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ORIGINAL RESEARCH

Antibacterial Activity of Passion Fruit Purple Variant (*Passiflora edulis Sims var. edulis*) Seeds Extract Against *Propionibacterium acnes*

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Background: Passion fruit is used commercially for consumption and in beverages. This plant exhibits various pharmacological properties and possesses a complex phytochemistry. In recent years, this plant has been shown to have potential antimicrobial activity. The seeds contain a high amount of piceatannol, which exhibits an inhibitory effect on *Propionibacterium acnes*. Therefore, the seed extract of purple variant, *Passiflora edulis Sims var. edulis*, may have potential antibacterial activity against *P. acnes*.

Aim: To evaluate the antibacterial activity of *P. edulis Sims var. edulis* seeds extract on *P. acnes.*

Methods: Seed extract was prepared by maceration and dissolved in DMSO in multiple concentrations. *Propionibacterium acnes* was cultured in *Brucella* blood sheep 5% agar for 24 hours and the agar disc diffusion method was used to evaluate the inhibitory effect of each concentration (1.25%, 2.5%, 5%, 10%, 20%, and 40%), in comparison with clindamycin and erythromycin. Zones of inhibition at 24 hours were measured and documented, then analyzed to obtain the mean inhibition zone (MIZ). The Mann–Whitney test was conducted to compare the antibacterial activity of the extract, clindamycin and erythromycin. A serial dilution assay of five different concentrations (5%, 10%, 15%, 20%, and 25%) in brain–heart infusion broth was performed to determine the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC).

Results: After 24 hours, all concentrations of *P.edulis Sims var. edulis* showed an inhibitory effect against *P. acnes*, with MIZs for of each concentration of 1.25% 6 mm, 2.5% 6.83 mm, 5% 8.5 mm, 10% 10.08 mm, 20% 14 mm, and 40% 16 mm. Clindamycin revealed comparable antibacterial effect to 5% seed extract, with an MIZ of 8 mm. However, erythromycin was found to be superior to the seed extract, with a 22.67 mm MIZ ($p<0.05$). The serial dilution assay revealed an MIC of 20% and MBC of 25%.

Conclusion: Seed extract of *P. edulis Sims var. edulis* had good antibacterial activity against *P. acnes*, and the effect increased with the concentration.

Keywords: *Passiflora edulis*, passion fruit, *Propionibacterium acnes*, antibacterial

Introduction

Plants have been used over thousands of years as a source of therapeutic medicine. Tropical fruits are largely known to have not only antioxidant and anti-inflammatory activities but also antibacterial properties, and have a long history of use as traditional herbal medicines.[1] *Passiflora edulis Sims* is a variety of passion fruit that is mostly used commercially for consumption, in refreshment drinks and in syrup production. This plant exhibits various pharmacological properties and possesses a complex

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phytochemistry. Most of the pharmacological investigations into *P. edulis* have addressed its central nervous system (CNS) activities, such as anxiolytic, anticonvulsant, and sedative actions.[2] In recent years, many studies have been conducted on the purple variant, *Passiflora edulis Sims var. edulis*, and revealed a high concentration of piceatannol. This polyphenol is largely found in the seeds and shows high antioxidant and potential antibacterial activity.[3,4]

Propionibacterium acnes is a Gram-positive anaerobic bacillus normally found on human skin and plays an important role in the pathogenesis of acne vulgaris. People with acne lesions have higher numbers of *P. acnes* compared with controls. Increased colonies are found as a result of sebum accumulation and infundibulum hyperkeratosis. Furthermore, *P. acnes* was found to be capable of inducing the release of chemoattractants, eliciting the inflammatory response. Therefore, the clinical manifestations appear to be the result of bacterially induced inflammation of plugged sebaceous glands.[4,5] Docherty et al reported that piceatannol has antibacterial activity against *P. acnes* and this is comparable with erythromycin and benzoyl peroxide, which are commonly used as first line topical antimicrobial therapy in acne vulgaris.[4] For this reason, an in vitro study is necessary to evaluate the antibacterial property of passion fruit extract, which contains a high concentration of piceatannol. Therefore, the aim of this study is to evaluate the inhibitory effect of *P. edulis Sims var. edulis* seed extract against *P. acnes*.

