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Antibacterial and Antibiofilm Activities of Sugar Palm Fruit Extract against *Propionibacterium acnes*

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Original Research Article

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Abstract: Acne vulgaris has been a skin disease that is caused by excessive oil on the skin that gives an optimal environment for acne-causing skin microbes, including *Propionibacterium acnes*. Sugar palm fruit (*Arenga pinnata*) has the potential for acne treatment due to its bioactive compounds that have been reported for exerting anti-inflammation and antioxidant activities. This research was focused on investigation of antibacterial and antibiofilm activities of sugar palm fruit extract (SPFE) for management of acne caused by *P. acnes*. Sugar palm fruit was extracted in methanol to produce SPFE and the chemical compounds of SPFE were identified by using pyrolysis gas chromatography-mass spectrometry (py-GC/MS). Bioefficacy of SPFE as antibacterial and antibiofilm agents was tested on inhibiting *P. acnes* growth, eradicating the existed *P. acnes* biofilms, and preventing *P. acnes* biofilm formation *in vitro*. Chromatographic profiling by py-GC/MS showed that SPFE consisted of major compounds, including levoglucosan and methyl-β-D-glucoside as pyran group. SPFE at 16 µg/mL effectively inhibited ~40% of *P. acnes* growth. For antibiofilm effect, SPFE was more effective for eradication effect on existed *P. acnes* biofilm compared to that of preventive effect on *P. acnes* biofilm formation. At 100 µg/mL, SPFE removed up to 50% of the existed *P. acnes* biofilms. Thus, SPFE may offer alternative candidate to treat acne vulgaris caused by *P. acnes* by inhibiting its growth and removing its existed biofilms.
Keywords: Sugar palm fruit extract, *Arenga pinnata*, antibacterial activity, antibiofilm activity, *Propionibacterium acnes*.

INTRODUCTION

Acne vulgaris is a common skin disease that occurs in adolescents. About 80-100% of 14 to 19 years old teenagers experience this skin disorder. Acne itself is caused due to excessive oil on the skin so as to provide a good environment for the growth of microbes that cause acne, namely *Propionibacterium acnes*. Parts of the body that acne usually occured are the face and the back, because in these areas, there are many oil glands. There has been much research done to solve the problem of acne, one of the most commonly used is the antibiotic therapy, but it may cause chronic side effects [1]. Fruits are an important source of bioactive compounds, such as ascorbic acid, flavonoids, phenolic compounds and pectins that may act as antimicrobial and antioxidant agents. Antioxidants can protect the skin from oxidative stress that can lead to inflammation in the form of acne [2].

Sugar palm fruits (*Arenga pinnata)*, known as kolang kaling in Indonesia, belong to a populer tropical fruit with potential health nutrition and natural ingredient contents. Riley *et al.* reported that sugar palm fruits are rich in carbohydrates and fibers, but less in lipid and protein [3]. It is also high in mineral contents including calcium (Ca) and phosphor (P), and a high Ca/P ratio is known to be associated with more health benefits, including bone mineralization [4]. In terms of its pharmacological effect, sugar palm fruits contained octadecenoic acid and resorcinol that could be applied for topical acne treatment [5, 6]. Our recent study also demonstrated that galactomannan, a polysaccharide compound extracted from sugar palm fruits, exerted potential cosmeceutical efficacy by inhibiting tyrosinase activity, blocking microphtalmia-associated transcription factor gene expression, and preventing photoaging biomarkers, such as matrix metalloproteinase 1 and 13 in cell culture systems [7]. In this study, we determined whether sugar palm fruit extract (SPFE) also possessed antibacterial and antibiofilm activities against *P. acnes in vitro* for management of acne vulgaris.

MATERIALS AND METHODS

Extraction and identification of sugar palm fruits

Sugar palm fruits were purchased from a traditional market in Jakarta (Indonesia). The fruits were extracted in methanol according to the modified method of Uribe *et al.* [8]. Fruit flesh was mashed and diluted in 80% methanol with solid/liquid ratio 1:4, followed by agitatation at 200 rpm in room temperatures with orbital shaker for 30 minutes. The mixture was then filtered with Whatman paper and centrifuged 10.000 ×g for 30 minutes twice. Subsequently, the extract was concentrated with rotary evaporator in 37°C, followed by freeze drying to obtain SPFE for further identification and assays.

