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Phytochemical properties of methanol and ethyl acetate extracts of cananga odorata flowers and their pharmacological activities

Sarah Niaci[1], Rosnani Nasution[1]*, Nurdin Saidi[1], Muhammad Bahi[1], Marianne Marianne [2]

[1.] Universitas Syiah Kuala, Banda Aceh, Indonesia
[2.] Sumatera Utara University, Medan, Indonesia

ABSTRACT

This work aimed to investigate the phytochemical contents of methanol as well as ethyl acetate extracts from Cananga odorata flower and their activities against a skin acne bacterium - Propionibacterium acnes. Herein, methanol extract of C. odorata was sequentially partitioned using n-hexane and ethyl acetate as solvents. Each extract was studied for its antibacterial activity against P. acnes bacteria. Antibacterial testing was conducted based on disc diffusion assay technique, where the discs were dripped with several variants of concentrations of C. odorata flower extract ranging from 0.5, 2, 4, 6, and 8%. The results showed that the ethyl acetate extract had a higher inhibition zone than the methanol extract. At a concentration of 0.5% ethyl acetate extract the diameter of the inhibition zone formed was 7.3 mm and at a concentration of 8% it was 14 mm. While the methanol extract at a concentration of 0.5% did not show antibacterial effect, but was active at a concentration of 8% with the inhibition diameter reaching 8.3 mm. The effect of extract concentration on the diameter of the inhibition zone is concentration-dependent. In conclusion, the methanol and ethyl acetate extracts of C. odorata flowers had weak inhibitory activities against P. acnes.

Keywords: Cananga odorata, biological activity, bacterial inhibition, Propionibacterium acnes, medicinal plant.

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Correspondence: Rosnani Nasution* ✉ rosnani@unsyiah.ac.id

Universitas Syiah Kuala, Banda Aceh, Indonesia

INTRODUCTION

Skin diseases such as acne are sometimes considered unimportant, even trivial when compared to other organ diseases. [1]. The cause of acne could be stem from 4 factors that may influence each other, namely changes in follicular keratinization, Propionibacterium acnes colonies, increased sebum production and inflammation. [2,3]. P. acnes bacteria could produce several inflammatory substances that induce acne development [4]. In general, acne appears due to the presence of P. acnes bacteria on the facial skin [5]. These bacteria will enter the pores of the skin that are clogged with dust and eventually cause acne. Before the bacteria growth becoming uncontrollable on the facial skin, prevention and treatment should be carried out.

The preventive treatment could be conducted either by applying healthy diet (i.e. consuming low-fat foods) or by using medicines. Generally, the commercial drugs use a lot of chemicals that contain sulfur and other astringent elements that potentially cause side effects on the skin [2]. Hence, the employment of natural ingredients in the treatment is required, where plant extracts have been reported to be capable of preventing the development of acne associated with P. acnes bacteria. [6, 7]. The utilization of natural compounds deriving from plants as curative agents are welcomed in general medical practices. [8, 9].

One of the medicinal plants that can be used to manufacture natural medicines and cosmetics is Cananga odorata – a member of family Annonaceae. Its flower has several properties including as a medicine for skin diseases, asthma, mosquito repellent, antibacterial, antioxidant and antidepressant [10]. C. odorata flowers contain flavonoids and saponins which are antibacterial and anti-inflammatory. Flavonoids are the most dominants in phenolic compounds possessing effective properties to hamper the growth of microorganisms [8,11]. Based on previous reports, the bark of the plant C. odorata has antifungal, antibacterial, and cytotoxic activities [12]. The stem bark of C. odorata has high bacteriostatic activity towards P. acnes bacteria [13]. Furthermore, C. odorata leaves contain saponins, flavonoids, and polyphenols which are thought to be useful as antimicrobials [14]. Lastly, C. odorata flower oil has antimicrobial, antioxidant, antifungal and antidepressant activities [15].

Several other studies related to C. odorata flowers have been carried out, including the extraction of C. odorata flowers as aromatherapy liquid soap [16], C. odorata oil extraction for insect

repellent lotion [17], and C. odorata flower essential oil extraction for perfume and mosquito repellent lotion [18]. The antibacterial activity test of C. odorata flowers against the growth of Staphylococcus aureus bacteria has also been reported. However, so far, researches on C. odorata flowers against P. acnes bacteria is still relatively scarce. This is the novelty of our study, where we investigated the biological activities of extracts from C. odorata against P. acnes bacteria. It is considered strategic since C. odorata flowers have high antibacterial activity, which allows them to be used as natural medicines and cosmetics [19].

MATERIAL AND METHOD

Material and Bioindicator

In this study, the apparatus used included glass utensils such as maceration containers, separating funnels, glass funnels, beakers, Erlenmeyer, ose needles, micro pipettes, gauze, discs, filter paper and other supporting tools, then a rotary evaporator (Buchi R-100), distillation apparatus (Ruchi), Ultraviolet spectrophotometer (AE-S60-2UP UV), incubator (Memmert), analytical balance (CPA22AS Sartorius), oven (Memmert), Laminar flow (Esco).