Materials and Methods

Seeds of *P. edulis Sims var. edulis* were collected from a farm in Brastagi, Karo, North Sumatera, Indonesia, from January to February 2019. The extract was prepared by maceration in the Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. First, the seeds were separated from the pulp, dried at 40°C, and mashed with a blender into powder. Then, the powder was soaked in 96% ethanol in a 2:3 ratio for 5 days, and evaporated at 40–50°C to produce the seed extract (Figure 1). The extract was dissolved in DMSO into multiple concentrations, of 1.25%, 2.5%, 5%, 10%, 20%, and 40% (Figure 2A).

Propionibacterium acnes used in this study was obtained from ATCC (strain 11827). In the first step, the antimicrobial activity of the plant extracts was evaluated using the agar disc diffusion method. The inoculum size of each clinical isolate was standardized matching a turbidity equivalent to a 3 McFarland standard. A sterile cotton swab was dipped into the suspension, and rotated several times on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. The swab was drawn over the entire surface of already prepared plates of Brucella blood sheep (BBS) 5% agar to achieve uniform distribution of bacteria (Figure 2B). Four discs impregnated with the extracts at various concentrations and one disc in the center with clindamycin as control were placed on the BBS 5% agar plates (Figure 2C). Three separate plates were prepared

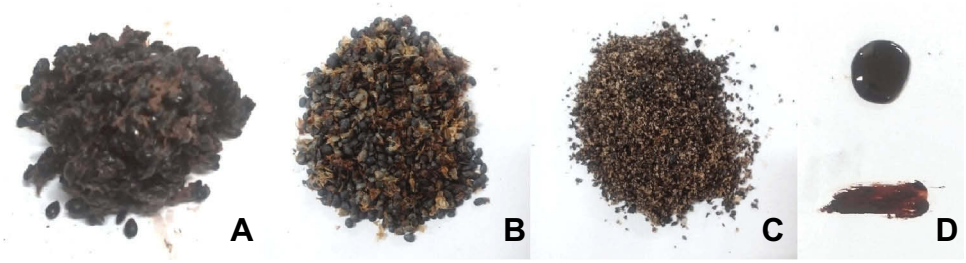

Figure 1 *Passiflora edulis Sims var. edulis*: (**A**) wet seeds, (**B**) dry seeds, (**C**) simplicia, and (**D**) seed extract.

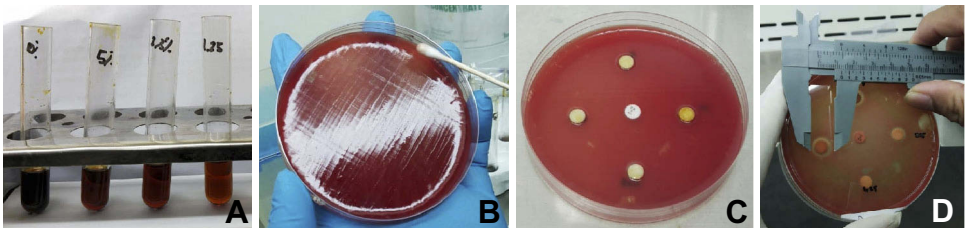

Figure 2 (**A**) Seed extract of *Passiflora edulis Sims var. edulis*; (**B**) isolated *P. acnes* swabbed over BBS 5% agar plate for 24 hours; (**C**) disc diffusion method of various extract concentrations compared with clindamycin (center); (**D**) measuring the inhibition zone using calipers.

in a similar manner for the positive controls of the bacterial strains. We also compared the 1% extract with erythromycin in conjunction with clindamycin as the most common topical antibiotic preparation used in acne vulgaris. Cultures were incubated in an anaerobic gas jar with an anaerobic gas pack, creating anaerobic conditions at 37°C, and the inhibition zone diameter was measured at 24 hours (Figure 2D). The entire procedure was repeated for three sets of plates. Data were analyzed to find the mean diameter of the zone of inhibition and the Mann–Whitney test was used to compare the inhibition zones of the extract, clindamycin and erythromycin.

The antibacterial assay was continued with a serial dilution method for several concentrations of the extract, namely 5%, 10%, 15%, 20%, and 25%. Brain–heart infusion (BHI) broth medium was prepared in dilution tubes. The cotton swab containing *P. acnes* was dipped into the dilution tubes and rotated several times. Then, each concentration of the extract was added to the dilution tubesand incubated anaerobically at 37°C for 24 hours (Figure 3). Thereafter, a sterile cotton swab was dipped into each dilution tube and swabbed over the entire surface of BBS 5% agar plates. The agar plates then were incubated anaerobically at 37°C for another 24 hours and *P. acnes* culture results were compared for each extract concentration to determine the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). The entire procedure was repeated for three sets of tubes.

