin infections to severe lifethreatening infections (Brooks et al.,2004).*C. asiatica* methanol extracthavingstrong inhibition activity against *P. aeruginosa and S. aureus* was previously reported by (Thangavel Arumugam et al. 2011).

CONCLUSION[U21]S

Premna pubescens and *Centella asiatica* extracts of showed antimicrobial activity against tested pathogens including antibiotic resistant strains. Further studies are being carried out in order to separate the individual components inplantextractsof *P. pubescens* and *C. asiatica* using column chromatographytodevelopbiopesticides as alternativetosynthetic agents. However, it isnecessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic propertie[U22]s.

ACKNOWLEDGEMENTS

We are thankful for constant encouragementandsupport from Biology Department, Mathematic and Natural Sciences, BOPTN Grants Funding [i-[23]Medan State University, Post-doctoral grants from the ministry of research, technology and higher education of Indonesia.

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REPLY TO REVIEWER'S COMMENTS SHEET

(Article No. 86564)

<7 days for implementing the changes>

Your paper has undergone first reviews. You received **two** reviews from **two** independent reviewers who don't know your identity. Their remarks are impartial, focused on the merit and academic quality of your paper. They are renowned professionals with huge experience in publishing and reviewing papers in your field of study. You may disagree with some of their remarks but keep in mind that the reviewers are experts in your chosen topic and they will help you improve your paper. It is normal even for experienced conservators and researchers to receive huge numbers of remarks from reviewers.

The authors are obliged by the publishing agreement to carry out all the changes advised by reviewers within a deadline set by the editor. Refusal or not providing the amended document on time will result in rejecting your paper for publication.

Overall comments:

Author is advised to re-write the full text carefully with the help of English Language Expert and correct the spelling, grammar, punctuation and vocabulary usage errors. Provide English Language Editing certificate

Serial No.	Part of the Manuscript	Reviewer's Comments	Response of Author
1	Corresponding Author	Who is the corresponding author? One author must be designated as "Corresponding Author". Name with contact details (E-mail address, full postal address and Telephone number) of corresponding author should be mentioned here clearly	Diky Setya Diningrat, dikysd@unimed.ac.id Jl. Willem Iskandar Pasar V Medan Estate, Medan, North Sumatra, Indonesia, +62 81361362400
2	Running Title	Provide the running title of the article as it is necessary according to the format of the journal.	It will be repaired based on the format journal
3	liveDNA	From January 1, 2017, its compulsory for all corresponding authors submitting papers to any Science Alert Journal to provide LiveDNA iDs (livedna.net) before final publication of their articles. With this standard identifier, you can create a	I will submit to LiveDNA

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4	Conflict of interest	A statement on conflicts of interest should be included in the manuscript. Either mention: 'none declared', or specify the authors' financial or other interests which should be known to the readers.	I will submit in cover letter
5	Author's contribution	There is a series of questions that will enable you to state the contributions of each author. Each author listed on the manuscript should have a real and concrete contribution to the submission. Every single person who contributed to the manuscript should be listed. More information about authorship can be collected from *Editorial Policies*	I will submit in cover letter
6	Significance statement	A statement about the significance of this research work should be included in the manuscript. The significance statement should provide the novelty aspect and significance of this research work with respect to the existing literature and more generally to the society. It should be a short summary which describe what this paper adds to and what was already known. **Start this statement with the following words:** This study discover the ---------- that can be beneficial for ..... **And the last sentence of this statement could be such as:** This study will help the researcher to uncover the critical areas of ---------- that many researchers were not able to explore. Thus a new theory on ---------- may be arrived at. **A Model Significance Statement:** This study discovers the possible synergistic effect of vitamin E, calcium, and vitamin D combination that can be beneficial for osteoporosis-induced ovariectomized rats. This study will help the researcher to uncover the critical area of postmenopausal bone loss that many researchers were not able to explore. Thus, a new theory on these micronutrients combination, and possibly other combinations, may be arrived at.	I will submit in cover letter
7	Abstract	Which Statistical method was being used to analyze data? Poorly written future recommendation should not be added in conclusion.	Statistical method used is ANAVA (analysis of variant) using software of

			SPSS 121 -Soxhlet used is the product of IWAKI SOXHLET-100 IWAKI soxhlet extractor 100 ML -methanol used is methanol compound P.A. 99,9% sigma-adrich
8	Introduction	References must be cited in the text in superscript digits at the end of sentence or paragraph before punctuation or full stop[1]. In case of two or more references, separate the superscript digits by comma[1,2,6]. Moreover, If there are more references but in continuous numbers then use dash between superscript digits[2-6].	Will be mentioned
9	Materials and Methods	When the study was carried out? mention the grading of chemich either analytical grade was used or other Please indicate both the manufacturer's name and location (including city, state, and country) for all specialized equipments, kits and reagents used in the experiment Which Statistical method was being used to analyze data?	Will be mentioned
10	keywords	highlighted keywords are not unique. Provide at least five key words. Key words should be unique and a concise summary of your paper's content and should capture the most important aspects of your paper	Will be provided
11	References	References must be in English language References are each must be numbered, ordered sequentially as they appear in the text	Will be repaired

		Some of the references are incomplete or not formatted properly. Such type of references are not acceptable. Go to Http://scialert.net and consult "references" from "guide to author" for having idea of reference categories and formatting	
	figures	Metion the unit in which it was measured Remove this figure as it caused repetition of the data that is represented in table 1	Will be mentioned
	acknowledgement	Funding source with relevant grant number should be mentioned in the Acknowledgement.	Will be mentioned
	conclusion	Poorly written conclude about the findings so that a reader should have a good idea of what you have investigated and discovered. future recommendation should be added separately	Will be added
	result	recheck it as lowest MIC value is 0 according to above table	0 is not the lowest MIC value because 0 has no bioactive compound activity in killing microbes on the contrast 66 MIC is the lowest one

Don't forget to make the changes

One of the biggest mistakes made by authors is to respond to all the comments, but forget to actually update the paper.
Don't be that person: one way to make sure you remember is to always include line numbers in your changes. That way, you actually have to make the change first before including the line numbers in your response.

How to prepare the revised paper?

1. Before you start editing the document, make sure Tracking changes is enabled! In MS Word go to Review menu and enable 'Track changes'.
2. You have to address every comment from both reviewers.
3. Mark your every change by adding a comment, excluding typos or similar minor editorial errors.

The comments should include a justification of the change (or lack of change!). In other words, answers to two questions:

- Why I made this change?
- What is the result?

If you decide to explain or justify why you believe your change (or lack of it) adds to the paper, always make sure to clearly indicate parts of paper you refer to (e.g. I mentioned this fact already in the Introduction, p. 1 paragraph 2. I believe bringing up this information there clarifies the issue from the very beginning).
Don't add comments with questions. Bear in mind that:

- Our editors won't answer them

- There will be only two stages of corrections – after major and minor review. There is no space for discussions with the reviewers.

If you remove something, ~~don't mark it like this~~. Instead, mark the whole paragraph by adding a comment with explanations of what you did and how it helps to improve the paper.
Summary: Track changes + put a comment with justification on every change you make

b. Cover letter
It is a good academic practice to attach a cover letter to submissions of new and revised papers to journals. Cover letter sent with the paper after major review usually includes:

- Courteous thanks to the editor, etc.
- General confirmation on carrying out all minor editorial corrections (typos etc.)
- Confirmation of implementing the requested changes. Explanations on general character of changes. You may mention 1-3 fundamental changes you made and explain how you dealt with it.
- A list of tasks given by the reviewer with precise indication where in the document you included the changes.
- Other comments those are important to the editor.

