

Antimicrobial activity of *Pycnarrhena cauliflora* (Miers.) Diels. methanol extract

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ABSTRACT

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Pycnarrhena cauliflora (Miers.) Diels., local name sengkubak, is one of indigenous plants from West Kalimantan that has been used as natural flavor. *Pycnorrhena cauliflora* is one of species of Menispermaceae family which is rich in bisbenzylisoquinoline alkaloids. This alkaloids are known to have various biological activities including antiprotozoal, antiplasmodial, antifungal and antibacterial activities. This study aimed to investigate antimicrobial activity of the *P. cauliflora* (Miers.) Diels. methanolic extracts against gram-positive and gram-negative bacteria. The methanolic extract of *P. cauliflora* (Miers.) Diels., root, leaf and stem were prepared by maceration. The disk-diffusion method was then used to determine the antimicrobial activity of the extracts against *Streptococcus pyogenes*, *S. mutants*, *Staphylococcus aureus*, *S. epidermidis*, *Salmonella typhi*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Escherichia coli* after 18-24 h incubation at 37 °C. Amoxicillin was used as positive control for gram-positive bacteria and ciprofloxacin was used as gram-negative bacteria. The inhibition zones were then measured in mm. Analysis were conducted in duplicates. The results showed in general the methanolic extracts of *P. cauliflora* (Miers.) Diels. root (inhibition zone diameter= 10-23 mm) were more active than that leaf (0-15 mm) and stem (0-17 mm) extracts against gram-positive bacteria. The zone inhibition diameter of amoxicillin as positive control was 8-42 mm. In addition, the methanolic extracts of *P. cauliflora* (Miers.) Diels. root (12-17 mm) were also more active than that leaf (0-12 mm) and stem (0-12 mm) extracts against gram-negative bacteria. The zone inhibition diameter of ciprofloxacin as positive control was 33-36 mm. In conclusion, the methanolic extract of *P. cauliflora* (Miers.) Diels. root is the most extract active against both gram-positive and gram-negative bacteria. Further study will be focused to isolate active compounds in the methanolic extract of the root.

ABSTRAK

Pycnarrhena cauliflora (Miers.) Diels., dengan nama local sengkubak, adalah salah satu tanaman asli Kalimantan Barat yang telah digunakan sebagai penyedap alami. *Pycnarrhena cauliflora* (Miers.) Diels. adalah salah satu spesies famili Menispermaceae yang banyak mengandung alkaloid bisbenzylisoquinolin. Alkaloid ini dikenal mempunyai berbagai aktivitas biologi termasuk antiprotozoal, antiplasmodium, antifungi dan antibakteria. *Pycnorrhena cauliflora*(Miers.) Diels. adalah salah satu spesies famili Menispermaceae yang banyak mengandung alkaloid bisbenzylisoquinolin. Alkaloid ini diketahui mempunyai berbagai aktivitas biologi seperti antiprotozoal, antiplasmodium, antifungi dan antibakteri. Penelitian ini bertujuan untuk mengkaji aktivitas antimikroba ekstrak metanol *Pycnarrhena cauliflora* (Miers.) Diels. terhadap bakteri gram positif dan negatif. Ekstrak metanol akar, batang dan daun *P. cauliflora* Miers. Diels. dibuat dengan maserasi. Metode difusi cakram agar digunakan untuk menetapkan aktivitas antibakteri ekstrak terhadap *Streptococcus pyogenes*, *S. mutants*, *Staphylococcus aureus*, *S. epidermidis*, *Salmonella typhi*, *Shigella flexneri*, *Pseudomonas aeruginosa* dan *Escherichia colisetelah* inkubasi selama 18-24 jam pada suhu 37 °C. Amoksisilin digunakan sebagai control positif untuk bakteri gram positif dan

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ciprofloksasin digunakan sebagai kontrol positif untuk bakteri gram negatif. Selanjutnya zona hambatan diukur dalam mm. Penetapan dilakukan duplo. Hasil penelitian menunjukkan secara umum ekstrak metanol akar *P. cauliflora* (Miers.) Diels. (diameter zona hambatan = 10-23 mm) lebih aktif dari pada daun (0-15 mm) dan batangnya (0-17 mm) melawan bakteri gram positif. Selain itu, ekstrak metanol akar *P. cauliflora* (Miers.) Diels. (12-17 mm) juga lebih aktif daripada daun (0-12 mm) dan batangnya (0-12 mm) melawan bakteri gram negatif. Diameter zona hambatan ciprofloksasin sebagai kontrol positif adalah 33-36 mm. Dapat disimpulkan, ekstrak metanol akar *P. cauliflora* (Miers.) Diels. merupakan ekstrak paling aktif melawan baik bakteri gram positif dan negatif. Penelitian lanjutan akan difokuskan untuk mengisolasi senyawa aktif dalam ekstrak metanol dari akar.