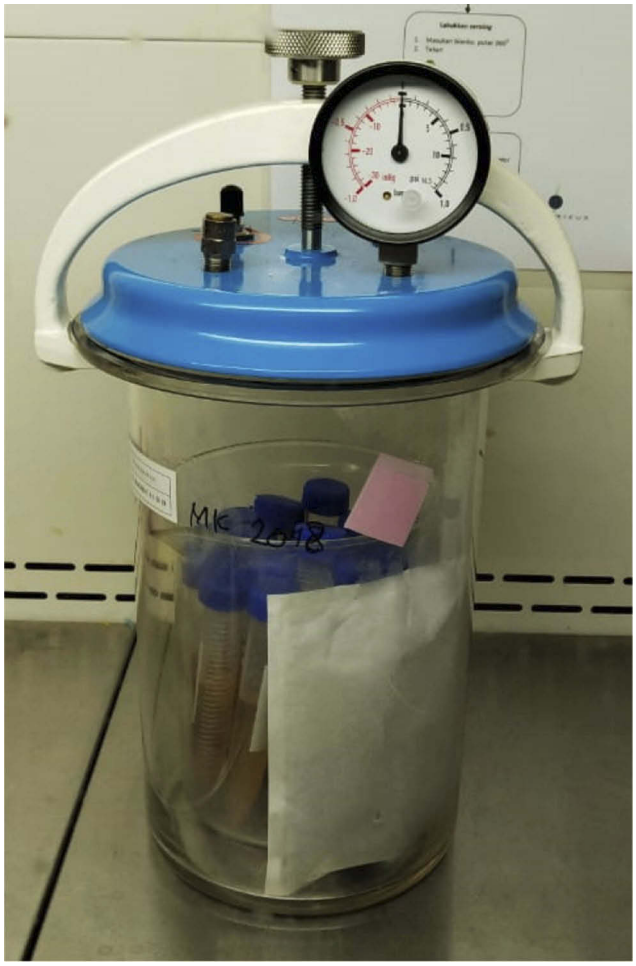

Figure 3 All the tested dilution tubes were put into an anaerobic jar with a gas pack.

Results

In the agar disc diffusion method, all concentrations of *P. edulis Sims var. edulis* showed inhibitory activity on *P. acnes* (Figure 4). The minimum concentration of the extract to have an antibacterial effect was 5%, with a mean inhibition zone (MIZ) of 8.5 mm, followed by 10% with MIZ 10.08 mm, 20% 14 mm, and 40% 16 mm (Table 1). These results are comparable with or even superior to clindamycin, which had an MIZ of 8 mm. There was no statistically significant difference in inhibition zones between *P. edulis Sims var. edulis*seed 5% extract and clindamycin (p=0.121). However, the antimicrobial activity of *P. edulis* seedextract was inferior to erythromycin, which had an MIZ of 22.67 mm (p=0.046) (Table 2).

In serial dilution assays, *P. acnes* growth was still found at concentrations of 5%, 10%, 15%, and 20%. The passion fruit purple variant seed extract showed more than 50% colony inhibition at a concentration of 20%. Furthermore, no bacterial growth was found at 25% concentration of the extract (Figure 5). Therefore, the MIC and MBC of *P. edulis Sims var. edulis* seed extract against *P. acnes* were 20% and 25%, respectively.

Discussion

Preliminary analysis of the crude extract from the leaf, stem, and fruit of *Passiflora edulis* revealed the presence of several biologically active compounds, such as carbohydrates, tannin, glycoside, flavonoid, saponin, alkaloids, volatile oil, phenol, resins, and balsam, which may have therapeutic value.[6] Furthermore, Johnson et al reported the antimicrobial properties of the leaf and callus of *P. edulis*, which varied in effectiveness depending on the solvent type owing to the variety of contained compounds, including saponins, steroids, tannins, phenolics, triterpenoids, alkaloids, and flavonoids.[7] Ripa et al observed that the chloroform leaf extracts were much more active than the petroleum ether extracts, having average zones of inhibition of 7–10 mm by the disc diffusion method.[8] These

