

Antibacterial Activity Against *Propionibacterium acnes* of *n*-Hexane Fractions from Siam Weed Leaves (*Chromolaena odorata*)

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ABSTRACT

The present study aims to separate the active fractions from the *n*-hexane extract of Siam weed (*Chromolaena odorata* (L.) R.M. King & H. Rob) leaves with potential as the antiacne agent. The present work includes isolation of active fractions guided by antiacne assay against *Propionibacterium acnes* and phytochemical analysis of the active fractions. Extraction was conducted by maceration using *n*-hexane. An agar well diffusion assay was applied to evaluate the antiacne activity of the extract at the concentration of 400 µg/well with *chloramphenicol* as a positive control. Column chromatography assisted by thin-layer chromatography of *n*-hexane extract gave six active fractions. Fractions TH5 and TH6 displayed the highest inhibition of 51% and 47% respectively. Further purification of the fraction yielded more than 14 subfractions. Phytochemical analysis indicated that fraction TH5 contains alkaloid and steroid while fraction TH6 contains alkaloid. The active fractions isolated from *C. odorata* have the potential to be developed as a natural antiacne.

Keywords: *Antiacne, Phytochemical, Fractions, Chromolaena odorata*

1. INTRODUCTION

The local community in East Kalimantan has a long history related to the use of plants as traditional medicine. In many areas, particularly rural areas, medicinal plants play an important role for most people in their healthcare and daily needs [1]. Medicinal plants have been implemented as a bio-resource of drugs, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs [2].

In relation to the use of medicinal plants in infection treatment, infectious disease is one of the health problems that cannot be solved completely. Of these, *Propionibacterium acnes*, natural skin and mucosal flora of the sebaceous follicles of the skin, is of major importance in the pathogenesis of acne and also in some other opportunistic infections [3,4]. *P. acnes* initiates disease by bacterial seeding, modification, and formation of biofilm [5]. The bacterial strain also causes acute inflammation, including inflammation in cultured prostate cells [6]. The use of drugs to overcome the diseases caused by *P. acnes* on the market has

weaknesses and some of them cause negative effects on health. On the other hand, the use of medicinal plants has the potential to be more economical health care with fewer negative effects compared to synthetic drugs. Therefore, the development and utilization of medicinal plants become very important to do including the search for new antiacne substances from various sources.

Chromolaena odorata is one of the medicinal plants that has long been used by the native ethnic of Kalimantan for skin infection and to stop bleeding. Previous investigations of the leaves of *C. odorata* revealed the presence of phenols, glycosides, steroids, triterpenes, flavonoids, alkaloids, tannin, steroid, and saponin [7]. It has been reported to have anti-inflammatory [8], antiplasmodial [9], antibacterial, antifungal, antioxidant [10], and antidiabetic activities [11]. It is then considered important to explore the potential contained in *C. odorata*, particularly search for the secondary metabolites contained in plants that have the potential to be developed as natural antibacterials.

This study aimed to separate the active fractions from the *n*-hexane extract of *C. odorata* leaves with

potential as a natural antiacne agent. This study includes isolation of active compounds guided by antiacne assay and phytochemical analysis of the active fractions.

2. MATERIALS AND METHODS

2.1. Plant Material

The leaves of *C. odorata* were collected from Samarinda, East Kalimantan, Indonesia and authenticated by the Laboratory of Forest Dendrology and Ecology, Faculty of Forestry, Mulawarman University, Indonesia.

2.2. Chemicals and Drugs

n-Hexane, ethyl acetate, acetone, methanol, silica gel, iodine powder, agar powder, alcohol, D-glucose, chloramphenicol, acetic acid anhydride, Dragendorff solution, Molisch reagent, sulfuric acid, lead sulfate, hydrochloric acid, and sodium hydroxide were purchased from a chemical supplier.

2.3. Extraction and Fractionation

About 300 g powder of *C. odorata* leaves was extracted with *n*-hexane for 48 h. The *n*-hexane filtrate was filtered using Whatman filter paper and then concentrated using a rotary vacuum evaporator at 30-40 °C temperature to obtain the *n*-hexane extract of *C. odorata*. The extract was then fractionated by column chromatography on silica gel and eluted with 200 ml each of *n*-hexane: ethyl acetate mixture of ratio (10: 0), (9: 1), (8: 2), (7: 3), (6: 4), (5: 5), (4: 6), (3: 7), (2: 8), (1: 9), and (0: 10). The filtrates were collected in the test tube and grouped by the thin layer chromatography profile.

2.4. Antibacterial Activity Assay

The antibacterial activity against *Propionibacterium acnes* was analyzed using agar diffusion method at a concentration of 400 µg/well. The microorganisms were obtained from the culture collection of the Laboratory of Forest Product Chemistry at the Mulawarman University. Briefly, 20 ml of sterilized media agar was poured into a sterile petri dish. 100 µl of the bacterial suspension was then swabbed on a petri dish. Wells (7 mm diameter) were cut into the agar plates using a sterile cork borer. About 100 µl of the sample from each concentration was poured into individual wells. The plates were incubated for ±18 – 24 h at 37 °C and after incubation, the diameter of the inhibition zones was measured in mm and recorded.

2.5. Phytochemical Analysis

Phytochemical analyses were carried out on the fractions of *C. odorata* leaves for the qualitative determination of the chemical constituents which included alkaloid, flavonoid, steroid, terpenoid, tannin, saponin, and carbohydrate. The procedure to analyze each bioactive compound was described by Harborne [12] and Kokate [13].