List of change after reviewed

1. Title '
   Before: Antimicrobial Profile of *Premna pubescens* Blume and *Centella asiatica* Extracts Against Bacteria and Fungi Pathogens
   After: Antimicrobial Profile of *Premna pubescens*. Blume and *Centella asiatica* Extracts Against Bacteria and Fungi Pathogens
2. Abstract:
   Before:
   Abstract
   Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine. *Premna pubescens*.Blume (Buasbuas) has been used to increase the body immunity and endurance. *Centella asiatica* (Pegagan) used for medicinal purposes. Native to China, India and Indonesia recently have had interest in herb for its possible health benefits.The aim of the study was to investigate in vitro antimicrobial activity of North Sumatera medicinal plants *P. pubescens* and *C. asatica* against the important pathogens. To investigate in vitro antimicrobial activity of aerial parts of *P. pubescens* and *C. asatica*, that popularly used as folk medicines. **Materials and Methods:** The organic solvent plant extracts are tested on the various microorganisms including bacteria and fungi using agardiffusion technique. **Results:** The length of the inhibition zone was measured in millimeters from the edge of the well to the inhibition zone. *P. pubescens* showed significant to moderate activity against (14 mm) *Pseudomonas marginalis* and (21 mm) *Streptococcus mutans* with 100 mg/ml DMSO plant drug concentration. The results of (MICs) values are lowest at 66 and highest at 152 mg/ml for *P. pubescens* whereas 0 to 155 mg/ml for *C. asiatica*. **Conclusion:** The extracts were assessed to validate the potential activity of the medicinal plants against microbes. These studies wereconducted to separate the individual componentsof *P. pubescens* and *C. asatica* using column chromatography to develop alternative synthetic agents. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties.
   After:

Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine. *Premna pubescens.* Blume (Buasbuas) has been used to increase the body immunity and endurance. *Centella asiatica* (Pegagan) used for medicinal purposes. This research is important performed to find out the antimicrobial capabilities of *P. pubescens* and *C. asiatica* methanol extracts. This research 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. asatica* against the important pathogens. **Materials and Methods:** The organic solvent plant extracts are tested on the various microorganisms including bacteria and fungi using agar diffusion technique. The data were analyzed with Anova statistics using SPSS software. **Results**: The length of the inhibition zone was measured in millimeters from the edge of the well to the inhibition zone. *P. pubescens* showed significant to moderate activity against (14 mm) *Pseudomonas marginalis* and (21 mm) *Streptococcus mutans* with 100 mg/ml DMSO plant drug concentration. The results of (MICs) values are lowest at 66 and highest at 152 mg/ml for *P. pubescens* whereas 0 to 155 mg/ml for *C. asiatica*. **Conclusion:** In general, based on the result of this research, it can be said that *P. pubescens* and *C. asatica* plants can be used as antibacterial and antifungal compounds.

3. Keyword:

   Before: *Premna pubescens. Blume, Centella asiatica*, antimicrobial, MIC, Inhibition zone

   After: *Premna pubescens.* Blume*, Centella asiatica*, antibacterial, antifungal, Minimal inhibitory concentration

4. Running title

   Before: -

   After:

Corresponding Author: Diky Setya Diningrat, Biology Department of Mathematics and Natural Sciences Faculty, Medan State University, Jl. Willem Iskandar Pasar V Medan, North Sumatera, Indonesia, Tel: +6181361362400 email: dikysd@unimed.ac.id

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

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

5. Citation

   Before: countries (Rios & Recio, 2005).

   After: countries[1].

6. Before: North Sumatera Indonesia has a rich heritage of knowledge on plant baseddrugs both for use in preventive and curative medicine (Khairiah et al., 2017).

   After: North Sumatra is very rich in plants that are believed to have medicinal properties for either prevention or treatment[7].

7. Before: Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines (Perwitasari et al., 2016; Manaf et al., 2017).

   After: Many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines, because of the side effects and the resistance that pathogenic microorganisms build against antibiotics[12,16].

8. Before: **Plant materials and extraction:** The whole plants of *P. Pubescenspubescens* 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 with 95% (v/v) Methanol as solvent for 12 h was performed. The resultant extraction was frozen and dried for 24°C/48 h. Yield of Methanol extracts: 30% (Villalobos et al., 2016).

   After: **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-adrich) as solvent for 12 h was performed. The resultant extraction was frozen and dried for 24°C/48 h. Yield of Methanol extracts: 30%[23].
9. Before: No mentioned of statistical analysis

   After: **Statistical analysis**: All data were statistically analyzed with SPSS software (version 16). One-way analysis of variance (ANOVA) was used to study significant difference among means with significance level at p=0.05[23].
10. Before:

RESULTS

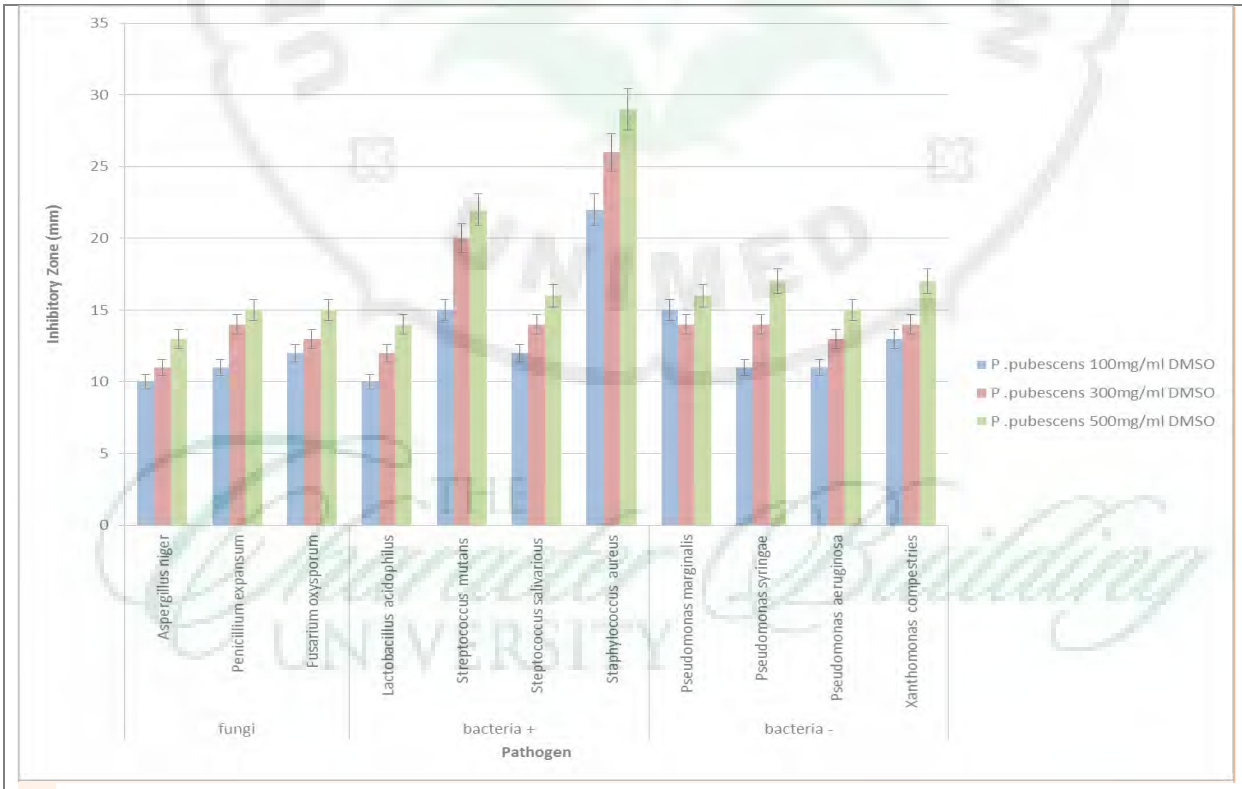
In the study of methanolic extract exhibited different degree of growth inhibition against the tested bacterial and fungal strains. ~~According to Fig. 1a, 2 band Table 1~~, methanolic extracts of *P. pubescens* and *C. asatica* exhibited considerable antimicrobial activity against testedmicrobial strains as shown in table 1 and figure 1a , b. *Premnapubescens* showed significant to moderate activity against *P. Marginalis*and *S. mutans*with 100 mg/ml DMSO plant drug concentration. *Centellaasiatica*issignificant against *P.syringae* andmoderate against other pathogens *F. oxysporum*, *L. acidophilus*, *S. ~~Salivarious~~salivarious*and *S. ~~Aureus~~aureus*with 100 mg/ml DMSO.