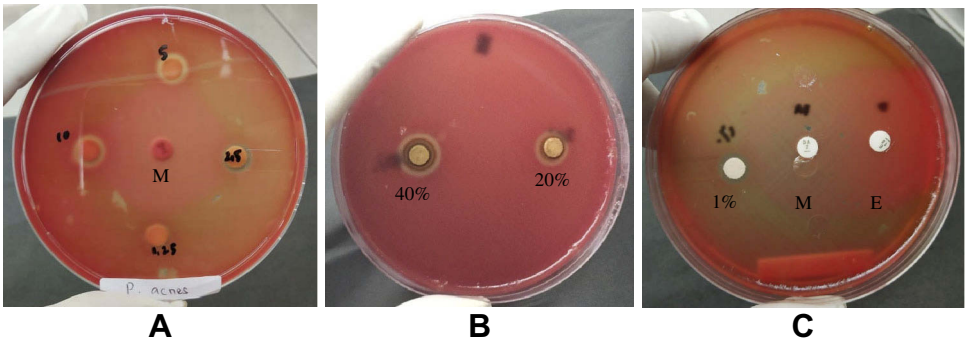

Figure 4 Agar disc diffusion method showing the inhibition zone of *Passiflora edulis Sims var. edulis* seed extract with concentrations of (**A**) 1.25%, 2.5%, 5%, and 10%, (**B**) 20% and 40%; (**C**) compared with clindamycin (M) and erythromycin (E).

studies showed that the stem extract has the highest activity against the growth of *Vibrio mimicus*, with a zone of inhibition of 17 mm. Besides this, the extract showed good activity against the growth of *Vibrio parahaemolyticus* (16 mm), *Shigella dysenteriae* (15 mm), and *Shigella boydii* (14 mm).[7,8] According to Akanbi et al, the fruit extract has significant antimicrobial activities in hexane and water solvents. The hexane extract showed MIZs of 16 mm for *Klebsiella pneumoniae*, and 14 mm for *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and the water extract an MIZ of 16 mm for *Salmonella paratyphi*. Furthermore, the hexane leaf extract showed MIZs of 16 mm for *S. paratyphi*, 15 mm for *S. aureus*, 14 mm for *P. aeruginosa*, and 13 mm for *K. pneumoniae*. The water extract showed degree of growth inhibition of 15 mm for *S. paratyphi* and 13 mm for *S. aureus*. The stem extracts showed greater and significant activities for the hexane and methanol extracts, with MIZs of 16 and 14 mm, respectively, for *K. pneumoniae*. No activity was observed against any of the tested bacteria for the ethyl acetate and water stem extracts. They concluded that the antimicrobial activities were dependent on the part of the plant used as well as the type of extract used.[6] However, there are no data available on the antibacterial effects of *P. edulis* seed extract on *P. acnes*.

In this study, the antimicrobial activity of *P. edulis Sims var. edulis* seed extract with ethanol solvent on *P. acnes* was evaluated using two different methods. The agar disc diffusion assay revealed that the seed extract with the minimum concentration of 5% already has antibacterial activity against *P. acnes*, which is comparable to clindamycin. A moderate inhibitory effect on *P. acnes* growth was observed with 10% concentration and this increased at higher concentrations. Hence, the 20% and 40% extracts of *P. edulis Sims var. edulis* seeds showed higher inhibitory activity against this Gram-negative anaerobic bacillus, which plays an important role in the pathogenesis of acne vulgaris. According to a 2019 publication that questioned the replicability of the agar disc diffusion method in evaluating the antimicrobial activity of plant extracts, serial dilution assays could be combined to determine the MIC and MBC of the plant extract, as in this study.[9] In serial dilution assays, inhibition of *P. acnes* colonies was observed at a concentration of 20%.

Table 1 Inhibition Zone of *Passiflora edulis Sims var. edulis* Seed Extract at Several Concentrations Compared with Clindamycin and Erythromycin

Disc	Zone of Inhibition (mm)			Mean Inhibition Zone (mm)
	1	2	3	
Extract				
1.25%	6	6	6	6
2.5%	7	6.5	7	6.83
5%	8.5	8	9	8.5
10%	10	10.25	10	10.08
20%	14	14	14	14
40%	16	16	16	16
Clindamycin	8	8	8	8
Erythromycin	22	23	23	22.67

Table 2 Comparison of *Propionibacterium acnes* Inhibition Zones in *Passiflora edulis Sims var. edulis* Seed Extract 5% with Clindamycin and Erythromycin

	n	Mean ± SD	*p*
*Passiflora edulis Sims var. edulis*seed extract 5%	3	8.5±0.5	
Clindamycin	3	8	0.121
Erythromycin	3	22.67±0.58	0.046