2.6. Data Analysis

All values obtained were expressed as means ± standard deviation. The test was calculated based on the average of three repetitions for then compared with the positive control as well as the negative controls so that it could be analyzed for the inhibition of the fractions was used and the comparison of each fraction.

3. RESULTS AND DISCUSSION

The extraction of *C. odorata* leaves with *n*-hexane solvent yielded 2.08 %. The crude extract was fractionated by column chromatography. The fractions were combined based on their TLC profile to afford six fractions TH1 (165 mg), TH2 (1.016 mg), TH3 (184 mg), TH4 (3.03 mg), TH5 (282 mg), TH6 (53 mg) which then were tested their activity against *P. acnes*.

C. odorata is a known invasive weed and readily spreads with ease inhabiting any available space. The ability of *C. odorata* to exhibit antimicrobial activities in the current research work indicates a potential for alternative use of the weed as raw materials for the production of medicine that can be used in diseases caused by *P. acnes* [10]. Activity-guided fractionation is indeed a very useful and effective tool when searching for and identifying new disease-control agents. The extraction and fractionation process of *C. odorata* leaves was depicted in Figure 1.

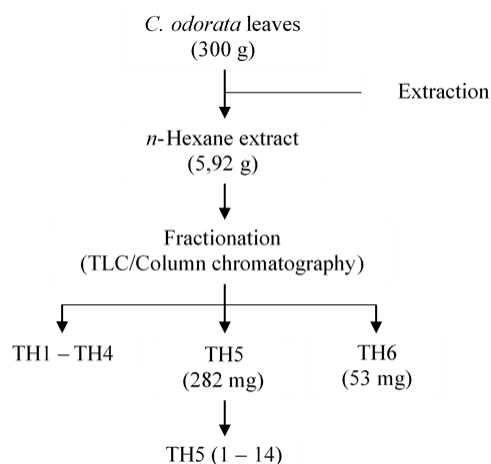


Figure 1 Extraction and fractionation of *Chromolaena odorata* leaves

The antiacne activity of the fractions of *C. odorata* was presented in Table 1. Each fraction can inhibit the growth of *P. acnes* at a concentration of 400 µg/well. Fractions TH5 and TH6 have the highest inhibition of 51% and 47% respectively while the lowest inhibition was at the TH3 fraction which was 33.59%. *Chloramphenicol* is used as a standard antibacterial agent and the zones of inhibition produced were far greater than the fractions. The result of this study is related to the presence of active compounds such as alkaloids, flavonoids, terpenoids, and steroids in the extract and these compounds may account for their antibacterial activity [7].

Table 1. The antibacterial activity of *Chrmolaena odorata* fractions against *Propionibacterium acnes* at a concentration of 400 µg/well

Fraction	Inhibition ± SD	
	(mm)	(%)
TH1	10.78±0.19	37.02±0.19
TH2	10.78±0.19	37.02±0.19
TH3	9.78±0.51	33.59±0.51
TH4	13.11±1.58	47.01±1.58
TH5	14.33±0.67	51.39±0.67
TH6	13.22±0.19	47.41±0.19

The TLC profile at fractions TH1-TH5 showed a good separation on *n*-hexane and ethyl acetate solvents in the ratio of 8: 2 on the appearance in Figure 2. Fraction TH6 shows a single spot that is almost pure.

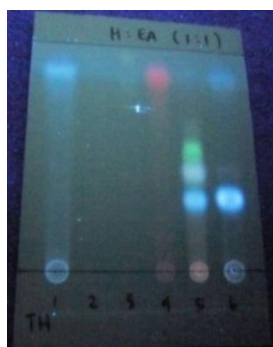


Figure 2 The Chromatogram of Fraction TH (1–5).

From the initial separation, the TH5 fraction was selected for further separation based on its inhibitory effect in *P. acnes*. Fractionation at the TH5 fraction yielded six subfractions. Fraction TH5-1, TH5-11, and TH5-14 in Figure 3 show a single spot that is almost pure. Further analysis required includes purification and identification of the compounds guided by the antiacne assay.

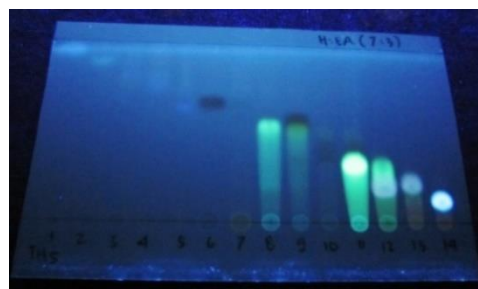


Figure 3 The chromatogram of fraction TH5 (1–14).

The chemical compound identification through phytochemical tests showed that some of the active fractions of *C. odorata* contain alkaloid and steroid and did not reveal any flavonoid, tannin, saponin, and carbohydrate compound. Phytochemical analysis indicated that fraction TH5 contains alkaloid and steroid while fraction TH6 contains alkaloid. The secondary metabolites detected in the fraction in our study show consistency with previous reports on the phytochemical composition of *n*-hexane extract of *C. odorata* [6]. Identification of alkaloids by Ikewuchi [14] showed that *C. odorata* leaf extract contained akuammidine, voacangine, lupanine, echitamine, angustifoline, echitamidine, augustamine, and crinamidine.

4. CONCLUSION

Column chromatography of *n*-hexane extract gave six active fractions, in which fractions TH5 and TH6 have the highest inhibition of 51% and 47% respectively. Phytochemical analysis indicated that fraction TH5 contains alkaloid and steroid while fraction TH6 contains alkaloid.

The active fractions isolated from *C. odorata* have the potential to be developed as a natural antiacne. Further purification, identification, and characterization of the active compounds would be our priority in future studies.

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