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Antibacterial Activity from *Croton Argyratus* Stem Bark Extract

Vienna Saraswaty[1]*, Henry Setiyanto[2], Jetty Nurhajati[3]

[1]Research Centre for Chemistry, Indonesian Institute of Sciences, Jl. Cisitu Sangkuriang Bandung 40135, Indonesia

[2]Analytical Chemistry Research Group, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung, Jl.Ganesha 10 Bandung 40132, Indonesia

[2]Biology Study Program, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jl.Raya Bandung-Sumedang KM.21, Jawa Barat, Indonesia

Abstract: Antibacterial activity of Croton argyratus stem bark extract was tested against four bacterial using the agar well diffusion methods. Methanol and acetone extract of C.argyratus exhibited antibacterial activity. The methanol extract from the stem bark gave antibacterial activity against B. subtilis and S. aureus. Acetone extract gave antibacterial activity against P. aeruginosa and S. aureus. Phytochemical screening showed that the chemical constituent of C.argyratus contains some terpenoid, triterpenoid and phenolic compound.
Keywords: croton argyratus, antibacterial, phytochemical.

Introduction

Research on antibacterial activity using medicinal plants is still interested.[1-5] Bacteria and other microorganisms that cause infections are remarkably resilient and can develop ways to survive drugs meant to kill or weaken them. Usually, resistance of antibiotic often led by the wrong and increasing use of antibiotics.[6] But recently, the mutation of microbe also plays a role. So, problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. One action which can be taken to solve this is by searching new lead compound from traditional plant.[5-6]

Croton argyratus is a tree belongs to the family of *Euphorbiaceae*. Locally, it is known as cheret budak, semelit sayor, akar cheret budak, campo rimbo or silver croton. The tree is common at South East Asia (Singapore, Malaysia, Indonesia, Phillipina). In Indonesia, the tree can be found at Lumut mountain at southeast Borneo, West Java, Sumatera.[7-11] Traditionally at Jambi (Indonesia), the root of this tree is used for antimalaria, anti fever, antidiarrhea and afterbirth cure.[12] Literature search shown that the tree is well known as antiplasmodial and have cytotoxic activity to human lung cancer.[9, 13] Detailed chemical study on the roots of *C.argyratus* revealed that the extract from roots contain styryldehydropyran, goniothalamin and a clerodane-type diterpene, and junceic acid.[13]

Corres. author: vien002@lipi.go.id

Our research was focused to antibacterial activity from methanol and acetone extract of *C.argyratus* stem bark and its phytochemical properties.

Experimental

Material and Methods

Material

Croton argyratus stem bark was collected from Cibodas Botanical Garden, Jawa Barat. Bacteria used i.e. *E. coli, Pseudomonas aeruginosa, B. subtilis, and S. aureus* are collection from ITB Bandung. Nutrient broth, Nutrient Agar are from Difco. Phytochemical screening agent used are from Merck with p.a grade. Organic solvent used was in technical grade, except DMSO in p,a grade from Merck. Autoclave was used for sterilization for all glass ware and media for antibacterial assay. All media and glass ware were sterilized for 15 minutes at 121 ^{0}C.

Methods

Sample preparation

C.argyratus stem bark was dried in oven blower for 3 days at 55 ^{0}C, the grilled. About 83 gr dry pulveres *C.argyratus* was maserated using methanol and acetone. The filtrate of extracts were evaporated under vacuum using Heidolph Rotary vapour (Germany) at 45 ^{0}C. The dry extract was collected, weighed and dissolved in DMSO at different concentration i.e 40%, 20%, 30%, 10%, 5% and 2.5% w/v before assay.

Antibacterial assay (Agar Well Diffusion Method)

Antibacterial assay was performed using *E. coli, Pseudomonas aeruginosa, B. subtilis, and S. aureus*. Various concentration of methanol and acetone extract were dissolved in DMSO. One ose of bacteria were inoculated first in Nutrient broth for 24 hours at 37^{o}C. Then the fresh bacteria were used for the assay. About 100 µl of suspension of bacteria was put in petri dish mixed with 20 ml sterile Nutrient Agar. After the agar freezed, the wells of sample were made using perforator with Ø of 6 mm. About 20 µl of sample was put in the well using eppendorph pipette. After 24 hours of incubation at 37^{o}C the inhibition zone was measured. As standard, tetracycline was used.