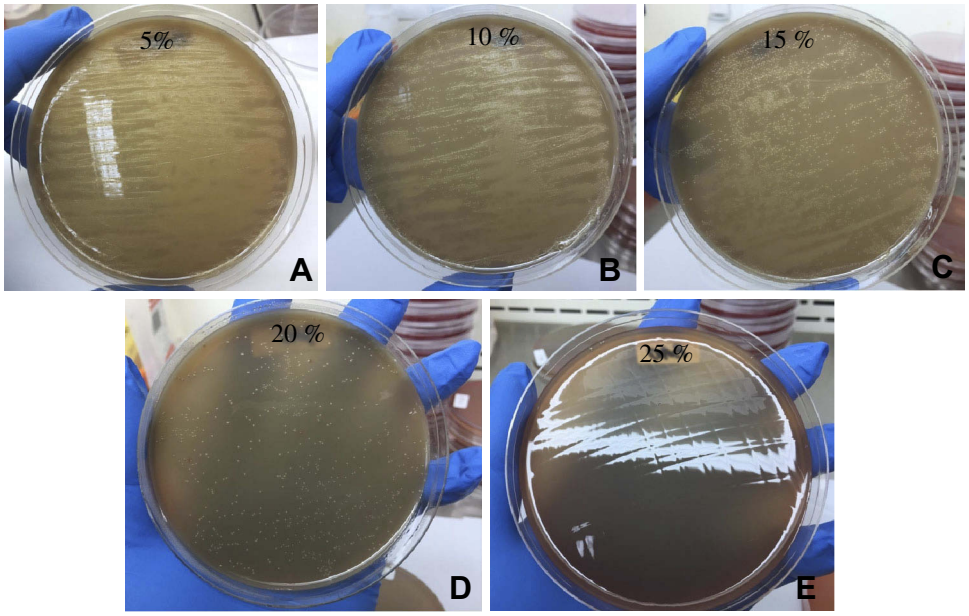

Figure 5 *Propionibacterium acnes* growth on BBS 5% agar plates with different extract concentrations: (**A**) 5%, (**B**) 10%, (**C**) 15%, (**D**) 20%, (**E**) 25%.

Bactericidal activity was noted with the 25% extract. The in vitro test results confirmed that *P. edulis Sims var. edulis* seeds showed potential antibacterial activity against *P. acnes*, with an MIC of 20% and MBC of 25%.

The high amount of piceatannol in the seeds of *P. edulis Sims var. edulis* was thought to play a significant role in the inhibitory effect on *P. acnes* growth. Matsui et al reported that 88% of total polyphenols of the fruit is found in the seeds, which comprised 33% of the freeze-dried seed compounds, and the majority of these is piceatannol (4.8 mg/g).[3] Our results are in accordance with a study on the antimicrobial effect of piceatannol on *P. acnes*, which also showed significant inhibitory activity in comparison with resveratrol, benzoyl peroxide, and erythromycin. The average IC_{50} and IC_{100} of piceatannol were 123 and 234 mg/L, respectively, for three strains of *P. acnes* (ATCC 25746, 29399, and 33179). Benzoyl peroxide treatment had an average IC_{50} of 164 mg/L and an average IC_{100} of 295 mg/L. On the other hand, the average IC_{50} for resveratrol was much lower, at 73 mg/L, and the average IC_{100} was 187 mg/L. Furthermore, with erythromycin, the average IC_{50} for all strains of *P. acnes* was only 1.5 mg/L and the IC_{100} was 11 mg/L.[4] Therefore, the seed extract of *P. edulis Sims var. edulis* in ethanol solvent has significant antimicrobial activity against *P. acnes*, and may be a promising alternative to clindamycin and erythromycin topical preparations, which are widely used as the first line antibacterial treatment for acne vulgaris.

Conclusion

The extract of *P. edulis Sims var. edulis* seeds showed good antimicrobial effects on *P. acnes* and the inhibitory activity increased with concentration of the extract. Comparable inhibitory effects with clindamycin and erythromycin may support the application of this extract in the management of acne vulgaris. Therefore, further clinical trials on the use of *P. edulis Sims var. edulis* seeds extract are necessary to show its efficacy in acne vulgaris patients.

Disclosure

The authors report no conflicts of interest in this work.

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