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Antimicrobial Profile of Premna pubescens. Blume and Centella asiatica Extracts Against Bacteria and Fungi Pathogens Martina Restuati and 1Diky SetyaDiningrat Biology Department, Faculty Mathematics and Natural Sciences, Medan State University, Medan 20221, North Sumatera, Indonesia 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 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 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. pubescensshowed 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
Corresponding Author: Diky Setya Diningrat, Biology Department of Mathematics and Natural Sciences Faculty, Medan State University, Jl. Willem IskandarPasar V Medan, North Sumatera, Indonesia, Tel: +6181361362400 email: dikysd@unimed.ac.id Orcid: 0000-0002-7195-1626 Scopus id: 56716422800 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 Papua1,2.

North Sumatra is very rich in plants that are believed to have medicinal properties for either prevention or treatment3. 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 drugs4.

It has alsobeenwidelyobservedandacceptedthatthe medicinal value of plants lies in the bioactive phytocomponents living in the plants5,6. Much work has been done on ethnomedicinal plants in North Sumatera Indonesia3,6,7,8. Medicinal plants represent a rich source of antimicrobial agents6,9,10,11. 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 resistance10,13. 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 Indonesia13. 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 milk14,15 and also increase appetite15,16.

Compounds derived from the plant have been found contain alkaloid, flavonoid, saponin, steroid, dan fenolik5,6. Antimicrobial activity of Premna pubescens. Blume was previously screened6. 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 ability6,8.

Antibacterial activity of C.asiatica was previously screened9. 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 37oC 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 105 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=0.0517,18. 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 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 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 intracellulardrugaccumulation19 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 arrows3,11. Several phytochemicals are identified in differentparts. P. pubescens flowers contain terpenes, multiflorenol, and cyclisadol20. The latex contains caoutchouc, calotropin, calotoxin, calactin, uscharin, trypsin, voruscharin, uzarigenin, syriogenin and proceroside15.

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 isovalerate21.

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-rutinoside21. The principal active medicines are asclepin and mudarin22.

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 solutions23. 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 infections21. C. asiatica methanol extracthavingstrong inhibition activity against P.

aeruginosa and S. aureus was previously reported24. CONCLUSION Premna pubescens and Centella asiaticaextracts 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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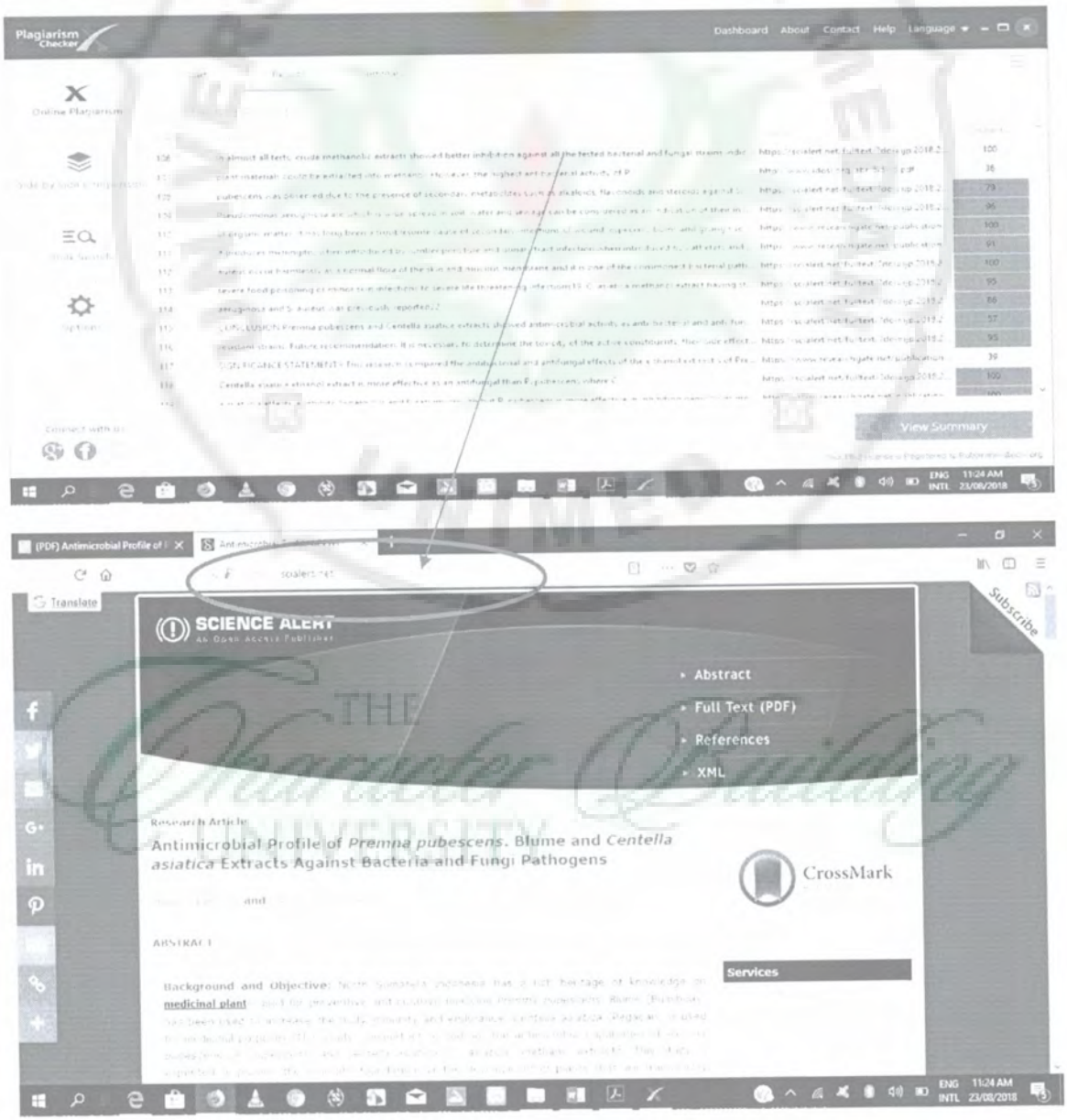