INTRODUCTION

Deforestation in Kalimantan to make place for an oil palm plantation may not only enhance the economical status and life style of the population but also leads to a loss of endogenous medicinal plants.^{1,2} *Pychnarrhena cauliflora* (Miers.) Diels. (Menispermaceae) is one of an endogenous plant from West Kalimantan that locally well known as *sengkubak*.³ In the past, *P. cauliflora* (Miers.) Diels. already existed and growth will in the forest around where the human live. Its leaves were traditionally used by Dayak and Melayu ethnics as a natural taste substance. *Pychnarrhena cauliflora* (Miers.) Diels. is quite difficult to cultivate due to its growth is very slow. Until now, no cultivation technique for *P. cauliflora* (Miers.) Diels. was developed. Recently, *P. cauliflora* (Miers.) Diels. is difficult to obtain from homestead and it can only be obtained from the upper montane forest.⁴ Therefore, modern taste substances such as monosodium glutamat have been used to replace the *P. cauliflora* (Miers.) Diels. leaves.

Although the leaves of *P. cauliflora* (Miers.) Diels. is traditionally used as seasonings and flavorings for a long time, its other utilizations have not been recognized, yet. Moreover, not much the biological activities of *P. cauliflora* have been reported. Thereby, exploration of the utilization and investigation of the biological activities of *P. cauliflora* (Miers.) Diels. are important in order to conserve this plant face extinction or genetic loss due to habitat destruction.

Pycnorrhena cauliflora (Miers) Diels. is one of species of Menispermaceae family which is rich in bisbenzylisoquinoline alkaloids. These alkaloids are known to have various biological activities including antiprotozoal,⁵⁻⁷ antifungal,⁷ antiplasmodial and antibacterial.⁸ As part of our research program consists in the evaluation of the biological activities of bisbenzylisoquinoline alkaloids from *P. cauliflora* (Miers.) Diels. We reported here antibacterial activities of methanolic extracts of various part of the *P. cauliflora* (Miers.) Diels. against gram-positive and gram-negative bacteria.

MATERIALS AND METHODS

Plant collection and extract preparation

The study was approved by the Health and Medical Research Ethic Committee, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta. The plant was collected in Pontianak, West Kalimantan and was identified by comparison with authentic specimens at Laboratory of Biology, Department of Pharmacognocny, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta. A voucher specimen was deposited at the laboratory. Methanolic extracts of *P. cauliflora* (Miers.) Diels. were prepared by maceration. The part of plant tested (either root, stem and leaf) was air dried (10 days at 33 °C) and powdered. The dried powder was then macerated in methanol at room temperature for 24 h. The macerate was filtered and collected.

The residue was then further macerated following the same procedure two time. The filtrates obtained were evaporated by rotary evaporator until dried extract obtained and then refreshed at 4 °C until antibacterial screening.

Test microorganisms

Total eight bacterial strains obtained from the collection of Department of Microbiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada were used for antimicrobial screening. Four of them were gram-positive bacteria i.e. *Staphylococcus aureus*, *S. epidermidis*, *S. mutants* and *S. pyogenes* and four of them were gram-negative bacteria i.e. *Salmonella typhi*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Escherichia coli*. All strains were maintained at 4 °C on nutrient broth medium.

Antibacterial screening

Antibacterial activity screening of methanolic extracts of *P. cauliflora* (Miers.) Diels. root, stem and leaf were conducted using the agar diffusion method. The bacterial strains were cultivated in brain heart infusion medium (BHI) broth and then incubated at 37 °C for 24 h. The bacteria were then standardized for their CFU/mL which was equivalent to 1×10^6 CFU/mL. Mueller Hinton agar plates were seeded with the test organisms and the plates left to dry for 5 min. After drying, wells were made in the agar using sterile cork borer measuring 9 mm in diameter. One hundred microliters (100 µL at concentrations of 20 mg/mL) of the methanolic extracts of *P. cauliflora* (Miers.) Diels. were dispensed into the labelled wells. The plates were then kept in room temperature for one hour for

the extract to diffuse into the medium. The plates were then incubated at 37 °C for 24 h. Amoxicillin at concentration of 25 µg/mL was use as positive control for gram positive bacteria and ciprofloxacin at concentration of 5 µg/mL for gram negative bacteria.

