

EFFECTIVENESS OF ANTIBACTERIAL EXTRACT OF KENOP (GOMPHRENA GLOBOSA) FLOWER EXTRACT AGAINST GROWTH OF *PROPIONIBACTERIUM ACNES* BACTERIA

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ABSTRACT

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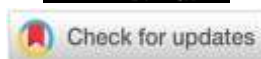
Acne,
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Abstract:

Acne is a chronic inflammation of the pilosebaceous tissue caused by the bacterium *Propionibacterium acne* (*P.acnes*) which often experiences antibiotic resistance. Research on the benefits of herbal plants in dealing with acne is still needed, one of which is the use of Knobs flowers (*Gomphrena globosa*) which is easily found and has potential as an antioxidant and antibacterial. This study aims to determine the effectiveness of the extract of the flower knob in inhibiting the growth of *P.acnes* bacteria. The design of this study was purely experimental with the Kirby-Bauer method using MHA (Mueller Hinton Agar) media. A positive control using clindamycin antibiotics and negative control using aquadest. The treatment group consisted of 25%, 50%, 75%, and 100% knob methanol extract with 3 replications. Data analysis using One Way ANOVA to find out the average difference between treatment groups. Based on the results of the study, it was found that the higher the concentration of the extract, the greater the inhibitory power as long as the extract had not reached saturated concentration ($p < 0.05$). There is a decrease in the inhibition zone diameter at 100% concentration because it is influenced by the diffusion rate of a compound and other factors.

Abstrak:

Jerawat merupakan inflamasi kronik pada jaringan pilosebaceous yang disebabkan bakteri *Propionibacterium acnes* (*P.acnes*) yang sering mengalami resistensi antibiotik. Penelitian terhadap manfaat tanaman herbal dalam menangani jerawat masih diperlukan, Salah satunya adalah pemanfaatan Bunga Kenop (*Gomphrena globosa*) yang mudah ditemui dan memiliki potensi sebagai antioksidan dan antibakteri. Penelitian ini bertujuan untuk mengetahui efektifitas ekstrak bunga kenop (*Gomphrena globosa*) dalam menghambat pertumbuhan bakteri *P.acnes*. Desain penelitian ini adalah eksperimental murni dengan metode Kirby-Bauer yang menggunakan media MHA (Mueller Hinton Agar). Kontrol positif menggunakan antibiotik klindamisin dan kontrol negatif menggunakan aquadest. Kelompok perlakuan terdiri dari ekstrak methanol bunga kenop 25%, 50%, 75% dan 100% dengan ulangan sebanyak 3 kali. Analisis data dengan One Way ANOVA untuk mengetahui beda rerata antara kelompok perlakuan. Berdasarkan hasil penelitian didapatkan bahwa semakin tinggi konsentrasi ekstrak maka daya hambatnya semakin besar selama ekstrak belum mencapai konsentrasi jenuh ($p < 0,05$). Terdapat penurunan diameter zona hambat pada konsentrasi 100% karena dipengaruhi kecepatan difusi suatu senyawa dan faktor lainnya.



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INTRODUCTION

Acne (*Acne vulgaris*) is a chronic inflammation of the pilosebaceous tissue caused by *Propionibacterium acnes* (*P.acnes*) bacteria which is characterized by the appearance of blackheads, pustules, lesions, papules, and nodules measuring 1-5 mm [1]. The incidence for acne were 26% in women, 12% of men with aged 40 years old, and 20% of adolescents [2]. Based on the literature, approximately 81% people with acne are influenced by genetic factors, and the rest is influenced by external factors such as air pollution, race, excessive fatty food, etc. It also easily appears on oily facial skin and during pre-menstruation on for women due to hormonal influences. Acne often affects areas of the face so that it interferes with daily activities, cause lack of confidences and 30% -50% of psychiatric disorders [3].

The pathogenesis of rupture, consists of follicular epidermal hyperproliferation, excessive sebum production, and the appearance of *P.acnes* bacteria. *Propionibacterium acnes* produces extracellular lipase, which replaces inflammation in the skin and overcomes acne [4]. The wall of the *P.acnes* bacteria consists of antigens that stimulate antibody formation. *Anti-Propionibacterium* anti-bodies increase the proinflammation cascade. This bacteria stimulates cytokine binding that binds to *Toll-Like Receptor 2* (TLR-2) on monocytes and poly-morphonuclear cells in the sebaceous follicle region, instead, using pro-inflammatory cytokines such as IL-1 α , IL-8, IL-12, and TNF- α [3]–[5].

Pharmacotherapy for *P.acnes* bacteria is often used is benzoyl peroxide, antibiotics, or retinoids, but retinoid often cause side effects such as erythema, itching, rashes, even pseudomembranous colitis and also experience resistance such as metronidazole and phosphomimic groups [6]. Clindamycin drug could be using against *P.acnes*. In addition, clindamycin was use as positive control that effective to inhibit the growth of *P.acnes* bacteria.