For identification, SPFE was run on gas chromatography mass spectrometry with pyrolysis system (py-GC/MS) using QP2010 to identify composition of chemical compounds. The SPFE (0.5 g) was injected to the capillary column (phase Rtx-5MS) with 60 m × 0.25 mmID film thickness. Pyrolysis temperature was set to 280°C. Helium was used as the carrier gas. For assays, SPFE was diluted in dimetyhl sulfoxide (DMSO) at various concentrations.

Preparation and growth of *Propionibacterium acnes* bacteria

P. acnes (ATCC 6919) were cultured in Brain Heart Infusion (BHI) media supplemented by 2% v/v fetal bovine serum (FBS). For optimal *P. acnes* biofilm growth, 3% w/v sucrose was added to media that had been supplemented by FBS. *P. acnes* were cultured anaerobically using anaerobic gas pack in an anaerobic jar at 37°C for 48 hours.

Antibacterial assay of SPFE against *P. acnes* growth

Antibacterial activity of SPFE was determined by minimum inhibitory concentration (MIC) using standard microdilution method [9]. A 100 µL of *P. acnes* culture was added to the 96-wells plate, followed by the addition of 100 µL of sample (SPFE) in various concentrations (0.25-500 µg/mL). Kanamycin (0.25 µg/mL) was used as a reference. The untreated *P. acnes* culture was used as control. Plate was anaerobically incubated at 37°C for 48 hours. Absorbance was measured at 595 nm using macroplate reader. Experiments was done in triplicate.

Antibiofilm assay of SPFE against *P. acnes* biofilms

Antibiofilm activity of SPFE was quantified based on 2 systems, including eradication of established *P. acnes* biofilms and prevention of *P. acnes* biofilm formation [9]. In general, biofilms were first grown in the 96-well plate by inoculating 100 µL of *P. acnes* culture and incubated anaerobically at 37°C. After incubation for 24 hours, supernatant containing planktonic cells was discarded and plate was washed with 100 µL of sterile phosphate buffered saline (PBS). Subsequently, a 100 µL of media is added to the plate and incubated for 48 hours. Supernatant was discarded and plate was washed with 100 µL of sterile PBS.

For eradication effect, the 96-well plate was coated with 150 µL artificial saliva**.** The plate was dried at 37°C overnight. A 20 µL *P. acnes* and 180 µL BHI broth were moved into the wells, and plate was incubated at 37°C for 48 hours. After the biofilm had been formed, 50 µL of SPFE sample (10-500 µg/mL) was added to each well and the plate was further incubated for 48 hours. Kanamycin (0.25 µg/mL) was used as a reference. The untreated *P. acnes* biofilm was used as control. After the incubation, the well was gently washed using PBS to discard the planktonic cells in the well, and the plate was dried in room temperature for an hour. The biofilm formed at the bottom of the well was dyed using a 100 µL crystal violet 0.4% w/v for 30 min. The well was destained using 200 µL ethanol 96%. A 100 µL ethanol was pipetted to a new well, and then the absorbance was measured at 595 nm using macroplate reader. Experiments was done in triplicate.

For prevention assay, the 96-well plate was coated with 150 µL artificial saliva (1% CMC, KH_2PO_4 10 mM, KCl 50 mM, $CaCl_2$ 1 mM, and $MgCl_2$ 0.1 mM) and 50 µL SPFE sample (10-500 µg/mL of final concentration). Kanamycin (0.25 µg/mL) was used as a reference. The untreated artificial saliva was used as control. The plate was dried at 37°C overnight. After the 96-well plate dried, 20 µL of *P. acnes* and 180 µL BHI broths were moved into the wells. The plate was incubated at 37°C for 48 hours. The plate was gently washed using PBS to discard the planktonic cell in the well and the well was dried in room temperature for an hour. The biofilm formed at the bottom of the well was dyed using 100 µL of crystal violet 0.4% w/v for 30 min. The well was detained using 200 µL of ethanol 96%. A 100 µL ethanol was pipetted to a new well and then the absorbation was measured on 595 nm using microplate reader. Experiments was done in triplicate.