Analysis

Descriptive analysis was carried out on the various methanolic extracts of *P. cauliflora* (Miers.) Diels. For the antimicrobial activity evaluation of the methanolic extracts, the inhibition zone diameters around the wells were measured in mm and recorded.⁹

RESULTS

Agar disk-diffusion method is the official method used in many clinical microbiology laboratories for antimicrobial activity testing. In this method, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, wells (about 9 mm in diameter) were made in the agar and the test extracts at certain concentration are dispensed into the wells. The the agar plates are then incubated under suitable conditions. The test extracts diffuse into the agar and inhibits bacterial growth and then the inhibition zone diameter is measured. FIGURE 1 presents the inhibition zone of methanolic extract of *P. cauliflora* (Miers.) Diels. root against gram-positive bacteria (*S. pyogenes*, *S. mutants*, *S. aureus*, *S. epidermidis*) and gram-negative bacteria (*S. typhi*, *S. flexneri*, *P. aeruginosa* and *E. coli*) after 18 to 24 h incubation at 37 °C, whereas the antibacterial activity is presented in TABLE 1.

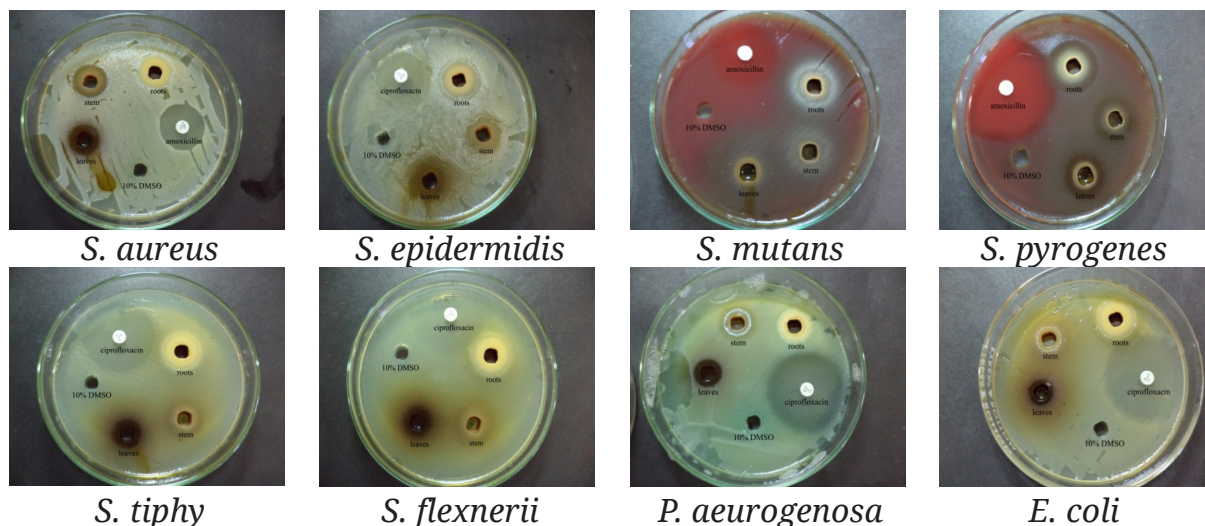


FIGURE 1. Inhibition zone of methanolic extract of *P. cauliflora* (Miers.) Diels. root after incubation with *S. pyrogenes*, *S. mutants*, *S. aureus*, *S. epidermidis*, *S. typhi*, *S. flexneri*, *P. aeruginosa* and *E. coli* for 18-24 h at 37 °C.