There are 30,000 species of medicinal plants in Indonesia, and Indonesian citizens use only 1,200 species [7]. One of the medicinal plants that are often used is the kenop flower (*Gomphrena globosa*). The Kenop flower has a length of 10-15 cm and is commonly used as ornamental plants and in Bali traditional ceremonies. Kenop flower (*Gomphrena globosa*) in the basic contains such as carbohydrates, antifungal, glycosides, phenols, koumarins, steroids, anthraquinones compounds, color pigments betacyanin, anti-cancer, analgesics, gomphrenin III which are anti-inflammatory and antioxidant substances [7], [8]. According to Kusmiati research in 2017, Kenop flower extract (*Gomphrena globosa*) contains antibacterial substances that can inhibit the growth of the *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhi* bacteria [8]. Antioxidants in *Gomphrena globosa* flowers such as tannins, flavonoids, saponins, quinones, and alkaloids block the mechanism of metabolism and interfere with the permeability of bacterial cells so that they inhibit bacterial growth. Furthermore, the research for effectiveness from Kenop flower against *P.acnes* bacteria not already yet. Therefore, we conducted a study to find out the effect of kenop extract on the inhibitory growth of *P.acnes* bacteria.

RESEARCH METHOD

This research is an experimental study carried out at the Microbiology Laboratory of Udayana University from July to September 2019. The study has received an ethical eligibility permit from the Research Ethics Commission (KEP) of the Faculty of Medicine, Udayana University, with letter number 1825 / UN14.2.2.VII.14 / LP / 2019. The independent variable in the study was the methanol extract of kenop flower (*Gomphrena globosa*), which was tested on *P.acnes* bacteria. The dependent variable is

the diameter of the inhibitory zone in *P.acnes* bacteria. Controlled variables were temperature and incubation time, that was repeated three times to ensure validity. There were five samples in one experiment, the concentration for samples are from Kenop flower extract such as, 25%, 50%, 75%, 100%, and also clindamycin for positive control.

Primary data obtained from the Kirby-Bauer experimental method with Muller-Hinton media. Secondary data was obtained from literature studies, journals, literature reviews, textbooks, and the internet. The steps in this study, firstly, was to manufacture a simplicial and methanol extract using the kenop (*Gomphrena globosa*) flower. Following that, was to make the bacterial culture using the Kirby-Bauer method, and lastly, would be to test for antibacterial activity, and to measure the data using the SPSS software application version 16.00. From 100 grams

kenop (*Gomphrena globosa*) flower simplicial, around 2 grams of a thick extract of the kenop (*Gomphrena globosa*) was obtained. The test solution consists of 4 groups, namely group 1 methanol extract concentration of 100% methanol kenop (*Gomphrena globosa*) flower, group 2 with 75% concentration, group 3 with 50% concentration, and the last group concentration was 25%. The Positive control solution used is clindamycin phosphate 1.2%, and the negative control used is distilled water. *Propionibacterium acnes* bacteria was isolated on blood agar media and incubated at 37°C for 24 hours, and its form and nature have been proven by gram staining.

RESULTS AND ANALYSIS

The results of the measurement of the inhibition of these bacteria are measured using calipers in millimeter (mm) (Table 1 and Figure 1).

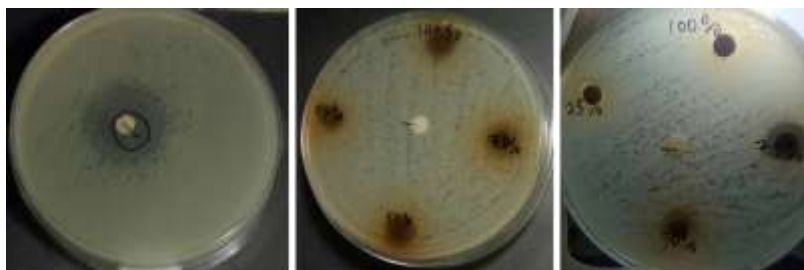


Figure 1. Potential of Kenop flowers (*Gomphrena globosa*) induced Bacterial *P.acnes* inhibition in MHA media.

Table 1.
The Result of Minimum Inhibitory Concentration (MIC) Extract of Kenop (*Gomphrena globosa*) flower with *Propionibacterium acnes* Bacteria.