STATISTICAL ANALYSIS

Data were expressed by computational analysis (SPSS 12.0), and the significance of the differences was assessed via a *t*-test. A value of $p < 0.05$ was taken as statistically significant.

RESULTS

Identification of chemical compounds in SPFE

The py-GC/MS chromatogram (Figure 1 and Table 1) showed that SPFE had 8 compounds with major pyran group including levoglucosan (78.90%) and methyl-β-D-glucoside (7.20%). Other major compound was detected as calditocaldarcheol (7.50%) that was grouped in oxonium salt.

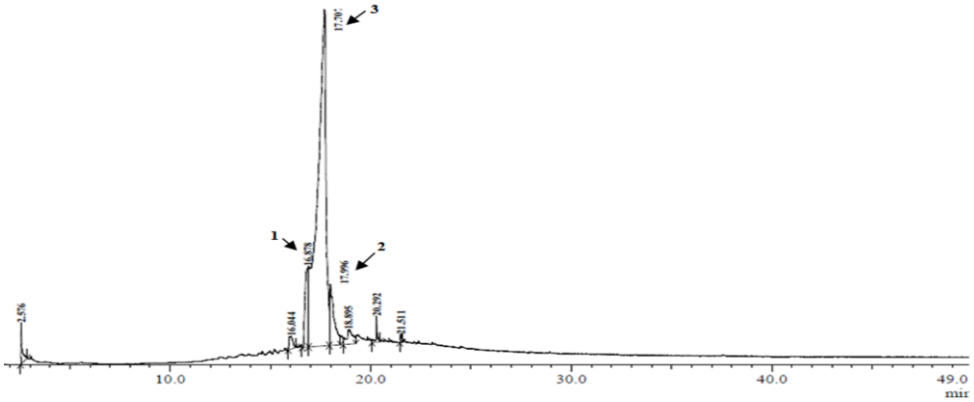


Fig-1: Chromatogram of chemical compounds in SPFE by py-GC/MS. Major compounds in SPFE were calditocaldarcheol (1), levoglucosan (2) and methyl-β-D-glucoside (3)

Table-1: Chemical compounds of SPFE

Retention time	Constituents	Group	Concentration (%)
2.576	Cyclopropane	Alkane	1.35
16.044	Quinitol	Diol	1.33
16.878	Calditocaldarcheol	Oxonium salt	7.50
17.707	Levoglucosan	Pyran	78.90
17.996	Methyl-β-D-glucoside	Pyran	7.20
18.895	β-D-glucofuranose	Furan	2.60
20.292	Hexadecenoic acid	Fatty acid	0.82
21.511	Octadecenoic acid	Fatty acid	0.31

Antibacterial activity of SPFE

To determine the effect of SPFE on the inhibition of *P. acnes* growth, various concentrations of SPFE (0,25-500 µg/mL) were tested against *P. acnes* culture in BHI broth. Our results demonstrated that SPFE exerted antimicrobial activity against *P. Acnes* with MIC value of >500 µg/mL. At 16 µg/mL, SPFE effectively inhibited ~50% of *P. acnes* growth (Figure 2). Meanwhile, kanamycin reference (0.25 µg/mL) inhibited up to 80% of *P. acnes* growth.

