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Phytochemical Screening and Antibacterial activity of Polyphenol rich Extract of Passion Fruit Pericarp (*Passiflora edulis* Sims) on *Propionibacterium acnes*

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ABSTRACT

Indonesia's primary hub for cultivating purple passion fruit is North Sumatera. The passion fruit was transformed into a beverage product, which led to the creation of passion fruit pericarp trash. The potential medicinal properties of the passion fruit pericarp, particularly its antibacterial impact, need to be further explored. This study aimed to assess the phytochemical composition and antibacterial properties of an ethanol extract derived from the pericarp of purple passion fruit against Propionibacterium acnes. The extraction was performed using the percolation method with 96% ethanol. The antibacterial activity against Propionibacterium acnes was assessed using the agar diffusion method with paper discs. The phytochemical screening of the simplicia and ethanol extract revealed the existence of flavonoid, glycoside, saponin, tannin, and steroid/triterpenoid compounds. The ethanol extract exhibited significant antibacterial activity against Propionibacterium acnes at a dosage of 300 mg/ml, demonstrating a dose-dependent impact.

Keyword: *Passiflora edulis* Sims, antibacterial, *Propionibacterium acnes*, Phytochemical.

ABSTRAK

Pusat utama budidaya buah markisa ungu di Indonesia adalah Sumatera Utara. Buah markisa ini diolah menjadi produk minuman, yang menghasilkan limbah perikarp (kulit buah) markisa. Potensi sifat obat dari perikarp markisa, terutama dampak antibakterinya, perlu dieksplorasi lebih lanjut. Penelitian ini bertujuan untuk menilai komposisi fitokimia dan sifat antibakteri dari ekstrak etanol yang berasal dari perikarp buah markisa ungu terhadap Propionibacterium acnes. Ekstraksi dilakukan dengan metode perkolasi menggunakan etanol 96%. Aktivitas antibakteri terhadap Propionibacterium acnes dinilai menggunakan metode difusi agar dengan cakram kertas. Skrining fitokimia dari simplisia dan ekstrak etanol mengungkapkan adanya senyawa flavonoid, glikosida, saponin, tanin, dan steroid/triterpenoid. Ekstrak etanol menunjukkan aktivitas antibakteri yang signifikan terhadap Propionibacterium acnes pada dosis 300 mg/ml, menunjukkan dampak yang bergantung pada dosis.

Keyword: Passiflora edulis *Sims*, antibakteri, *Propionibacterium acnes*, Fitokimia

1. Introduction

Indonesia, being a tropical country, is home to an extensive variety of plants that have long been utilized for various aspects of human life, including as herbal medicines. The use of medicinal plants in Indonesia dates back centuries, well before the advent of modern healthcare services and pharmaceuticals. This tradition of using herbal medicine is deeply embedded in the nation's culture and continues to be widely practiced across communities [1] . Despite the long history and cultural significance, the effectiveness and safety of many

traditional medicines remain inadequately studied. Therefore, it is crucial to explore, research, develop, and optimize these natural medicinal resources, which are valuable national assets. One such plant with significant medicinal potential is the purple passion fruit (*Passiflora edulis* Sims.). This fruit is rich in various phytochemicals that contribute to its potential health benefits. The leaves of the purple passion fruit contain glycosides, tannins, flavonoids, saponins, and alkaloids [2]. Similarly, the stems also contain glycosides, flavonoids, saponins, and alkaloids, while the fruit itself is rich in glycosides, tannins, flavonoids, and alkaloids. These compounds are known for their diverse biological activities, including antibacterial properties.

Previous studies have demonstrated that the methanol extract of purple passion fruit leaves exhibits antibacterial activity against a range of bacteria, including *Staphylococcus aureus, Staphylococcus faecalis, Bacillus subtilis, Escherichia coli, Proteus vulgaris*, and *Salmonella typhi* [3]. This finding underscores the potential of purple passion fruit as a source of natural antibacterial agents, which could be harnessed to develop alternative treatments for bacterial infections.

North Sumatra, a region in Indonesia, is a major producer of purple passion fruit. The fruit is primarily processed into beverage products, resulting in the generation of significant amounts of passion fruit pericarp waste. This byproduct has been largely overlooked, despite its potential medicinal value. Recent research suggests that the pericarp of the purple passion fruit may possess antibacterial properties, making it a promising candidate for further investigation [4]. The research gap lies in the limited understanding and exploration of the antibacterial properties of purple passion fruit pericarp. While studies have focused on the leaves, stems, and fruit, the pericarp remains under-researched despite its potential medicinal applications. Given the increasing interest in natural and sustainable medicinal resources, it is crucial to examine the antibacterial activity of the ethanol extract of purple passion fruit pericarp. One specific area of interest is its potential efficacy against Propionibacterium acnes, a bacterium associated with acne vulgaris. Acne is a common skin condition that affects millions of people worldwide, and there is a growing demand for effective, natural treatments with fewer side effects compared to conventional antibiotics.