TABLE 1. Antibacterial activity presented as inhibition zone diameter (mm) of methanolic of *P. cauliflora* (Miers.) Diels. root, leaf and stem against gram-positive and gram-negative bacteria

Bacterial	Root	Leaf	Stem	Control
Gram-positive				Amoxicillin
<i>S. aureus</i>	15	0	15	22
<i>S. epidermidis</i>	19	0	14	8
<i>S. mutants</i>	10	0	0	26
<i>S. pyrogenes</i>	23	15	17	42
Gram-negative				Ciprofloxacin
<i>S. typhi</i>	15	0	0	33
<i>S. flexneri</i>	12	0	0	36
<i>P. aeruginosa</i>	15	12	12	31
<i>E. coli</i>	17	10	10	34

Among three methanolic extract of *P. cauliflora* (Miers.) Diels tested, the root extract was active against all both test gram-positive and gram-negative bacteria (TABLE 1). In general, this root extract was more active that that leaf and stem extract against gram-positive bacteria with the inhibition zone ranging from 10 to 23 mm, 0 to 15 mm and 0 to 17 mm, respectively. However, in general it was less active compared to amoxicillin as positive control with the inhibition zone ranging from 8 to 42 mm, except against *S. epidermidis*. The inhibition zone diameter of the root extract against

S. epidermidis (19 mm) was higher than that amoxicillin (8 mm). The methanolic extracts of *P. cauliflora* (Miers.) Diels. root in general were also more active against gram-negative bacteria than that leaf and stem extract against gram-negative bacteria with the inhibition zone ranging from 12 to 17 mm, 0 to 12 mm and 0 to 12 mm, respectively. However, it was less active compared to ciprofloxacin as positive control with the inhibition zone ranging from 33 to 36 mm. In addition, the methanolic extract of *P. cauliflora* (Miers.) Diels. root was more active against the gram-positive bacteria with

the inhibition zone ranging from 15 to 23 mm compared to that the gram-negative bacteria with the inhibition zone ranging from 12 to 17 mm.

DISCUSSION

As an indigenous plant that cannot be cultivated, the research publications concerning biological activities of *P. cauliflora* (Miers.) Diels. are limited. *Pychnarrhena cauliflora* (Miers.) Diels. was reported to have antioxidant,^{10,11} cytotoxic,^{12,13} antibacterial activities.¹⁴ In this study, it was reported that methanolic extracts of the *P. cauliflora* (Miers.) Diels. had antibacterial activity against both gram-positive and gram-negative bacteria which it has not been reported in the previous studies. The highest active extract was demonstrated from the methanolic root extract.

Pychnarrhena cauliflora (Miers.) Diels. is one of species of Menispermaceae family which is rich in bisbenzylisoquinoline alkaloids. These alkaloids are well known to have various biological activities including antiprotozoal,⁵⁻⁷ antifungal,⁷ antiplasmodial and antibacterial.⁸ Bisbenzylisoquinoline has been isolated from another species of *Pychnarrhena* species i.e. *P. ozanta*. In the previous study, alkaloids were identified in the methanolic root extract of *P. cauliflora* (Miers.) Diels., whereas they were not identified neither in the methanolic stem or leaf extracts of *P. cauliflora* (Miers.) Diels.¹⁵ It supported the results of this study that the root extract is the most active extract compared to that leaf and stem extracts.

It is also predicted that alkaloids contained in the methanolic root extract of *P. cauliflora* (Miers.) Diels. that have antibacterial activity. Moreover, these alkaloids may be bisbenzylisoquinoline groups. The antibacterial activity of bisbenzylisoquinoline alkaloids isolated from Menispermaceae family have been reported in the previous studies. Cocsoline, a bisbenzylisoquinoline isolated from *Epinetrum villosum*, displayed antibacterial activity

against various gram-positive and gram-negative bacteria with MIC values ranging from 15.62 to 1,000 µg/mL.¹⁶ Tetrandrine and fangchinoline, two bisbenzylisoquinoline isolated from *Stephania tetrandra*, showed significantly active against methicillin resistant *Staphylococcus aureus* (MRSA) and ESBL-producing *E. coli* especially for tetrandrine.¹⁷ Further study will be focused to isolate bisbenzylisoquinoline alkaloids or other active compounds from the methanolic root extract from *P. cauliflora* (Miers.) Diels.

CONCLUSION

The methanolic extract of *P. cauliflora* (Miers.) Diels. root is the most active extract compared to that stem and leaf extracts against both the test gram positive and gram-negative bacteria. Further study will be conducted to isolate antibacterial active compounds from the methanolic extract of *P. cauliflora* (Miers.) Diels. root.

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