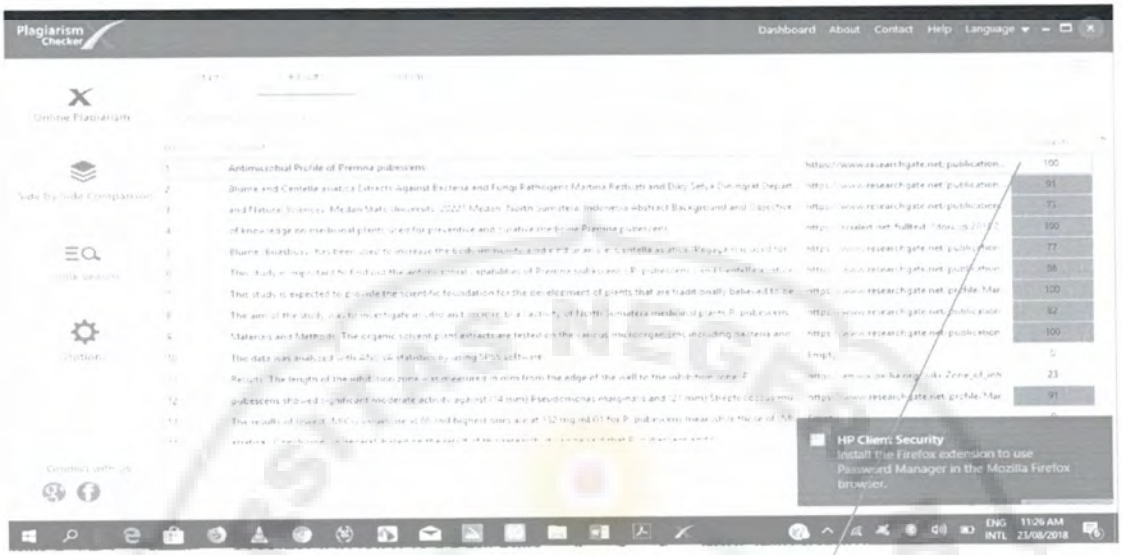
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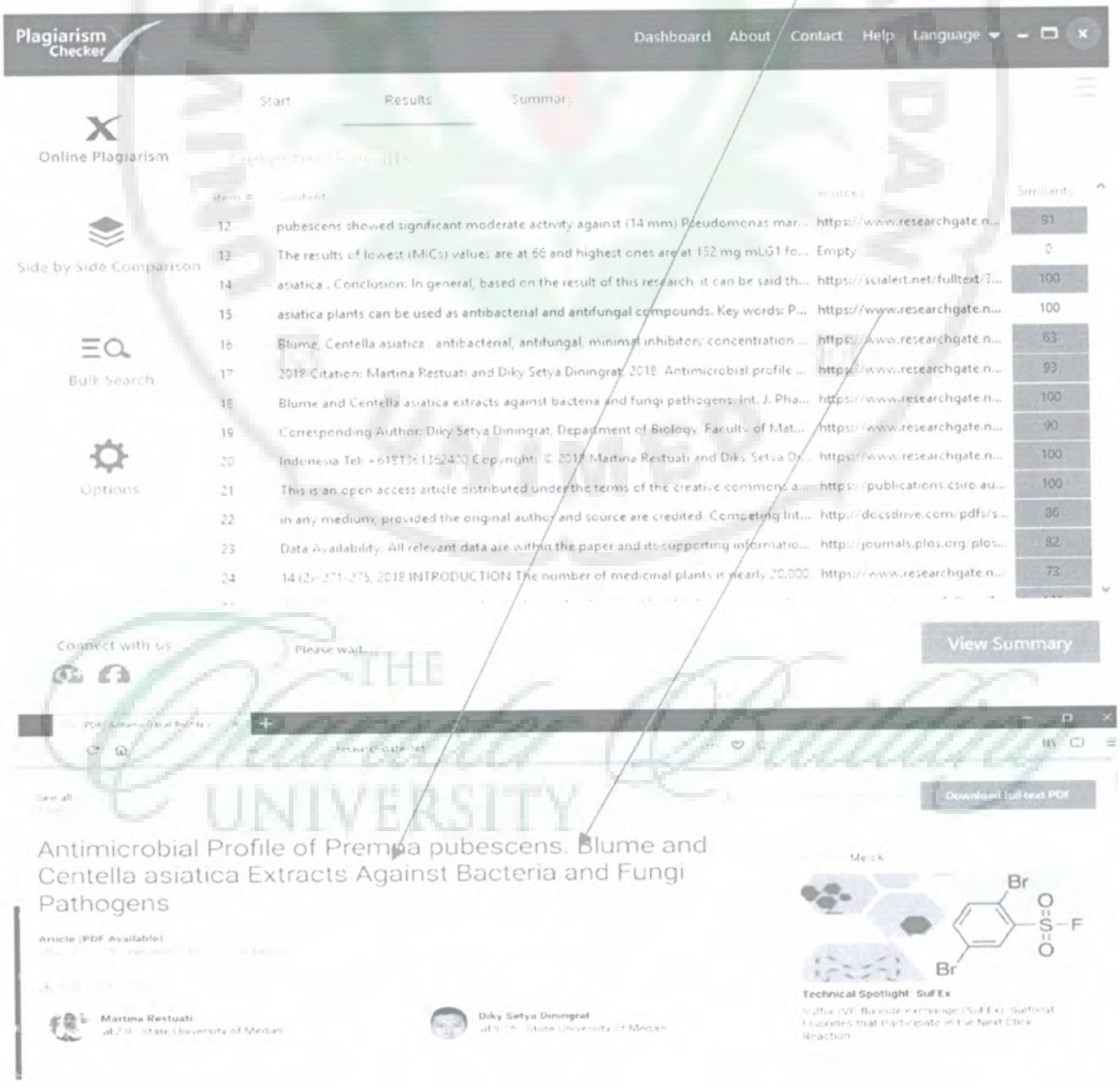
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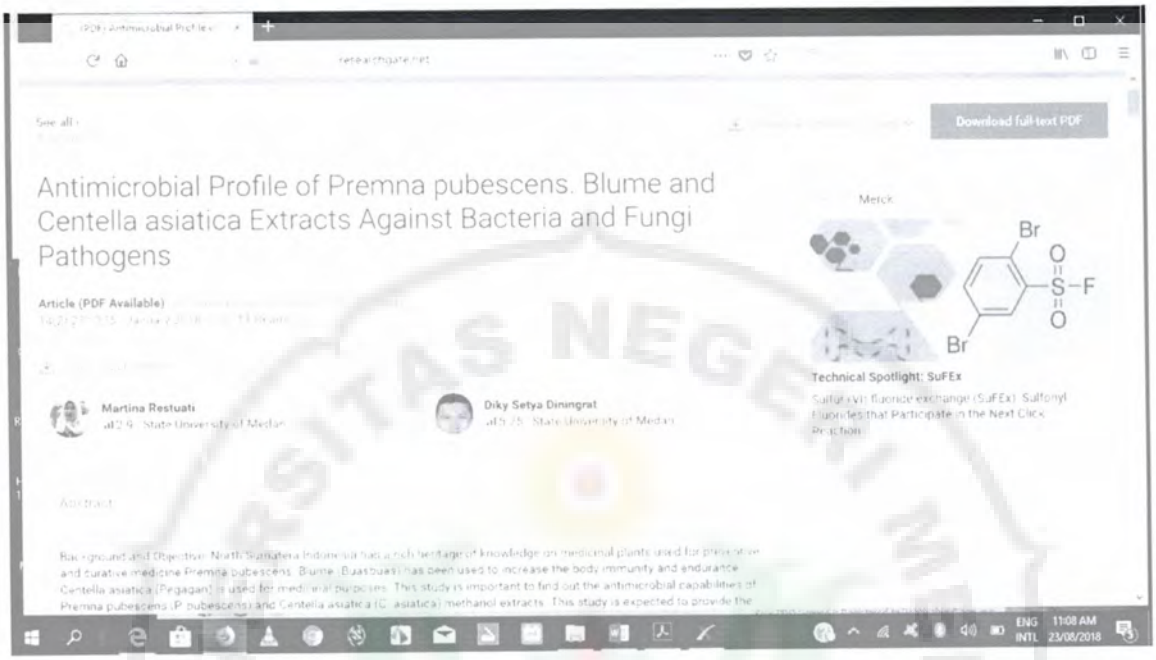