As for the materials, this work required n-hexane, ethyl acetate and methanol solvents; the solvents used for extraction were technical solvents. Phytochemical reagents, Dragendorff (Bi $(NO_3)_3$), Mayer (potassium tetra iodo mercurate), Wagner (I_2 in KI), Liebermann-Burchard (glacial acetic acid-H_2SO_4(P)). Mueller Hinton Agar (MHA) and Nutrient Broth (NB) media. Otherwise stated, all chemicals were procured from Merck (Selangor, Malaysia) and analytical grade. Drugs used as positive control were purchased from PT. Kimia Farma (Jakarta, Indonesia).

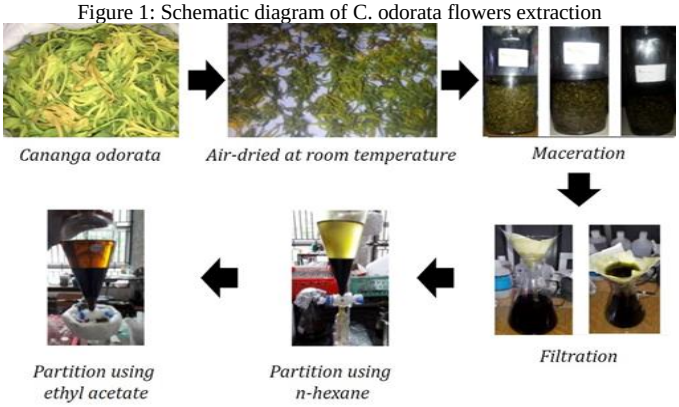
C. odorata flower samples were collected on Friday, August 23rd, 2019 from Ie Masen, Banda Aceh, Aceh, Indonesia. The determination of C. odorata plant has been carried out at the Herbarium Laboratory of the Department of Biology, Universities Syiah Kuala University.

The bioindicator used was the bacterium P. acnes (ATCC 27853) obtained from the Microbiology Laboratory, Sumatera Utara University, Medan, Indonesia.

Extraction of C. odorata

Collected fresh C. odorata flowers weighing 5 kg was cleaned using running water and dried at room temperature. Dried C. odorata flowers (1.5 kg) were cut into small pieces and macerated using methanol for 1x24 hours repeatedly for 3 days. Thereafter, the sample was filtered to obtain the filtrate, and followed by evaporation in a rotary evaporator to obtain methanol extract. The methanol extract was partitioned with increasing polarity employing n-hexane and ethyl acetate, where each soluble was labeled as n-hexane extract and ethyl acetate extract. Afterward, each extract was investigated for its antibacterial activity against P. acnes. The overall process of the extraction could be seen in the schematic diagram presented below (Figure 1). Qualitative phytochemical screenings were conducted according to the previous reports. [20,21].

Figure 1: Schematic diagram of C. odorata flowers extraction



Antibacterial Test of C. odorata Flower Extracts against P. acnes

Antibacterial testing of C. odorata flower extract was conducted through disc diffusion assay, as suggested by published work [22]. Bacterial suspension was made by adding 1-2 oses of P. acnes (ATCC 27853) bacteria into NB liquid medium after which it was incubated for 24 hours with 100 rpm shaking using a shaker. The test bacteria suspension was equalized with the turbidity of the McFarland solution 0.5-0.8.

A total of 20 µL bacterial suspension was dripped onto MHA media which had been priorly solidified in a petri dish, then leveled using a spreader. The entire plate was allowed to stand for a while for the bacteria to reach their logarithmic phase. On the agar media, a 6 mm diameter disc paper was placed on which the test solution was dripped with a concentration variant of 0.5, 2, 4, 6, and 8% (w/v) of obtained extracts from C. odorata flower, the solvent of each extract as a negative control and a solution of tetracycline antibiotics. with a concentration of 100 g/mL as a positive control, each was dripped on a different paper disc of 30 µL. Furthermore, the petri dishes were incubated at 37ºC for 18 hours.

RESULT AND DISCUSSION

Phytochemical Profile of The Extract

Phytochemical tests were done to evaluate the phytoconstituents of C. odorata flowers. The secondary metabolites tested included alkaloids, terpenoids, steroids, flavonoids, saponins and phenols. According to Table 1, it could be seen the methanol extract of C. odorata flower contained secondary metabolites of alkaloids, terpenoids, saponins and phenols. Meanwhile, extract partitioned with ethyl acetate was found to contain terpenoids, saponins, and phenols. Alkaloids and steroids were mostly present in a form of plant salts, thus becomes more soluble in methanol extract that has higher polarity. This is the reason why alkaloids and steroids were not found in ethyl acetate sample. Based on previous reports, C. odorata flowers contained phytocompounds such as flavonoids, saponins, along with volatile oil components of

monoterpene and polyphenol compounds [23].