Table 1. Antimicrobial activity ofmethanolic extracts*Premna pubescens*~~.~~ Blume and*Centella asiatica*

Pathogen		*Premna pubescens*~~.~~ Blume				*Centellaasiatica*			
		A	B	C	MIC	A	B	C	MIC[U1]
Fungi	*Aspergillus*	10	11	13	153	15	18	20	66

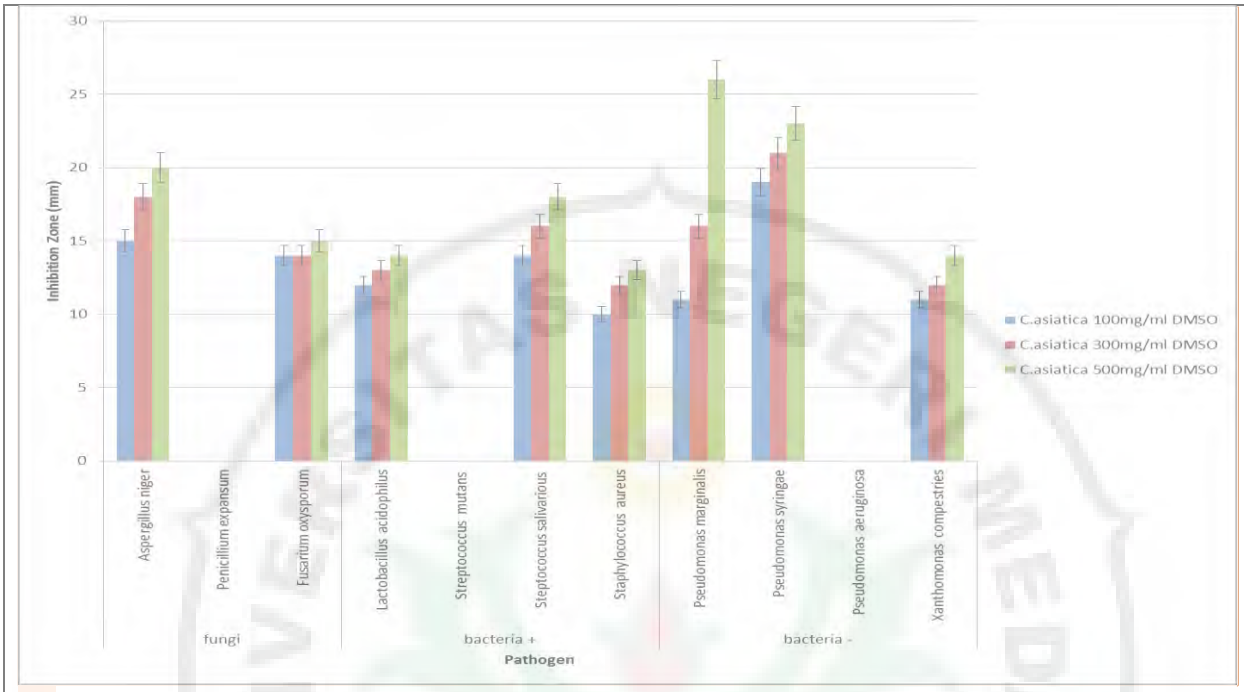
	niger								
	Penicillium expansum	11	14	15	101	0	0	0	0
	Fusarium oxysporum	12	13	15	105	14	14	15	96
Bacteria (+)	*Lactobacillus acidophilus*	10	12	14	121	12	13	14	156
	Streptococcus mutans	15	20	22	101	0	0	0	0
	Steptococcus salivarious	12	14	16	101	14	16	18	128
	Staphylococcus aureus	22	26	29	67	10	12	13	148
Bacteria (-)	*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

(0) Value indicates no activity,Volume per well: 50µl, Borer size used: 6mm used Plant Methanolic extract concentrations (A =100, B=300, and C=500 mg/ DMSO ml) MIC-Minimum inhibitory concentration



[U2]

Fig.1a.Antimicrobial activity of methanol extracts*Premna pubescens*-Blume



[U3]

Fig. ~~2~~1b. Antimicrobial activity of methanol extracts*C. asiatica*

After:

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. asatica* exhibited considerable antimicrobial activity against tested microbial strains. *Premna pubescens* showed significant to moderate activity against *P. marginalis* and *S. mutans* with 100 mg/ml DMSO plant drug 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 DMSO.

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

Pathogen		*Premna pubescens*. Blume				*Centella asiatica*			
		A	B	C	MIC (mg/ml)	A	B	C	MIC (mg/ml)
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 (+)	*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 (-)	*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

(0) Value indicates no activity,Volume per well: 50µl, Borer size used: 6mm used Plant Methanolic extract concentrations (A =100, B=300, and C=500 mg/ DMSO ml) MIC-Minimum inhibitory concentration

11. Before:

DISCUSSION

These extracts are harmless [U4] and nonphytotoxic; it has been proved that extracts have inhibitory effects on germination and on the viability of fungal spores as well.

After:

DISCUSSION

These extracts has been proved that extracts have inhibitory effects on germination and on the viability of fungal spores as well.

12. Before:

CONCLUSIONS

Premna pubescens and Centella asiatica extracts of showed antimicrobial activity against tested pathogens including antibiotic resistant strains. Further studies are being carried out in order to separate the individual components inplantextractsof P. pubescens and C. asiatica using column chromatographytodevelopbiopesticides as alternativetosynthetic agents. However, it isnecessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties.

After:

.

CONCLUSION

Premna pubescens and *Centella asiatica* extracts of 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 pharmaco-kinetic properties.

13. Before:
    ACKNOWLEDGEMENTS

    We are thankful for constant encouragementandsupport from Biology Department, Mathematic and Natural Sciences, BOPTN Grants Funding Medan State University, Post-doctoral grants from the ministry of research, technology and higher education of Indonesia.
    After:
    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.
14. Reference format:
    Before:

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After:

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REPLY TO REVIEWER'S COMMENTS SHEET

(Article No. 86564)

<7 days for implementing the changes>

Your paper has undergone first reviews. You received **two** reviews from **two** independent reviewers who don't know your identity. Their remarks are impartial, focused on the merit and academic quality of your paper. They are renowned professionals with huge experience in publishing and reviewing papers in your field of study. You may disagree with some of their remarks but keep in mind that the reviewers are experts in your chosen topic and they will help you improve your paper. It is normal even for experienced conservators and researchers to receive huge numbers of remarks from reviewers.

The authors are obliged by the publishing agreement to carry out all the changes advised by reviewers within a deadline set by the editor. Refusal or not providing the amended document on time will result in rejecting your paper for publication.

Overall comments:

Author is advised to re-write the full text carefully with the help of English Language Expert and correct the spelling, grammar, punctuation and vocabulary usage errors. Provide English Language Editing certificate

Serial No.	Part of the Manuscript	Reviewer's Comments	Response of Author
1	Corresponding Author	Who is the corresponding author? One author must be designated as "Corresponding Author". Name with contact details (E-mail address, full postal address and Telephone number) of corresponding author should be mentioned here clearly	Diky Setya Diningrat, dikysd@unimed.ac.id Jl. Willem Iskandar Pasar V Medan Estate, Medan, North Sumatra, Indonesia, +62 81361362400
2	Running Title	Provide the running title of the article as it is necessary according to the format of the journal.	It will be repaired based on the format journal
3	liveDNA	From January 1, 2017, its compulsory for all corresponding authors submitting papers to any Science Alert Journal to provide LiveDNA iDs (livedna.net) before final publication of their articles. With this standard identifier, you can create a	I will submit to LiveDNA

		profile of your research activities to distinguish yourself from other researchers with similar names, and make it easier for your colleagues to find your publications. To get LiveDNA, please go to the link: http://livedna.net/form.php	
4	Conflict of interest	A statement on conflicts of interest should be included in the manuscript. Either mention: 'none declared', or specify the authors' financial or other interests which should be known to the readers.	I will submit in cover letter
5	Author's contribution	There is a series of questions that will enable you to state the contributions of each author. Each author listed on the manuscript should have a real and concrete contribution to the submission. Every single person who contributed to the manuscript should be listed. More information about authorship can be collected from *Editorial Policies*	I will submit in cover letter
6	Significance statement	A statement about the significance of this research work should be included in the manuscript. The significance statement should provide the novelty aspect and significance of this research work with respect to the existing literature and more generally to the society. It should be a short summary which describe what this paper adds to and what was already known. **Start this statement with the following words:** This study discover the ---------- that can be beneficial for ..... **And the last sentence of this statement could be such as:** This study will help the researcher to uncover the critical areas of ---------- that many researchers were not able to explore. Thus a new theory on ---------- may be arrived at. **A Model Significance Statement:** This study discovers the possible synergistic effect of vitamin E, calcium, and vitamin D combination that can be beneficial for osteoporosis-induced ovariectomized rats. This study will help the researcher to uncover the critical area of postmenopausal bone loss that many researchers were not able to explore. Thus, a new theory on these micronutrients combination, and possibly other combinations, may be arrived at.	I will submit in cover letter
7	Abstract	Which Statistical method was being used to analyze data? Poorly written future recommendation should not be added in conclusion.	Statistical method used is ANAVA (analysis of variant) using software of

			SPSS 121 -Soxhlet used is the product of IWAKI SOXHLET-100 IWAKI soxhlet extractor 100 ML -methanol used is methanol compound P.A. 99,9% sigma-adrich
8	Introduction	References must be cited in the text in superscript digits at the end of sentence or paragraph before punctuation or full stop[1]. In case of two or more references, separate the superscript digits by comma[1,2,6]. Moreover, If there are more references but in continuous numbers then use dash between superscript digits[2-6].	Will be mentioned
9	Materials and Methods	When the study was carried out? mention the grading of chemich either analytical grade was used or other Please indicate both the manufacturer's name and location (including city, state, and country) for all specialized equipments, kits and reagents used in the experiment Which Statistical method was being used to analyze data?	Will be mentioned
10	keywords	highlighted keywords are not unique. Provide at least five key words. Key words should be unique and a concise summary of your paper's content and should capture the most important aspects of your paper	Will be provided
11	References	References must be in English language References are each must be numbered, ordered sequentially as they appear in the text	Will be repaired

		Some of the references are incomplete or not formatted properly. Such type of references are not acceptable. Go to Http://scialert.net and consult "references" from "guide to author" for having idea of reference categories and formatting	
	figures	Metion the unit in which it was measured Remove this figure as it caused repetition of the data that is represented in table 1	Will be mentioned
	acknowledgement	Funding source with relevant grant number should be mentioned in the Acknowledgement.	Will be mentioned
	conclusion	Poorly written conclude about the findings so that a reader should have a good idea of what you have investigated and discovered. future recommendation should be added separately	Will be added
	result	recheck it as lowest MIC value is 0 according to above table	0 is not the lowest MIC value because 0 has no bioactive compound activity in killing microbes on the contrast 66 MIC is the lowest one

Don't forget to make the changes

One of the biggest mistakes made by authors is to respond to all the comments, but forget to actually update the paper.
Don't be that person: one way to make sure you remember is to always include line numbers in your changes. That way, you actually have to make the change first before including the line numbers in your response.