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Technical Spotlight: SuFEx
Sulfur (VI) fluoride exchange (SuFEx): Sulfonyl Fluorides that Participate in the Next Click Reaction
Abstract
Background and Objective: North Sumatera Indonesia has a rich heritage of knowledge on medicinal plants used for 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
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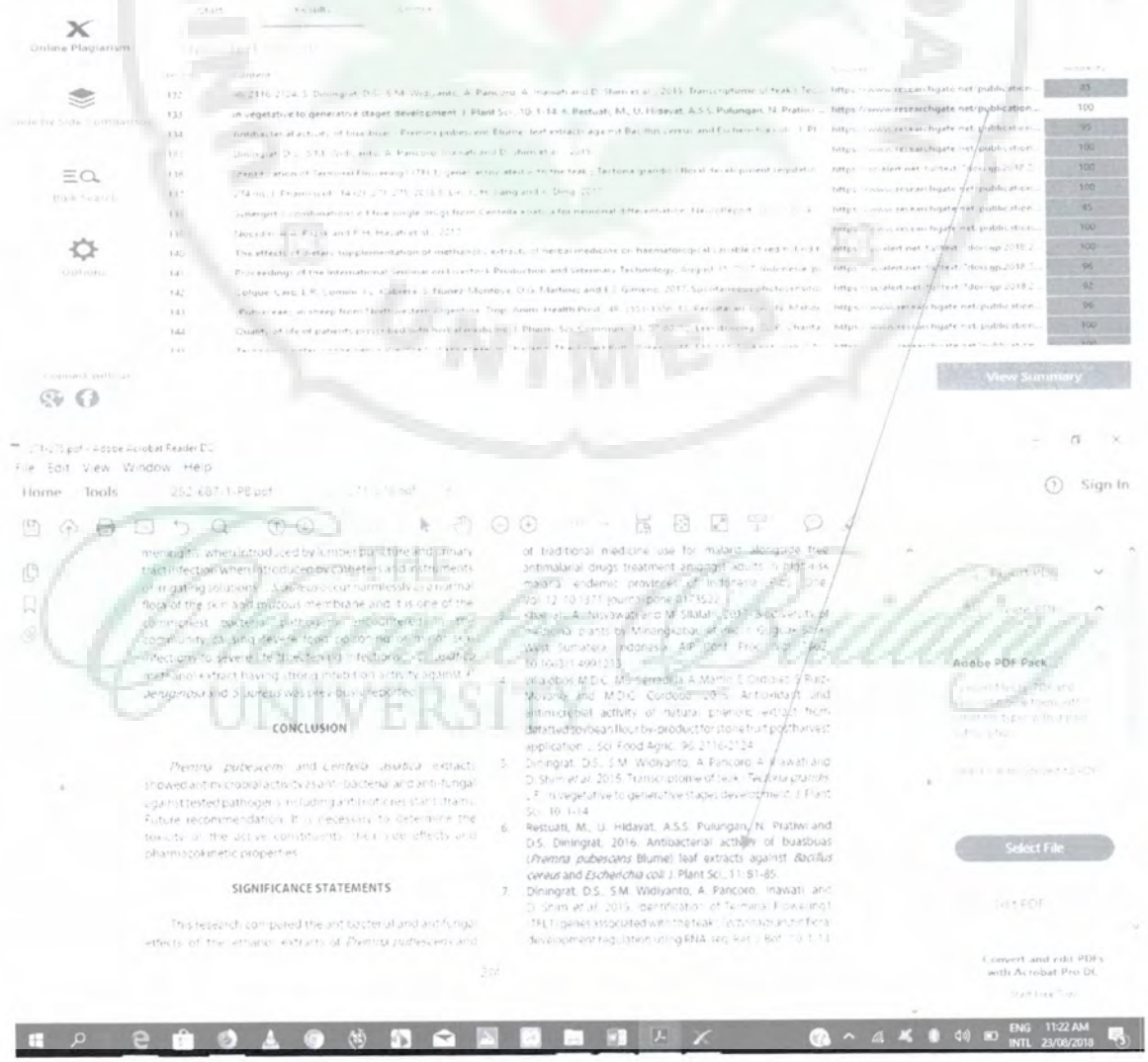
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