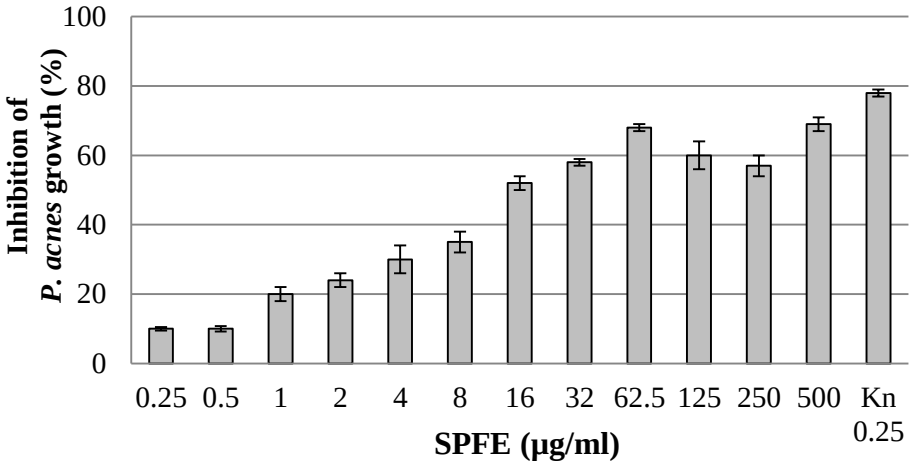


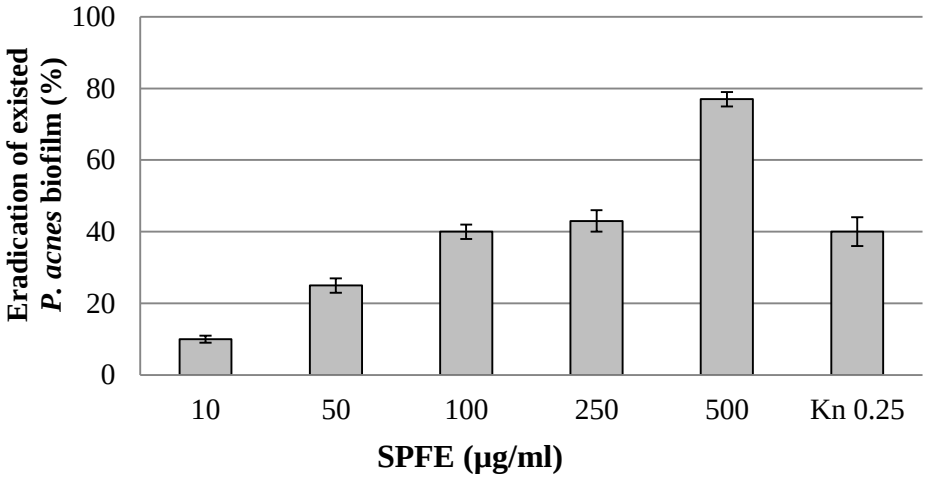
Fig-2: Antibacterial activity of SPFE against *P. acnes* growth *in vitro*. Kn (kanamycin) was used as a reference (0.25 µg/mL). Data were shown as mean ± SD from triplicate experiments. p<0.05 against control (untreated *P. acnes*)

Antibiofilm activity of SPFE

For antibiofilm effect, SPFE was more effective for eradication effect on existed *P. acnes* biofilm compared to that of preventive effect (Figure 3). At 100 µg/mL, SPFE killed up to ~40% of the existed *P. acnes* biofilm, and the increased concentration of SPFE (500 µg/mL) also significantly removed up to 80 of the existed *P. Acnes* biofilm. In the prevention system, SPFE up to up to 500 µg/mL only prevented ~30% of *P. acnes* biofilm formation. Kanamycin reference at 0.25 µg/mL showed slight antibiofilm activity with a 30-40% inhibition on

both the existed *P. acnes* biofilm and *P. acnes* biofilm formation.

(A)



(B)