How to prepare the revised paper?

1. Before you start editing the document, make sure Tracking changes is enabled! In MS Word go to Review menu and enable 'Track changes'.
2. You have to address every comment from both reviewers.
3. Mark your every change by adding a comment, excluding typos or similar minor editorial errors.

The comments should include a justification of the change (or lack of change!). In other words, answers to two questions:

- Why I made this change?
- What is the result?

If you decide to explain or justify why you believe your change (or lack of it) adds to the paper, always make sure to clearly indicate parts of paper you refer to (e.g. I mentioned this fact already in the Introduction, p. 1 paragraph 2. I believe bringing up this information there clarifies the issue from the very beginning).
Don't add comments with questions. Bear in mind that:

- Our editors won't answer them

- There will be only two stages of corrections – after major and minor review. There is no space for discussions with the reviewers.

If you remove something, ~~don't mark it like this~~. Instead, mark the whole paragraph by adding a comment with explanations of what you did and how it helps to improve the paper.
Summary: Track changes + put a comment with justification on every change you make

b. Cover letter
It is a good academic practice to attach a cover letter to submissions of new and revised papers to journals. Cover letter sent with the paper after major review usually includes:

- Courteous thanks to the editor, etc.
- General confirmation on carrying out all minor editorial corrections (typos etc.)
- Confirmation of implementing the requested changes. Explanations on general character of changes. You may mention 1-3 fundamental changes you made and explain how you dealt with it.
- A list of tasks given by the reviewer with precise indication where in the document you included the changes.
- Other comments those are important to the editor.

List of change after reviewed

1. Title '
   Before: Antimicrobial Profile of *Premna pubescens* Blume and *Centella asiatica* Extracts Against Bacteria and Fungi Pathogens
   After: Antimicrobial Profile of *Premna pubescens*. Blume and *Centella asiatica* Extracts Against Bacteria and Fungi Pathogens
2. Abstract:
   Before:
   Abstract
   Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine. *Premna pubescens*.Blume (Buasbuas) has been used to increase the body immunity and endurance. *Centella asiatica* (Pegagan) used for medicinal purposes. Native to China, India and Indonesia recently have had interest in herb for its possible health benefits.The aim of the study was to investigate in vitro antimicrobial activity of North Sumatera medicinal plants *P. pubescens* and *C. asatica* against the important pathogens. To investigate in vitro antimicrobial activity of aerial parts of *P. pubescens* and *C. asatica*, that popularly used as folk medicines. **Materials and Methods:** The organic solvent plant extracts are tested on the various microorganisms including bacteria and fungi using agardiffusion technique. **Results:** The length of the inhibition zone was measured in millimeters from the edge of the well to the inhibition zone. *P. pubescens* showed significant to moderate activity against (14 mm) *Pseudomonas marginalis* and (21 mm) *Streptococcus mutans* with 100 mg/ml DMSO plant drug concentration. The results of (MICs) values are lowest at 66 and highest at 152 mg/ml for *P. pubescens* whereas 0 to 155 mg/ml for *C. asiatica*. **Conclusion:** The extracts were assessed to validate the potential activity of the medicinal plants against microbes. These studies wereconducted to separate the individual componentsof *P. pubescens* and *C. asatica* using column chromatography to develop alternative synthetic agents. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties.
   After:

Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine. *Premna pubescens.* Blume (Buasbuas) has been used to increase the body immunity and endurance. *Centella asiatica* (Pegagan) used for medicinal purposes. This research is important performed to find out the antimicrobial capabilities of *P. pubescens* and *C. asiatica* methanol extracts. This research 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. asatica* against the important pathogens. **Materials and Methods:** The organic solvent plant extracts are tested on the various microorganisms including bacteria and fungi using agar diffusion technique. The data were analyzed with Anova statistics using SPSS software. **Results**: The length of the inhibition zone was measured in millimeters from the edge of the well to the inhibition zone. *P. pubescens* showed significant to moderate activity against (14 mm) *Pseudomonas marginalis* and (21 mm) *Streptococcus mutans* with 100 mg/ml DMSO plant drug concentration. The results of (MICs) values are lowest at 66 and highest at 152 mg/ml for *P. pubescens* whereas 0 to 155 mg/ml for *C. asiatica*. **Conclusion:** In general, based on the result of this research, it can be said that *P. pubescens* and *C. asatica* plants can be used as antibacterial and antifungal compounds.

3. Keyword:

   Before: *Premna pubescens. Blume, Centella asiatica*, antimicrobial, MIC, Inhibition zone

   After: *Premna pubescens.* Blume*, Centella asiatica*, antibacterial, antifungal, Minimal inhibitory concentration

4. Running title

   Before: -

   After:

Corresponding Author: Diky Setya Diningrat, Biology Department of Mathematics and Natural Sciences Faculty, Medan State University, Jl. Willem Iskandar Pasar V Medan, North Sumatera, Indonesia, Tel: +6181361362400 email: dikysd@unimed.ac.id

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

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

5. Citation

   Before: countries (Rios & Recio, 2005).

   After: countries[1].

6. Before: North Sumatera Indonesia has a rich heritage of knowledge on plant baseddrugs both for use in preventive and curative medicine (Khairiah et al., 2017).

   After: North Sumatra is very rich in plants that are believed to have medicinal properties for either prevention or treatment[7].

7. Before: Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines (Perwitasari et al., 2016; Manaf et al., 2017).

   After: Many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines, because of the side effects and the resistance that pathogenic microorganisms build against antibiotics[12,16].

8. Before: **Plant materials and extraction:** The whole plants of *P. Pubescenspubescens* 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 with 95% (v/v) Methanol as solvent for 12 h was performed. The resultant extraction was frozen and dried for 24°C/48 h. Yield of Methanol extracts: 30% (Villalobos et al., 2016).

   After: **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-adrich) as solvent for 12 h was performed. The resultant extraction was frozen and dried for 24°C/48 h. Yield of Methanol extracts: 30%[23].
9. Before: No mentioned of statistical analysis

   After: **Statistical analysis**: All data were statistically analyzed with SPSS software (version 16). One-way analysis of variance (ANOVA) was used to study significant difference among means with significance level at p=0.05[23].
10. Before:

RESULTS

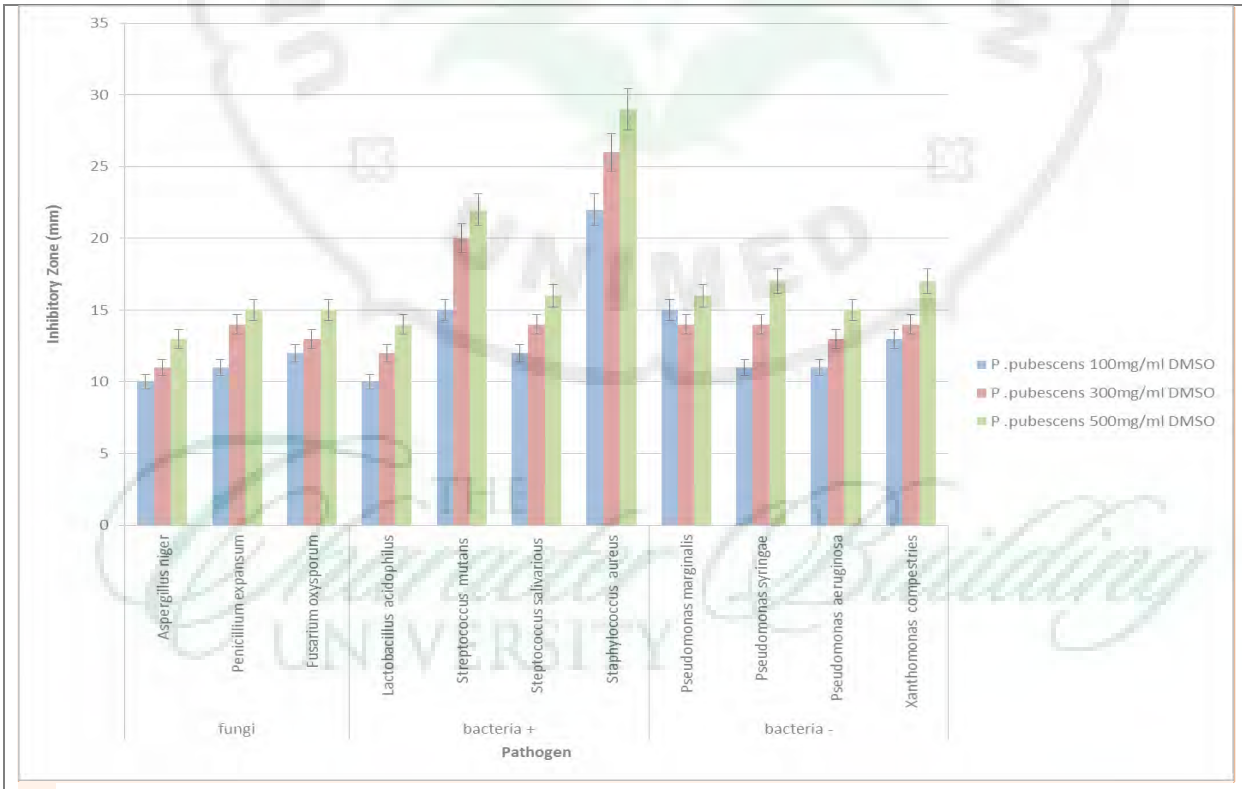
In the study of methanolic extract exhibited different degree of growth inhibition against the tested bacterial and fungal strains. ~~According to Fig. 1a, 2 band Table 1~~, methanolic extracts of *P. pubescens* and *C. asatica* exhibited considerable antimicrobial activity against testedmicrobial strains as shown in table 1 and figure 1a , b. *Premnapubescens* showed significant to moderate activity against *P. Marginalis*and *S. mutans*with 100 mg/ml DMSO plant drug concentration. *Centellaasiatica*issignificant against *P.syringae* andmoderate against other pathogens *F. oxysporum*, *L. acidophilus*, *S. ~~Salivarious~~salivarious*and *S. ~~Aureus~~aureus*with 100 mg/ml DMSO.

Table 1. Antimicrobial activity ofmethanolic extracts*Premna pubescens*~~.~~ Blume and*Centella asiatica*

Pathogen		*Premna pubescens*~~.~~ Blume				*Centellaasiatica*			
		A	B	C	MIC	A	B	C	MIC[U1]
Fungi	*Aspergillus*	10	11	13	153	15	18	20	66

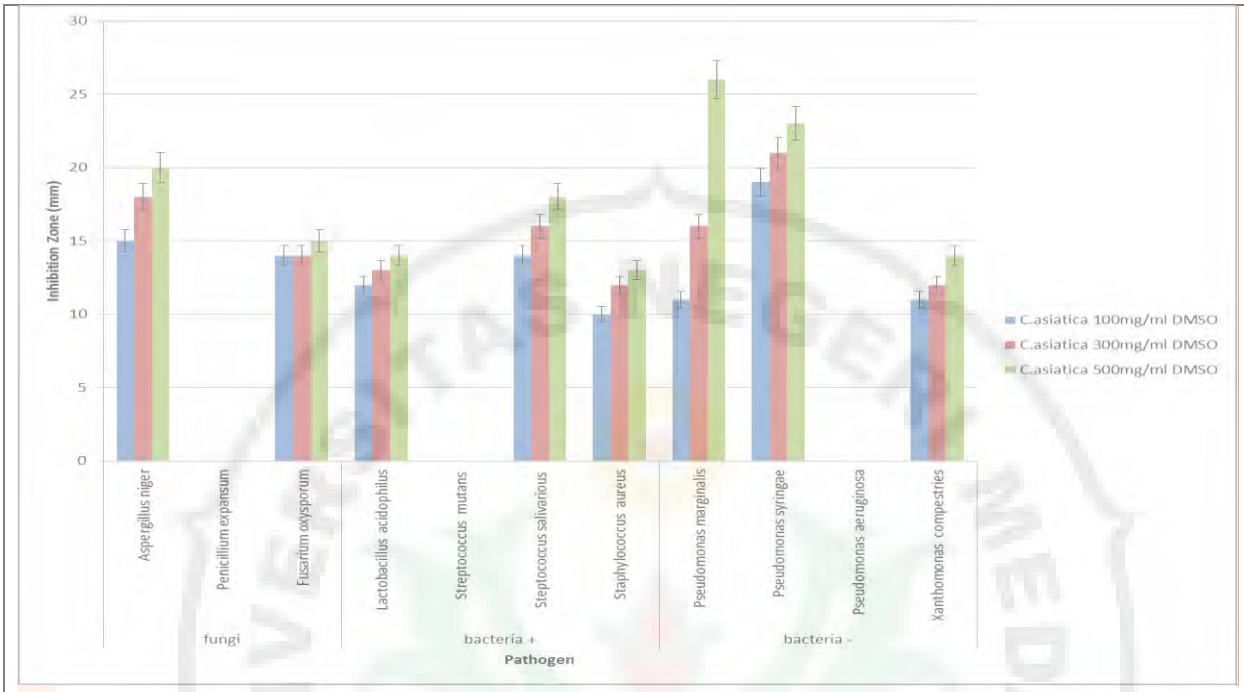
	niger								
	Penicillium expansum	11	14	15	101	0	0	0	0
	Fusarium oxysporum	12	13	15	105	14	14	15	96
Bacteria (+)	*Lactobacillus acidophilus*	10	12	14	121	12	13	14	156
	Streptococcus mutans	15	20	22	101	0	0	0	0
	Steptococcus salivarious	12	14	16	101	14	16	18	128
	Staphylococcus aureus	22	26	29	67	10	12	13	148
Bacteria (-)	*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

(0) Value indicates no activity,Volume per well: 50µl, Borer size used: 6mm used Plant Methanolic extract concentrations (A =100, B=300, and C=500 mg/ DMSO ml) MIC-Minimum inhibitory concentration



[U2]

Fig.1a.Antimicrobial activity of methanol extracts*Premna pubescens*-Blume



[U3]

Fig. ~~2~~1b. Antimicrobial activity of methanol extracts*C. asiatica*

After:

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. asatica* exhibited considerable antimicrobial activity against tested microbial strains. *Premna pubescens* showed significant to moderate activity against *P. marginalis* and *S. mutans* with 100 mg/ml DMSO plant drug 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 DMSO.

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

Pathogen		*Premna pubescens*. Blume				*Centella asiatica*			
		A	B	C	MIC (mg/ml)	A	B	C	MIC (mg/ml)
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 (+)	*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 (-)	*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

(0) Value indicates no activity,Volume per well: 50µl, Borer size used: 6mm used Plant Methanolic extract concentrations (A =100, B=300, and C=500 mg/ DMSO ml) MIC-Minimum inhibitory concentration

11. Before:

DISCUSSION

These extracts are harmless [U4] and nonphytotoxic; it has been proved that extracts have inhibitory effects on germination and on the viability of fungal spores as well.

After:

DISCUSSION

These extracts has been proved that extracts have inhibitory effects on germination and on the viability of fungal spores as well.

12. Before:

CONCLUSIONS

Premna pubescens and Centella asiatica extracts of showed antimicrobial activity against tested pathogens including antibiotic resistant strains. Further studies are being carried out in order to separate the individual components inplantextractsof P. pubescens and C. asiatica using column chromatographytodevelopbiopesticides as alternativetosynthetic agents. However, it isnecessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties.

After:

.

CONCLUSION

Premna pubescens and *Centella asiatica* extracts of 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 pharmaco-kinetic properties.

13. Before:
    ACKNOWLEDGEMENTS

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    After:
    ACKNOWLEDGEMENTS

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14. Reference format:
    Before:

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

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Running title

Antibacterial and Antifungal Effect of *Premna pubescens*. Blume and *Centella asiatica* Ethanol Extracts

Author contribution

Name of the author and e-mail ID	Types of contribution
Dr. Diky Setya Diningrat	Dr. Diky Setya Diningrat researched extract test on microbe
Dr. Martina Restuati	Dr. Martina Restuati researched extract of bioactive compound from *Premna pubescens*. Blume and *Centella asiatica*

Significance statement [H1]

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.