Groups	Repetition			Mean±SD (mm)
	I (mm)	II (mm)	III (mm)	
Positive Control	15 (+)	16 (+)	16 (+)	15.6±0.023 (+)
Negative Control	0 (-)	0 (-)	0 (-)	0 (-)
P1: 100% of Knobs flowers Extract	6.75 (+)	8 (+)	7 (+)	7.25±0.127 (+)
P2: 75% of Knobs flowers Extract	11 (+)	12 (+)	11 (+)	11.33±0.313 (+)
P3: 50% of Knobs flowers Extract	8 (+)	10 (+)	10 (+)	9.33±0.042 (+)
P4: 25% of Knobs flowers Extract	6 (+)	6 (+)	7,5 (+)	6.5±0.052 (+)

*Note: + (Positive inhibits bacterial replication) & - (Negatively inhibits bacterial replication)

Table 2.
The Result Comparison to Different
Concentration Group by One Way
ANOVA Analysis.

Group		Mean Difference	p
P1	P2	2,83	0.00
	P3	4,83	0.00
	P4	0,75	0.232
P2	P3	2,00	0.006
	P4	2,08	0.004
P3	P4	4,08	0.000

*Note: P1 (concentration of 100%), P2 (concentration of 75%), P3 (concentration of 50%), P4 (concentration of 25%)

The normality test uses Shapiro-wilk and shows that the data is normally distributed so the average difference test is using One Way ANOVA because the average difference is more than two groups (Table 1). Based on Table 2, it can be concluded that the higher the extract, the greater the inhibitory power, as long as the extract has not reached saturated concentration (CI = 95% and $p < 0.05$). The lowest extract concentration needed to cause inhibition is 25%. The average inhibition on control + 15.6 mm, control - 0 mm, concentration of 100% 7.25 mm, concentration 75% 11.33 mm, concentration 50% 9.33 mm and concentration 25% 6.5 mm.

The inhibitory zone from concentration of 75% is classified as a strong inhibitory zone while the inhibitory zones of 100%, 50%, and 25% are classified as moderate. This is consistent with research conducted by Manaf and colleagues in 2013, which stated that the provisions of the antibacterial strength of a plant are inhibitory zones.[9] If the inhibition zone is equal to 20 mm, the inhibition zone is sensitive, the inhibition zone of 10-20 mm is classified as sensitive, 5-10 mm is classified as moderate, and the inhibition zone below 5 mm is classified as resistance [9], [10]. According to Hidayah's statement in 2016, a microbe is declared sensitive to antimicrobials if the anti-

microbial substances in plant extracts have a inhibition zone of 12-24 mm [10]. The results show that only kenop (*Gomphrena globosa*) flower extract with a concentration of 75% are sensitive to *P.acnes* bacteria with minimum inhibitory zone 11.3 mm which is close to a minimum standard of antimicrobials that are sensitive to bacteria. Clindamycin antibiotic as the positive control is bacteriostatic and bactericidal and also has a narrow spectrum antibiotic group because it only works on gram-positive bacteria. Its performance is influenced by the concentration of the drug, the causative organism and the area of infection of the organism [6], [10]–[12].

In general, the increase in diameter of the inhibitory zone of the extract in bacterial activity increases with the concentration of the extract being concentrated. However, this study found a decrease in inhibition zone diameter at a concentration of 100% due to differences in the diffusion rate of antibacterial compounds on agar media, types and concentrations of antibacterial compounds, so as to give a difference in zone diameters within a certain period of time [6], [10], [13], [14].

The phenol component in flavonoids denaturizes enzymes and amino acids and activates essential enzymes in *P. acnes* bacteria [6], [11]. It caused bacterial cells nutrient leakage due to damage to cells membrane hydrophobic bonds such as protein and phospholipid bonds thus inhibiting the bacterial replication [10].

Tannin compounds bind to the building blocks of H + bacterial protein walls. That could effect the PH becomes acidic and makes the protein denature, thereby inhibiting bacterial development. Tannins also inhibit DNA topoisomerase and reverse transcriptase enzymes so that bacterial cells cannot replicate [8], [11], [15], [16].

Alkaloid compounds interfere with the peptidoglycan constituent in bacterial cells which cause the cell wall layer not to be formed as a whole due to a disruption in the process of active transport function, selectively the permeability function and protein composition, so that it will cause the cell death slowly while saponin compounds work by inhibiting and even killing bacteria by decreasing cell surface tension, causing the bacteria to undergo lysis[8],[9],[14],[16].

CONCLUSION

Kenop flower (*Gomphrena globosa*) extract has good bacterial inhibition, especially from concentration of 75% with a sensitive inhibitory zone category. The conclusion of this study is Knobs flowers has a great potential anti-bacterial against *P.acnes* bacterial. Further research needs to be done on the toxicity and bactericidal effects of kenop (*Gomphrena globosa*) flower extracts on other microorganisms. One of the potential results that can be done, is by making the extract of the kenop (*Gomphrena globosa*) flower into an acne herbal medicine which will continue to be available in phytopharmacology capsulation after several tests in subsequent studies.

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