Preliminary phytochemical screening assay

Preliminary phytochemical screening assay was performed as described in Harborne.[15]

Results and Discussion

We investigated the percentage yield of extract by using two different polar organic solvent i.e methanol and acetone. The percentage yield of extract in Tab. 1 almost similar. The extract gained in acetone was higher than in methanol.

Table I. Yield of Extraction.

Solvent	Acetone	Methanol
Yield (%w/w)	5	4.43

It is for the first time we reported that *C.argyratus* extract from collection of Cibodas botanical garden had antibacterial activity. The antimicrobial activity was studied by the agar diffusion method and the results are shown in table 2. The inhibition was observed against 4 bacteria i.e. *B. subtilis, S. aureus, P. aeruginosa* and *E. coli*. The acetone extract of *C.argyratus* stem bark give antibacterial activity against *P. aeruginosa* and *S. aureus*. And the methanol extract giving antibacterial activity against B. subtilis and S. aureus. Tab.2 presented that the methanol extract of *C.argyratus* stem bark gave a moderate sensitivity to *B. subtilis* and *S. aureus* with inhibition zone produced were in the range of 8 mm to 10 mm with 10% to 40 % w/v concentration and the acetone extract also afforded similar result against to *P. aeruginosa* and *S. aureus*, except the concentration were in 5% to 40% w/v.

Table II. Antibacterial activity Assay

Bacteria	Inhibition zone (mm)					
% Concentration (w/v)	2,5	5	10	20	30	40
Methanol extract						
B. Subtilis	-	-	8	9	10	10
S. aureus	-	-	8	9.3	10.3	10.3
P. aeruginosa	-	-	-	-	-	-
E. coli	-	-	-	-	-	-
Acetone extract						
B. Subtilis	-	-	-	-	-	-
S. aureus	-	7	7	10	10	10
P. aeruginosa	-	12	12	13	13	13
E. coli	-	-	-	-	-	-
Tetracyclin (Standard) (%w/v)	0.015625	0.03125	0.0625	0.125	0.25	0.5
S. aureus	45.9	45.7	40.6	38.6	38.1	35.2
Bacillus subtilis	17.7	18.8	20.9	21.6	23.5	25.6
Escherichia coli	10.8	10.6	12.6	14.4	15.7	15.2
Pseudomonas aeruginosa	18.8	19.3	23.8	26.7	29.3	31.9

(-) : (negative)

There is no significant difference of antibacterial activity at concentration of 20% to 40 %. The usage of 2.5% w/v concentration didn't give any sensitivity among the four bacteria tested. This investigation proposed that the methanol extract of stem bark *C.argyratus* can be used for the preventive action to avoid infection caused by *S. aureus* and *B. subtilis* at 5% w/v concentration. In contrast, the traditional usage of *C.argyratus* mentioned that the root can be used for the cure of diarrhea. Commonly, diarrhea often caused by *E. coli*. Our investigation at stem bark of this plant didn't give similar activity. This might be because of different part of source of the plant which we're tested in our Laboratory, further investigation of the root extract should be performed later.

Based on the antibacterial activity in different concentration, we also predicted that the minimum inhibitory concentration of methanol extract from the stem bark of *C.argyratus* against *B. subtilis* and *S. aureus* was in the range of 5% -10% w/v concentration and 2.5%-5% w/v to against *S. aureus* and *P. aeruginosa* for acetone extract.

Phytochemical Constituent

Table III. Preliminary Phytochemical Screening

Compound Group	Present or Absent	Colour Intensity
Tannin	Absent	
Terpenoid	Present	+++ (strong)
Flavonoid	Absent	
Triterpenoid	Present	++ (moderate)
Steroid	Absent	
Phenolic	Absent	
Saponin	Absent	
Alkaloid	Present	+ (low)

From Phytochemical screening tabulated in TABLE. III, the stem bark of *C.argyratus* showed the presence of terpenoid, triterpenoid and alkaloid compound. Based on colour intensity in qualitative identification, terpenoid showed strongest colour. So, we recommended that the major chemical constituent of *C.argyratus* stem bark was terpenoid. Detailed study of *C.argyratus* root which performed by Norizan Ahmat A. H. et.al afforded a styryldehydropyrone, (+)-goniothalamin and a clerodane-type diterpene, (-)-junceic acid was in the line with this research.[13] The bioactive compound of antibacterial strongly proposed was clerodane diterpenoid due to its cytotoxic activity against brine shrimp.[14]

Conclusions

Data from this experiment have provided useful information as the basic of scientific research on medicinal use of *C.argyratus*. However, there is need further research to carry out in advance to elucidate structure of bioactive compound.

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