Abstract

Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge onmedicinal 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 research is important to find out the antimicrobial capabilities of *P. pubescens* and *C. asiatica* methanol extracts.This research 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. asatica* 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 were analyzed with Anova statistics by using SPSS software. **Results:** The length of the inhibition zone was measured in millimeters from the edge of the well to the inhibition zone. *P. pubescens*showed significantmoderate activity against (14 mm) *Pseudomonas marginalis* and (21 mm) *Streptococcus mutans* with 100 mg/ml DMSO plant drug concentration. The results of lowest (MICs) values are at 66 and highest ones are at 152 mg/ml for *P. pubescens* meanwhile

those of (MICs) values are 0 to 155 mg/ml for *C. asiatica*. **Conclusion:**In general, based on the result of this research, it can be said that *P. pubescens* and *C. asatica* plants can be used as antibacterial and antifungal compounds.

Keywords: *Premna pubescens*. Blume, *Centella asiatica*, antibacterial, antifungal, Minimal inhibitory concentration

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LiveDNA: I attached my registration form

Competing Interest: The authors have declared that no competing 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 countrie. Nearly 6-7 thousand species of medicinal plants from around about 17-18 thousand flowering plants are known as traditional medicineand 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 variouspartsof different plants were used toeliminate 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 alsobeenwidelyobservedandacceptedthatthe 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[3,6,7,8]. Medicinal plants represent a

rich source of antimicrobial agents[6,9,10,11]. 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[10,13].

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[13]. In North Sumatera Indonesian traditionally medicinal system, it has been used toincrease 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[14,15] and also increase appetite[15,16]. 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, vallaarai Kula kud, Bai Bua Bok and Brahmi. In North Sumatera Indonesia is well-known as "Pegagan" this termis used to improve the mental ability[6,8]. Antibacterial activity of *C.asiatica* was previously screened[9]. 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 paper there sults of such studies are reported in order to orient future investigations towards the finding of potent, less toxic to human healt hand safe antimicrobial agents from natural sources.

MATERIALS AND METHODS

Plant materials and extraction: This research project was conducted fromJuly 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%[17,18].

Test microorganisms: Microbial strains of clinical, plant and aquatic origin i.e. *Asperigellus niger*, *Pencillium expansum*, *Fusariumoxysporum*, *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^{o}C overnight. The cells were harvestedby centrifuging at 4000 rpm for 5 min, washed with normal saline, spin at 4000 rpm for 5 min again and diluted innormal saline to obtain 5 x 10^{5} cfu/mL.

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 three to five

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 50µl of the extract concentrations of 100, 300and 500 mg/ ml DMSO and allow diffusion for 45 minutes. The solvents used to reconstitutethe extracts were similarly analyzed. The plates were incubated at 37°C for 24 hours for bacteria. The procedure above is also allowed for fungal assays, however media used is not nutrient agar but potato dextrose agarand incubated at 25°C for 48 hours. 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[17,18].

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. asatica* exhibited considerable antimicrobial activity against tested microbial strains. *Premna pubescens* showed significant moderate activity against *P. marginalis* and *S. mutans* with 100 mg/ml 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 DMSO.

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

Pathogen		*Premna pubescens*. Blume				*Centella asiatica*			
		A	B	C	MIC (mg/ml)	A	B	C	MIC (mg/ml)
Fungi	*Aspergillus niger*	10	11	13	153	15	18	20	66
	Penicillium expansum	11	14	15	101	0	0	0	0
	Fusarium	12	13	15	105	14	14	15	96

	oxysporum								
Bacteria (+)	*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 (-)	*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

(0) Value indicates no activity,Volume per well: 50µl, Borer size used: 6mm used Plant Methanolic extract concentrations (A =100, B=300, and C=500 mg/ DMSO ml) MIC-Minimum inhibitory concentration

The results of lowest MICs value are at 66 and highest at 153 mg/ml for *P. pubescens* meanwhile those of highest ones are at 0,155 mg/ml for *C. asatica*. 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. asatica* 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 clearas around the wells. However, plant extract was unable to exhibit antibacterial activity against tested bacterial

strains. These bacterial strains may have some kinds ofresistance mechanisms e.g. enzymatic inactivation, target sites modificationand decrease of intracellulardrugaccumulation[19] 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[3,11]. Several phytochemicals are identified in differentparts. *P. pubescens* flowers contain terpenes, multiflorenol, and cyclisadol[20]. The latex contains caoutchouc, calotropin, calotoxin, calactin, uscharin, trypsin, voruscharin, uzarigenin, syriogenin and proceroside[15]. Chemical constituents of *P. pubescens* flowersarelupeol, uscharin, proceroside, proceragenin (cardenolide), syriogenin, taraxast-20(30)-en-3-(4-methyl-3-pentenoate), 3-thiazolinecardenolide, gigantin, giganteol, isogiganteol, uscharidin, uzarigenin voruscharin a, calotropeol, 3-epimoretenol, a-lactuceryl acetate and alactuceryl isovalerate[21]. Rootbarkof *P. pubescens* contains triterpenes, a new norditerpenyl esternamed as calotropterpenyl ester, and two unknown pentacyclic triterpinoids named as calotropursenylacetate and calotropfriedelenylacetate, akundarolisovalerate, mundarol isovalerate and quercetin, 3-rutinoside[21]. The principal active medicines are asclepin and mudarin[22].

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 iswide-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, 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[23]. *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 lifethreatening infections[21]. *C. asiatica* methanol extracthavingstrong inhibition activity against *P. aeruginosa and S. aureus* was previously reported[24].

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 isnecessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties.

ACKNOWLEDGEMENTS

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Antimicrobial Profile of *Premna pubescens.* Blumeand *Centella asiatica*Extracts AgainstBacteria and FungiPathogens

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Running title

Antibacterial and Antifungal Effect of *Premnapubescens*. Blume and *Centellaasiatica* Ethanol Extracts

Author contribution

Name of the author and e-mail ID	Types of contribution
Dr. Diky Setya Diningrat	Dr. Diky Setya Diningrat researched extract test on microbe
Dr. Martina Restuati	Dr. Martina Restuati researched extract of bioactive compound from *Premnapubescens*.Blume and *Centellaasiatica*

Significance statement [H1]

The significance of research compares antibacterial and antifungal compounds effect from 2 species of plant commonly believed to be traditional efficacious medicine in north Sumatra.

Abstract

Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge onmedicinal plantsused for preventiveand 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 research is important to find out the antimicrobial capabilities of *P. pubescens*and *C. asiatica* methanol extracts.This research 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 vitroantimicrobial activity of North Sumatera medicinal plants *P. pubescens* and *C. asatica* against the main pathogens.**Materials and Methods:**The organic solvent plant extracts are tested on the various microorganisms including bacteria and fungi by using agardiffusion technique. The data were analyzed with Anova statistics by using SPSS software. **Results:** The length of the inhibition zone was measured in millimeters from the edge of the well to the inhibition zone. *P. pubescens*showed significantmoderate activity against (14 mm) *Pseudomonas marginalis* and (21 mm) *Streptococcus mutans* with 100 mg/ml DMSO plant drug concentration. The results of lowest (MICs) values are at 66 and highest ones are at 152 mg/ml for *P. pubescens*meanwhile those of (MICs) values are 0 to 155 mg/ml for *C. asiatica*. **Conclusion:**In general, based on the result of this research, it can be said that *P. pubescens* and *C. asatica*plants can be used as antibacterial and antifungal compounds.

Keywords: *Premna pubescens*. Blume, *Centella asiatica*, antibacterial, antifungal, Minimal inhibitory concentration

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Competing Interest: The authors have declared that no competing 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 countrie. Nearly 6-7 thousand species of medicinal plants from around about 17-18 thousand flowering plants are known as traditional medicineand 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 variouspartsof different plants were used toeliminate 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 alsobeenwidelyobservedandacceptedthatthe 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[3,6,7,8]. Medicinal plants represent a rich source of antimicrobial agents[6,9,10,11]. 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[10,13].

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[13]. In North Sumatera Indonesian traditionally medicinal system, it has been used toincrease 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[14,15]and also increase appetite[15,16].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)commonlynames Pegagan, Asiatic Pennywort, North Sumatera Indonesian Pennywort, Luei Gong Gen, Takip, kohol, Antanan, Pegagan, Pegaga, vallaarai Kula kud, Bai Bua Bok and Brahmi. In North Sumatera Indonesia iswell-known as "Pegagan" this termis used to improve the mental ability[6,8]. Antibacterial activity of *C.asiatica* was previously screened[9].The aim of the studywasto investigate in vitroantimicrobialactivityofNorth Sumatera Indonesiamedicinalplants *P. pubescens* and *C. asiatica* against the main pathogens. Inthispapertheresultsofsuch studies are reported in order to orient future investigations towards the findingof potent,lesstoxictohumanhealthandsafe antimicrobialagentsfromnaturalsources.