2. Materials and Methods

2.1. Materials used

The study utilized purple passion fruit pericarp, nutrient agar, nutrient broth, and distilled water as the ingredients.

2.2. Identification of plants

The ripe fruit of Passiflora edulis Sims were gathered from the Central Market in Medan, Indonesia. The sample was detected at the Indonesian Institute of Science, specifically at the Research Center of Biology in Bogor, Indonesia.

2.3. Passion Fruit pericarp extraction

300 grams of powdered dried leaves were extracted using a maceration process with a mixture of ethanol and water (70:30, v/v). The extraction process required continuous agitation at a temperature of 25°C. The combination underwent filtration after a duration of 24 hours. This process was duplicated twice, resulting in a cumulative total of three extractions. The samples were combined and then centrifuged at a speed of 3500 rpm for 10 minutes at room temperature. The liquid component was condensed through the use of a rotary evaporator at a temperature of 38°C, resulting in the production of the hydroethanolic extract (HESc). The HESc sample was subjected to chloroform extraction (1:1 v/v; 3 times) to separate it, and then the water phase was extracted using ethyl acetate (1:1 v/v; 3 times). The ethyl acetate fraction was subjected to evaporation under decreased pressure and subsequent freeze-drying, leading to the formation of an extract high in polyphenols, referred to as PESc [5].

2.4. Analysis of the chemical compounds

The chemical secondary metabolites of alkaloids, flavonoids, glycosides, tannins, saponins, triterpenoids, and steroids were analyzed in the ethanol extract and phytochemical simplicia of Passion Fruit pericarp [6].

2.5 Measurement of total phenolic

The quantification of the extract's overall phenolic content was conducted using the Folin–Ciocalteu technique. The crude extract (1 mg/mL) was diluted to 3 mL with distilled water, mixed with 0.5 mL of Folin–Ciocalteu reagent for 3 minutes, and then 2 mL of 20% (w/v) sodium carbonate was added. The absorbance was measured at a wavelength of 650 nm after allowing the mixture to remain undisturbed in darkness for an additional 60 minutes. The total phenolic content was determined by employing the calibration curve and the outcomes were presented in milligrams of gallic acid equivalent per gram of dry weight [7].

2.6 Measurement of total flavonoids

The overall flavonoid content was assessed using the aluminum chloride colorimetric technique. Initially, 50 µL of crude extract (1 mg/mL ethanol) was diluted to 1 mL with methanol. This mixture was then combined with 4 mL of distilled water and 0.3 mL of a 5% NaNO2 solution. Subsequently, the combination was left undisturbed for a duration of 5 minutes prior to the addition of 0.3 mL of a solution containing 10% AlCl3. After the addition of 2 mL of a 1 mol/L NaOH solution, the volume was then modified to 10 mL using double-distilled water. The measurement of absorbance at a wavelength of 510 nm was conducted after allowing the mixture to remain undisturbed for a duration of 15 minutes. The final results were quantified as milligrams of quercetin equivalent per gram of dry weight. The total flavonoid concentration was determined by utilizing a calibration curve [8].

2.7. Bacterial Inoculum Preparation

Bacterial colonies were obtained from the culture stock using a sterile ose needle and then placed in a test tube containing 10 ml of nutrient broth media. The turbidity of the solution was measured at a wavelength of 580 nm until it reached a transmittance of 25%, which corresponded to 106 CFU (Colony Forming Units) [9].

2.8. Procedure for preparing the extract solution test

A precise amount of 1 gram of ethanol extract derived from the pericarp of passion fruit was measured using an analytical balance. The extract was diluted in 2 ml of DMSO solvent in a vial, resulting in an extract concentration of 500 mg/ml. The solvent was diluted to various quantities ranging from 400 to 6.25 mg/ml [10].

2.9. *Conducting an In Vitro Antibacterial test*

An inoculum of bacteria, measuring 0.1 ml, was placed in a petri dish. Then, 20 ml of nutrient agar was added and the mixture was allowed to achieve a temperature of 45°C. It was then homogenized and left undisturbed until the agar solidified. In addition, little circular pieces of paper (with a diameter of 6 mm) were soaked in a solution containing the extract at different concentrations. The soaked paper discs were then dried and placed on top of the agar media. The media was cultured at a temperature range of 36-37 °C for a duration of 18-24 hours. The caliper was used to measure the diameter of the inhibitory area surrounding the paper disc. The experiment was conducted thrice [11].

3. Results and Discussion

3.1. Phytochemical screening result of dried sample and ethanol extract of purple passion fruit

The qualitative test of phytochemical properties ondried sample and ethanol extract of purple passion fruit pericarp showed in table 1.