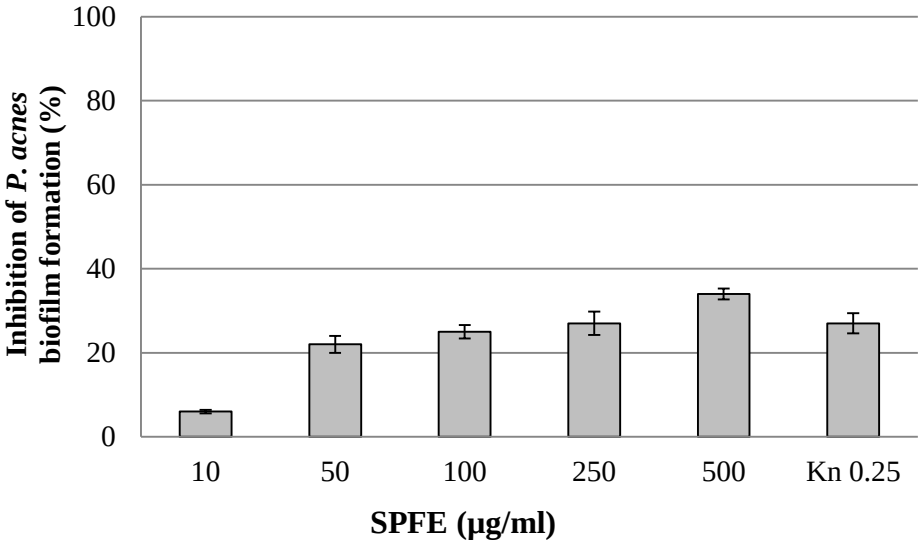


Fig-3: Antibiofilm activity of SPFE with eradication effect on the existed *P. acnes* biofilm (A) and preventive effect on *P. acnes* biofilm formation (B) *in vitro*. Kn (kanamycin) was used as a reference (0.25 µg/mL). Data were shown as mean ± SD from triplicate experiments. p<0.05 against control (untreated *P. acnes*)

DISCUSSION

Acne vulgaris has been a skin problem for most people particularly the teenagers due to the feeling of insecure of their appearance. Diet on sugar palm fruit is potential to apply for acne vulgaris treatment, because it has active compounds such as polyphenols that exert anti-inflammation and antioxidant activities [6]. Sugar palm fruit also improves our health due to its high carbohydrate content including fiber and low in protein and lipid contents [3].

In this study, our results demonstrated that identification of chemical compounds in SPFE by py-GC/MS revealed that SPFE was rich in pyran group including levoglucosan and methyl-β-D-glucoside (Figure 1 and Table 1). In line with our study, Sahari *et al.* also reported that major compounds in sugar palm fruit were β-1,6,-anhydro-D-glucopyranoside (levoglucosan) and 2-furaldehyde (furfural) [10].

For antibacterial activity, SPFE did not exert potential MIC value (>500 µg/mL), but at 16 µg/mL, SPFE inhibited >50% of *P. acnes* growth (Figure 2). These data indicate that SPFE may effectively possess antibacterial activity against *P. acnes*. Kanamycin was used as a reference due to its widespectrum antibiotic property against gram-positive bacteria. The MIC of kanamycin was ranged from 64-128 µg/mL [11]. Other studies also reported that sargafuran isolated from marine brown alga (*Sargassum macrocarpum*) and Indian sarsaparilla fruit extract *(Hemidesmus indicus)* exerted antibacterial activity against *P. acnes* with MIC values of 15 µg/mL and 51 µg/mL [12, 13].

In terms of antibiofilm efficacy, SPFE was found to be more effective for eradication effect (up to 80%) against the existed *P. acnes* biofilm compared to that of preventive effect. Unfortunately, kanamycin reference only showed less antibiofilm activity in both eradication and prevention effects against *P. acnes* biofilms. Dror *et al.* reported that various mechanisms of several antibiofilm agents may affect the different mechanisms of these agents on eradicating and preventing biofilms [14]. Our results indicate that antibiofilm activity of SPFE on biofilm eradication may correlate to its ability on degradation of exopolysaccharides matrix polymer components in biofilm and/or interfering with signaling molecules inside the biofilm [15, 16]. In addition, SPFE may not have the ability to interfere with the adhesion of biofilm to solid surfaces or to inhibit the bacteria quorum sensing, thus, biofilm formation could not be prevented [17].

CONCLUSION

Our results demonstrated that major compounds in SPFE were levoglucosan and methyl-β-D-glucoside as pyran group. SPFE exerted antibacterial effect against *P. acnes* growth and antibiofilm activity via eradicating the existed *P. acnes* biofilms. SPFE may have potential to be further developed as a promising material for combating acne vulgaris.

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