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No inhibition was observed with controlwhich 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 indicatingthat 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*.*Pseudomonasaeruginosaare* which iswide-spread in soil, water and sewage can be considered as an indication oftheirinvolvementinthenaturalprocess of mineralization of organic matter. It has long been a troublesome cause of secondary infections of wound, especially burns, giving rise to blue

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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 isnecessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties.

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Antimicrobial Profile of *Premna pubescens.* Blumeand *Centella asiatica*Extracts AgainstBacteria and FungiPathogens

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Running title[u1]

Author contribution

Name of the author and e-mail ID	Types of contribution
Dr. Diky Setya Diningrat	Dr. Diky Setya Diningrat researched extract test on microbe
Dr. Martina Restuati	Dr. Martina Restuati researched extract of bioactive compound from *Premnapubescens*.Blume and *Centellaasiatica*

Significance statement [u2]

The significance of research compares anti-microbial compound from 2 species of plant commonly believed to be traditional efficacious medicine in north Sumatra. Microbe is used as completely as representing ordinary pathogen which functions in human life and it comes from group of positive and negative Gram bacteria as well as fungus group

Abstract

Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge onmedicinal plantsused for preventiveand 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 research is important to find out the antimicrobial capabilities of *P. pubescens*and *C. asiatica* methanol extracts.This research 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 vitroantimicrobial activity of North Sumatera medicinal plants *P. pubescens* and *C. asatica* against the main pathogens.**Materials and Methods:**The organic solvent plant extracts are tested on the various microorganisms including bacteria and fungi by using agardiffusion technique. The data were analyzed with Anova statistics by using SPSS software. **Results:** The length of the inhibition zone was measured in millimeters from the edge of the well to the inhibition zone. *P. pubescens*showed significantmoderate activity against (14 mm) *Pseudomonas marginalis* and (21 mm) *Streptococcus mutans* with 100 mg/ml DMSO plant drug concentration. The results of lowest (MICs) values are at 66 and highest ones are at 152 mg/ml for *P. pubescens*meanwhile those of (MICs) values are 0 to 155 mg/ml for *C. asiatica*. **Conclusion:**In general, based on the result of this research, it can be said that *P. pubescens* and *C. asatica*plants can be used as antibacterial and antifungal compounds.

Keywords: *Premna pubescens*. Blume, *Centella asiatica*, antibacterial, antifungal, Minimal inhibitory concentration

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Competing Interest: The authors have declared that no competing 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 countrie. Nearly 6-7 thousand species of medicinal plants from around about 17-18 thousand flowering plants are known as traditional medicineand officially recognized as medicine system in Indonesia, i.e., North Sumatra, Borneo, Celebes and Papua[1,3,18,19].[u3]

North Sumatra is very rich in plants that are believed to have medicinal properties for either prevention or treatment[7]. Since ancient civilization, the variouspartsof different plants were used toeliminate pain,control suffering and counteract disease. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs[21]. It has alsobeenwidelyobservedandacceptedthatthe medicinal value of plants lies in the bioactive phytocomponents living in the plants[3,13]. Much work has been done on ethnomedicinal plants in North Sumatera Indonesia[19,7,4,13]. Medicinal plants represent a rich source of antimicrobial agents[11,12,13,14]. 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[12,16].

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[10]. In North Sumatera Indonesian traditionally medicinal system, it has been used toincrease 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[8,25]and also increase appetite[1,2].Compounds derived from the plant have been found contain alkaloid, flavonoid, saponin, steroid, dan fenolik[3,13]. Antimicrobial activity of *Premna pubescens.* Blume was previously screened[13].

Centella asiatica(Family Mackinlayaceae)commonlynames Pegagan, Asiatic Pennywort, North Sumatera Indonesian Pennywort, Luei Gong Gen, Takip, kohol, Antanan, Pegagan, Pegaga, vallaarai Kula kud, Bai Bua Bok and Brahmi. In North Sumatera Indonesia iswell-known as "Pegagan" this termis used to improve the mental ability[5,6]. Antibacterial activity of *C.asiatica* was previously screened[11].The aim of the studywasto investigate in vitroantimicrobialactivityofNorth Sumatera Indonesiamedicinalplants *P. pubescens* and *C. asiatica* against the main pathogens. Inthispapertheresultsofsuch studies are reported in order to orient future investigations towards the findingof potent,lesstoxictohumanhealthandsafe antimicrobialagentsfromnaturalsources.

MATERIALS AND METHODS

Plant materials and extraction: This research project was conducted fromJuly 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-adrich) as solvent for 12 h was performed. The resultant extraction was frozen and dried for 24°C/48 h. Yield of Methanol extracts: 30%[23].

Test microorganisms: Microbial strains of clinical, plant and aquatic origini.e. *Asperigellusniger*, *Pencilliumexpansum*, *Fusariumoxysporum*,*Xanthomonas compestries*, *Lactobacillus acidophilus*, *Pseudomonasmarginalis*,*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^{o}C overnight. The cells were harvestedby centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 min again and diluted innormal saline to obtain 5 x 10^{5} cfu/mL.

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 three to five 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 50µl of the extract concentrations of 100,

300and 500 mg/ ml DMSO and allow diffusion for 45 minutes. The solvents used to reconstitutethe extracts were similarly analyzed. The plates were incubated at 37°C for 24 hours for bacteria. The procedure above is also allowed for fungal assays, however media used is not nutrient agar but potato dextrose agarand incubated at 25°C for 48 hours. 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 $p=0.05$[1,23].

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. asatica* exhibitedconsiderable antimicrobial activity against testedmicrobial strains. *Premnapubescens*showedsignificant moderate activity against *P. marginalis*and *S. mutans* with 100 mg/ml DMSO medicinal plant concentration.*Centellaasiatica*issignificant against *P.syringae* andmoderate against other pathogens *F. oxysporum*, *L. acidophilus*, *S. salivarious* and *S. aureus* with 100 mg/ml DMSO.

Table 1. Antimicrobial activity ofmethanolic extracts*Premna pubescens*. Blume and*Centella asiatica*

	Pathogen	*Premna pubescens*. Blume				*Centellaasiatica*			
		A	B	C	MIC (mg/ml)	A	B	C	MIC (mg/ml)
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 (+)	*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 (-)	*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

(0) Value indicates no activity,Volume per well: 50µl, Borer size used: 6mm used Plant Methanolic extract concentrations (A =100, B=300, and C=500 mg/ DMSO ml) MIC-Minimum inhibitory concentration

The results of lowest MICs value are at 66 andhighest at 153 mg/ml for *P. pubescens* meanwhilethose of highest ones are at 0,155 mg/ml for *C.asatica*.The variation of antimicrobial activity of our extracts might be due to the distribution of antimicrobial substanceswhich is varied from fraction to fraction of the crude extract. No inhibitions were observed with *P. pubescens* on *P. expansum* and *C. asatica* 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 extractscauses the production of growthinhibition zones that appear as clearas around the wells. However, plant extract was unable to exhibit antibacterial activity against tested bacterial strains. These bacterial strains may have some kinds ofresistance mechanisms e.g.enzymatic inactivation, target sites modificationand decrease of intracellulardrugaccumulation[20]or the concentration of thecompoundusedmay notbe sufficient.

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

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Running title[U1][WU2]

Author contribution[U3]

Name of the author and e-mail ID	Types of contribution
Dr. Diky Setya Diningrat	Dr. Diky Setya Diningrat researched extract test on microbe
Dr. Martina Restuati	Dr. Martina Restuati researched extract of bioactive compound from *Premna pubescens*. Blume and *Centella asiatica*

Significance statement [U4]

This research identified extract activity of Premna and Centella on microbe however it is not new research, this covers previous research published by the same author, but the latest and different one is the following:

1. kinds of microbe are equated
2. Conducting comparison activity of bioactive compound between Premna and Centella on the same microbe.

Therefore, The findings can be used as reference and development of bioactive compound on pharmacy industry

It compares anti-microbial compound from 2 species of plant commonly believed to be traditional efficacious drug in north Sumatra. Microbe is used as completely as representing ordinary pathogen which functions in human life and it comes from group of positive and negative Gram bacteria as well as fungus group

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 research is important to find out the antimicrobial capabilities of *P. pubescens* and *C. asiatica* methanol extracts. This research 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. asatica* 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 were analyzed with Anova statistics by using SPSS software. **Results:** The length of the inhibition zone was measured in millimeters 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 DMSO plant drug concentration. The results of lowest (MICs) values are at 66 and highest ones are at 152 mg/ml for *P. pubescens* meanwhile those of (MICs) values are 0 to 155 mg/ml for *C. asiatica*. **Conclusion:** In general, based on the result of this research, it can be said that *P. pubescens* and *C. asatica* plants can be used as antibacterial and antifungal compounds.