Table 1. Phytochemical screening of dried sample and ethanol extract purple passion fruit pericarp

No.	Phytochemical properties	Simplicia	Ethanol extract
1.	Alkaloids	-	-
2.	Flavonoids	+	+
3.	Glycosides	+	+
5.	Saponin	+	+
6.	Tannin	+	+
7.	Steroids/Triterpenoids	+	+

where:

(+) positive: contains a class of compounds

(-) negative: does not contain compounds

Table 1 showed that the simplicia and ethanol extract of purple passion fruit pericarp had several compound such as alkaloids, flavonoids, glycosides, anthraquinone glycosides, saponin, tanin and steroids/triterpenoids. Flavonoids, tannin, saponins and steroids / triterpenoids are phytochemical compounds that have potential effect as antibacterial and antiviral agents [12].

3.2 Total Phenol, Flavonoid and In Vitro Antioxidant Activity

Spectrophotometer UV-VIS performed the quantitative analysis of total phenol, flavonoid and in vitro antioxidant activity of passion fruit pericarp

. The results are presented in Table 2

Table 2. total phenol, flavonoid and in vitro antioxidant activity of passion fruit pericarp

Sample	Mean Total Phenol Content (mg GAE/g sample)	Mean Total Flavonoid Content (mg QE/g sample)

Polyphenol rich extract of passion fruit pericarp	32.645 ± 0.863	14.72± 0.85

Passion fruit pericarp is a rich source of phenolic compounds and flavonoids, which are associated with various health benefits, including potential antibacterial activity. Studies have shown that passion fruit pericarp contains high levels of total phenolic compounds, known for their antioxidant properties and their ability to protect against oxidative stress and inflammation. As shown in Table 2, the total phenol content was measured at 32.645 ± 0.863 mg GAE/g sample, while the total flavonoid content was 14.72 ± 0.85 mg QE/g sample.

3.3 Antibacterial test of purple passion fruit pericarp ethanol extract

The results of the measurement of the inhibiting diameter of bacterial growth area Propionibacterium acnes can be seen in the table 3.

Table 3. Antibacterial acticity of ethanol extract of purple passion fruit pericarp

No.	**Concentration (mg/ml)**	**Diameter of Inhibition area (mm)**			
		P acnes			
		D1	**D2**	**D3**	**Mean**
1.	500	17,3	16,9	17,4	17,20
2.	400	15,9	15,6	15,9	15,80
3.	300	14,8	14,5	14,8	14,70
4.	200	13,4	13,5	13,6	13,50
5.	100	12,4	12,7	12,6	12,57
6.	75	10,4	10,4	10,3	10,37
7.	50	7,2	7,1	7,3	7,20
8.	25	6,5	6,2	6,5	6,40
9.	12,5	-	-	-	-
10	6,25	-	-	-	-
11.	Blank	-	-	-	-

where:

D : diameter (mm)

The concentration of the extract that meets the requirements set by Ditjen POM (1995) is one that shows an effective inhibition zone of approximately 14-16 mm. The antibacterial activity of the ethanol extract against Propionibacterium acnes demonstrated effective inhibition at a concentration of 300 mg/ml, with an inhibition diameter of 14.70 mm. The minimum inhibitory concentration (MIC) of the ethanol extract against Propionibacterium acnes was found to be 25 mg/ml. The ethanol extract of passion fruit pericarp effectively inhibited the growth of *Propionibacterium acnes* due to its phytochemical properties, which include strong antibacterial compounds such as flavonoids, tannins, saponins, and steroids/triterpenoids.

Tannins are phenolic compounds widely distributed in vascular plants. These compounds and their derivatives act as antibacterials by disrupting the function of the cytoplasmic membrane. At low concentrations, phenolic compounds can damage the cytoplasmic membrane, causing leakage of important metabolites necessary for the bacterial enzyme system. At higher concentrations, they can cause more extensive damage to the cytoplasmic membrane and cellular proteins [13].

Saponins have been recognized as effective antimicrobials in recent years. Their mechanism of action involves reducing surface tension, which increases cell permeability or causes cell leakage, leading to the discharge of intracellular compounds [14]. Steroids and triterpenoids also exhibit antibacterial activities, with several studies reporting their efficacy against various bacteria [15].

The cell wall structure of Gram-positive bacteria is relatively simple, consisting of a single layer with low lipid content (1-4%), making it easier for bioactive compounds to penetrate the cell [16]. In contrast, the cell wall of Gram-negative bacteria is more complex, comprising three layers: an outer layer of lipoprotein, a middle layer of lipopolysaccharide that acts as a barrier to antibacterial bioactive materials, and an inner layer of peptidoglycan with high lipid content (11-12%) [17-18].

4. Conclusion

Antibacterial activity of ethanol extract of purple passion fruit pericarp on *Propionibacterium acnes* shows an effective inhibition at the concentration of 300 mg / ml, and the effect shows dose dependent manner.

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