Table 1. Phytochemical screening of the extracts from C. odorata flowers

Secondary metabolites		Methanol	Ethyl acetate
Alkaloids	Dragendorff	+	-
	Mayer	-	-
	Wagner	-	-
Steroids		+	-
Terpenoids		+	+
Flavonoids		-	-
Saponins		+	+
Phenols		+	+

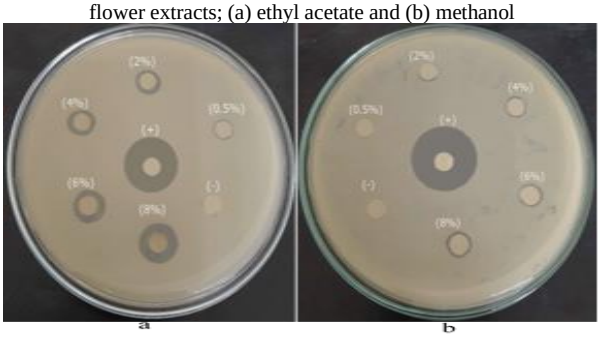
(+) = detected; (-) = not detected

The results of phytochemical testing carried out in this study are in line with several previous reports [16,24-26]. In accordance with previous reports that the bark of C. odorata contains flavonoids, tannins and contains essential oils such as linalool and eugenol [24]. C. odorata leaves are reported to contain flavonoid compounds, saponins and polyphenols. [16,24]. Meanwhile, C. odorata flowers have been reported to contain essential oils such as α-caryophyllene, caryophyllene, benzyl benzoate, germacrene D, as well as α-linalool, [25]. Furthermore, it was also reported that the content of flavonoid compounds, tannins, saponins, and steroids in C. odorata flowers was also reported. [26].

Antibacterial Activities of The Extract

Antibacterial activity test of C. odorata flower extract against P. acnes bacteria was carried out to evaluate the inhibitory effect of the extract against the tested bacteria. The selection of this test bioindicator is because P. acnes is one of the causes of human acne [5]. The bacterial test method was conducted by the disc diffusion assay by placing the disc that had been dripped with C. odorata flower extracts of several concentrations on the test medium. The testing process was repeated three times. The results of the inhibition zone of the flower extract of C. odorata against P. acnes bacteria can be seen in Figure 2, and the results of these investigations can be seen in the following Table 2. As for the n-hexane extract, the results would be reported separately.

Figure 2: Inhibition zone to assess the antibacterial activities of C. odorata flower extracts; (a) ethyl acetate and (b) methanol



Overall, our findings suggest the ethyl acetate extract showed greater inhibitory activity than the methanol extract. Ethyl acetate extract with a concentration of 0.5% showed an inhibition zone diameter of 7.3 mm and at a concentration of 8% it was 14 mm. While the methanol extract at a concentration of 0.5% did not show an inhibition zone, but the largest concentration showed an inhibition zone diameter of 8.3 mm.

Table 2. Inhibition zone of methanol and ethyl acetate extracts from C. odorata flowers against P. acnes

Samples	Inhibition zone (mm)				
	0.50%	2%	4%	6%	8%
Ethyl acetate extract	7.33 ± 0.57	8.33 ± 0.57	8.67 ± 0.57	11 ± 1	14 ± 1
Methanol extract	0 ± 0	6.3 ± 0.57	7±0	7.3 ± 0.57	8.3 ± 0.57
Tetracycline	20 ± 1				
Solvent	0 ± 0				

Tetracycline is an antibiotic used as a positive control which is one of the antibiotics that can treat various types of bacterial infections, one of which is P. acnes. Positive control produces an inhibition zone of 20-22 mm. The negative control used was the solvent of each extract, which did not show the formation of an inhibition zone, this proves that the solvent used to extract the sample of C. odorata flower had no effect on the growth of P. acnes bacteria. The antimicrobial activity was categorized as strong having inhibition zone diameter > 20 mm, moderate (16 - 20 mm), weak (10-15 mm), very weak < 10 mm [27]. Therefore, in this study, the ethyl acetate extract with a concentration of 0.5-8% had a weak zone of inhibition category, while the methanol extract had a very weak zone of inhibition. Differences in the antibacterial inhibition are due to the presence of different phytocompounds drawn into each extract sample. Weaker inhibition activity produced by methanol extract was ascribed to the presence of inert and inactive compounds, leading to the reduction of the concentration of the active compounds. Consequently, ethyl acetate extract containing less impurities (after the partitioning) would yield higher antibacterial activity. Nonetheless, the antibacterial activities could be further enhanced using topical deliver strategies including masks prepared using various biopolymers [28-32] and lotions [30].

CONCLUSION

Phytochemical screening revealed that methanolic extracts from C. odorata flowers contained alkaloids, terpenoids, saponins, and phenols. As for the ethyl acetate extract, it contained terpenoids, saponins, and phenols. The P. acnes inhibitions by the methanol and ethyl acetate extracts were very weak and weak, respectively.

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