Keywords: *Premna pubescens*. Blume, *Centella asiatica*, antibacterial, antifungal, Minimal inhibitory concentration

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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 countrie. 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[18,19].

North Sumatra is very rich in plants that are believed to have medicinal properties for either prevention or treatment[7]. 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[21]. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents living in the plants[3,13]. Much work has been done on ethnomedicinal plants in North Sumatera Indonesia[19,7,4,13]. Medicinal plants represent a rich source of antimicrobial agents[11,12,13,14]. 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[12,16].

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[10]. 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[8,25] and also increase appetite[1,2]. Compounds derived from the plant have been found contain alkaloid, flavonoid, saponin, steroid, dan fenolik[3,13]. Antimicrobial activity of *Premna pubescens.* Blume was previously screened[13].

Centella asiatica (Family Mackinlayaceae) commonly names Pegagan, Asiatic Pennywort, North Sumatera Indonesian Pennywort, Luei Gong Gen, Takip, kohol, Antanan, Pegagan, Pegaga, vallaarai 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[5,6]. Antibacterial activity of *C. asiatica* was previously screened[11]. 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 paper the results of such studies are reported in order to orient future investigations towards the finding of potent, less toxic to human health and 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-adrich) as solvent for 12 h was performed. The resultant extraction was frozen and dried for 24°C/48 h. Yield of Methanol extracts: 30%[23].

Test microorganisms: Microbial strains of clinical, plant and aquatic origini.e. *Asperigellus niger*, *Pencillium 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 37ºC overnight. The cells were harvested by centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 min again and diluted in normal saline to obtain 5 x 10^5 cfu/mL.

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 three to five 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 50µl of the extract concentrations of 100, 300 and 500 mg/ ml DMSO and allow diffusion for 45 minutes. The solvents used to reconstitute the extracts were similarly analyzed. The plates were incubated at 37°C for 24 hours 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 hours. 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 $p=0.05$[1,23].

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. asatica* exhibited considerable antimicrobial activity against tested microbial strains. *Premna pubescens* showed significant moderate activity against *P. marginalis* and *S. mutans* with 100 mg/ml 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 DMSO.

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

Pathogen		*Premna pubescens*. Blume				*Centella asiatica*			
		A	B	C	MIC (mg/ml)	A	B	C	MIC (mg/ml)
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 (+)	*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 (-)	*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

(0) Value indicates no activity,Volume per well: 50µl, Borer size used: 6mm used Plant Methanolic extract concentrations (A =100, B=300, and C=500 mg/ DMSO ml) MIC-Minimum inhibitory concentration

The results of lowest MICs value are at 66 and highest at 153 mg/ml for *P. pubescens* meanwhile those of highest ones are at 0,155 mg/ml for *C. asatica*. 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. asatica* 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[20] 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[7,14]. Several phytochemicals are identified in different parts. *P. pubescens* flowers contain terpenes, multiflorenol, and cyclisadol[22]. The latex contains caoutchouc, calotropin, calotoxin, calactin, uscharin, trypsin, voruscharin, uzarigenin, syriogenin and proceroside[2]. Chemical 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[24]. Root bark of *P. pubescens* contains triterpenes, a new norditerpenyl ester named as calotropterpenyl ester, and two unknown pentacyclic triterpinoids named as calotropursenyl acetate and calotropfriedelenyl acetate, akundarol isovalerate, mundarol isovalerate and quercetin, 3-rutinoside[24]. The principal active medicines are asclepin and mudarin[9].

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 aeruginosaare* 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, 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[15]. *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[17]. *C. asiatica* methanol extract having strong inhibition activity against *P. aeruginosa and S. aureus* was previously reported[6].

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 pharmaco-kinetic properties.

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Pegaga, vallaarai 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[5,6]. Antibacterial activity of *C. asiatica* was previously screened[11]. 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 paper the results of such studies are reported in order to orient future investigations towards the finding of potent, less toxic to human health and 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-adrich) as solvent for 12 h was performed. The resultant extraction was frozen and dried for 24°C/48 h. Yield of Methanol extracts: 30%[23].

Test microorganisms: Microbial strains of clinical, plant and aquatic origini.e. *Asperigellus niger*, *Pencillium 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, spun at 4000 rpm for 5 min again and diluted in normal saline to obtain 5 x 10^5 cfu/mL.

Determination of antimicrobial activity: The antimicrobial assay of both plant crude extracts was conducted by using the agar well -diffusion method (Balouiri et al., 2016) 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 three to five 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 50µl of the extract concentrations of 100, 300 and 500 mg/ ml DMSO and allow diffusion for 45 minutes. The solvents used to reconstitute the extracts were similarly analyzed. The plates were incubated at 37°C for 24 hours 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 hours. 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[23].

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. asatica*

exhibited considerable antimicrobial activity against tested microbial strains. *Premna pubescens* showed significant moderate activity against *P. marginalis* and *S. mutans* with 100 mg/ml 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 DMSO.

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

Pathogen		*Premna pubescens*. Blume				*Centella asiatica*			
		A	B	C	MIC (mg/ml)	A	B	C	MIC (mg/ml)
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 (+)	*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 (-)	*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

(0) Value indicates no activity,Volume per well: 50µl, Borer size used: 6mm used Plant Methanolic extract concentrations (A =100, B=300, and C=500 mg/ DMSO ml) MIC-Minimum inhibitory concentration

The results of lowest MICs value are at 66 and highest at 152 mg/ml for *P. pubescens* meanwhile those of highest ones are at 0,155 mg/ml for *C. asatica*. 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. asatica* 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[20] 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[7,14]. Several phytochemicals are identified in different parts. *P. pubescens* flowers contain terpenes, multiflorenol, and cyclisadol[22]. The latex contains caoutchouc, calotropin, calotoxin, calactin, uscharin, trypsin, voruscharin, uzarigenin, syriogenin and proceroside[2]. Chemical 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[24]. Root bark of *P.*

pubescens contains triterpenes, a new norditerpenyl ester named as calotropterpenyl ester, and two unknown pentacyclic triterpinoids named as calotropursenyl acetate and calotropfriedelenyl acetate, akundarol isovalerate, mundarol isovalerate and quercetin, 3-rutinoside[24]. The principal active medicines are asclepin and mudarin[9].

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 aeruginosaare* 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, 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[15]. *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[17]. *C. asiatica* methanol extract having strong inhibition activity against *P. aeruginosa and S. aureus* was previously reported[6].

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 pharmaco-kinetic properties.

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

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Running title[U1]

Author contribution[U2]

Significance statement [U3]

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 research is important to find out the antimicrobial capabilities of *P. pubescens* and *C. asiatica* methanol extracts. This research 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. asatica* 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 were analyzed with Anova statistics by using SPSS software. **Results:** The length of the inhibition zone was measured in millimeters 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 DMSO plant drug concentration. The results of lowest (MICs) values are at 66 and highest ones are at 152 mg/ml for *P. pubescens* meanwhile those of (MICs) values are 0 to 155 mg/ml for *C. asiatica*. **Conclusion:** In general, based on the result of this research, it can be said that *P. pubescens* and *C. asatica* plants can be used as antibacterial and antifungal compounds.

Keywords: *Premna pubescens*. Blume, *Centella asiatica*, antibacterial, antifungal, Minimal inhibitory concentration

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Competing Interest: The authors have declared that no competing 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[18] [AG5]. 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[19].

North Sumatra is very rich in plants that are believed to have medicinal properties for either prevention or treatment[7]. 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[21]. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents living in the plants[3,13]. Much work has been done on ethnomedicinal plants in North Sumatera Indonesia[19,7,4,13]. Medicinal plants represent a rich source of antimicrobial agents[11,12,13,14]. 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[12,16].

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[10]. 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[8,25] and also increase appetite[1,2]. Compounds derived from

the plant have been found contain alkaloid, flavonoid, saponin, steroid, dan fenolik[3,13]. Antimicrobial activity of *Premna pubescens.* Blume was previously screened[13].

Centella asiatica (Family Mackinlayaceae) commonly names Pegagan, Asiatic Pennywort, North Sumatera Indonesian Pennywort, Luei Gong Gen, Takip, kohol, Antanan, Pegagan, Pegaga, vallaarai 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[5,6]. Antibacterial activity of *C. asiatica* was previously screened[11]. 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 paper the results of such studies are reported in order to orient future investigations towards the finding of potent, less toxic to human health and safe antimicrobial agents from natural sources.

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pathogens encountered in the community causing severe food poisoning or minor skin infections to severe life threatening infections[17]. *C. asiatica* methanol extract having strong inhibition activity against *P. aeruginosa and S. aureus* was previously reported[6].

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 pharmaco-kinetic properties.

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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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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.

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