

Reevaluation of methanol extract from *Phoenix dactylifera* var. *sukkari* fruit's potential against acne-inducing bacteria

Reza Anindita^{1*}, Intan Kurnia Putri¹, Maya Uzia Beandrade¹,
Dede Dwi Nathalia¹, Melania Perwitasari¹

¹ Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga,
Jl. Pengasinan, Bekasi, West Java 17113, Indonesia

*corresponding author: rezaaninditaa@gmail.com

ABSTRACT

One of the natural ingredients that is often developed as an antibacterial product is the date palm (*Phoenix dactylifera*). However, scientific evidence regarding the effectiveness of the antibacterial compounds contained in dates needs to be evaluated, considering that several previous studies have reported that dates are more dominant as antioxidants than as antibacterials. Therefore, it is necessary to reevaluate the antibacterial ability of the methanolic extract of the sukkari variety date palm against the three test bacteria that cause acne, namely *P. acnes*, *S. epidermidis* and *S. aureus*. This research aimed to determine the sensitivity of the three bacteria tested in response to the methanol extract of the sukkari dates. This research design is experimental. The samples tested were sukkari and the tested bacteria were *P. acnes*, *S. epidermidis* and *S. aureus*. Dates were extracted by maceration method and made in concentrations of 50%, 60%, 70%, 80% and 90%. Positive control was chloramphenicol antibiotic while negative control was sterile distilled water. All treatments including control (+) and (-) were given to the test bacteria using the Kirby Baur method to see the diameter of the zone of inhibition of the test bacteria growth. The data was processed in tabular form and compared with the 2016 CLSI standard to see the sensitivity response of the test bacteria. Results of this research is the sukkari dates extract with concentrations of 50%, 60%, 70%, 80% and 90% was able to inhibit the growth of *P. acnes*, *S. epidermidis* and *S. aureus* bacteria with an interval of 7.0-10.0 mm in diameter of the zone of inhibition. All the tested bacteria showed a sensitivity response in the resistant category, so the sukkari dates fruit extract is not the main choice to be developed as a natural anti-acne product.

Keywords: dates, *Phoenix dactylifera*, *Propionibacterium acnes*, *Staphylococcus epidermidis*, sukkari

INTRODUCTION

One of the natural ingredients used as raw material for making skin care products as an anti-acne is the fruit of the date palm (*Phoenix dactylifera*). This is because dates contain phytochemical compounds that have the potential to inhibit acne-triggering bacteria such as *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* (Meer et al., 2017). However, this potential needs to be re-evaluated in vitro, considering that several studies have reported that the content of dates is more dominant as an antioxidant than as an antibacterial.

Re-evaluation or re-testing the potential of the content of phytochemical compounds in dates as antibacterial causes of acne is very much needed to see the effectiveness of dates in inhibiting acne-causing bacteria, bearing in mind that there are findings from previous studies

which prove that the simplicia treatment of dates, both fruit and seeds, shows a less effective category in inhibiting bacterial growth. cause of acne. The existence of scientific evidence regarding the effectiveness of dates as an antibacterial agent that causes acne will provide information on the accuracy of the efficacy of dates in anti-acne skin care products for the community so that it has an impact on increasing sales of these products.

Several studies have led to the need to conduct research on the reevaluation of dates, including the research of Masfiah & Rahayu (2019) which showed that giving a 100% concentration of methanol extract of dates was not able to inhibit the growth of *E. coli* and *K. pneumoniae* bacteria, other studies showed that giving the extract Aquades of date palm fruit has not been effective in inhibiting *S. aureus* bacteria (Ammar et al., 2009; Maged & Abbas, 2013; Saleh

& Otaibi, 2013; Al-Zoreky & Al-Taher, 2015; Adedayo et al., 2020; Albab et al., 2020). Another study conducted by Utami et al. (2021) also published the same results, namely the ethanol extract of dates was not effective in preventing the reproduction of methicilin Resistant bacteria – *Staphylococcus aureus* (MRSA).

Referring to the problem of scientific evidence that dates are less effective in inhibiting pathogenic bacteria and the use of more *S. aureus* as a test bacterium in previous studies, even though the three bacteria that are known to trigger acne are *P. acnes*, *S. epidermidis*, and *S. aureus*, it is necessary to re-evaluate the date fruit extract test against *P. acnes*, *S. epidermidis* and *S. aureus* bacteria as causes of acne.

The novelty value of this study compared to previous studies is the use of *P. acnes*, *S. epidermidis*, and *S. aureus* as test bacteria that cause acne. The urgency of this study was to determine the sensitivity response of acne-causing bacteria to the phytochemical compounds contained in the methanol extract of dates at concentrations of 50%, 60%, 70%, 80%, and 90%.

The target of this research is expected to be able to provide information to the public or consumers about the efficacy of dates that have the potential to inhibit acne-causing bacteria.

METHOD

Types and research design

This research was conducted at the Microbiology Laboratory S-1 Pharmacy STIKes Mitra Keluarga in February-March 2022. This type of research was quantitative using an experimental research design with treatment in the form of date palm methanol extract concentrations of 50%, 60%, 70%, 80 %, 90%, chloramphenicol 30 µg as a positive control, and sterile distilled water as a negative control. All treatments were given to 3 test bacteria namely *P. acnes*, *S. epidermidis* and *S. aureus* with three repetitions (Hastjarjo, 2019).

Tools and materials

The tools used included petri dishes (Pyrex, USA), beakers (Pyrex, USA), test tubes (Pyrex, USA), ose needles (ROFA, Indonesia), P100 micropipette (Socorex, Switzerland), hot plates and Stirer (IKA C-MAG HS7, Germany), automatic autoclave (Hirayama HG-80, 76L, Japan), incubator (Memmerth IN-30, Germany), Laminar Air Flow (LAF) (ESCO, Singapore), rotary evaporator (IKA-RV -3 V, Germany) 6 Hole water bath (HH, China), digital analytical balance (Acuplus, China), VM 300 micropipette and vortex mixer (Gemmy, Taiwan).

The materials used included dates of the Sukkari variety (Pondok Herbal Bekasi), pure isolate *P. acnes* ATCC: 11827, *S. epidermidis* ATCC: 12228, *S. aureus* ATCC: 6538 (microbiology laboratory, University of Indonesia), chloramphenicol antimicrobial susceptibility discs (oxid, Germany), Antimicrobial Susceptibility Blank Disks (Oxoid, Germany), sterile cotton swabs (One Med, Indonesia) Media Nutrient Agar (NA) (Merck, Indonesia), Media Nutrient Broth (NB), Mueller Hinton Agar (MHA) (Merck , Indonesia), Technical Methanol, 0.9% NaCl Pro Analyst (Merck, Indonesia) and Technical Aquades (ROFA, Indonesia).

Selection, collection, and preparation of samples

The natural product sample in this study was the Sukkari type of date palm weighing 3 kilograms (kg) purchased from Pondok Herbal Bekasi. Samples were tested organoleptically which included shape, smell, color, taste, and other characteristics (Raja et al., 2019). Determination of the Sukkari date fruit was carried out at the Research Center for Plant Conservation and Botanical Gardens, Indonesian Institute of Sciences (LIPI), Bogor, West Java, as evidenced by a certificate of plant determination. The dates to be extracted are separated from the seeds and then chopped to minimize the surface of the dates so that it is easier during the drying process, the chopped dates are in the oven at 80°C for 1x10 hours. Dried date palm simplicia is

blended until smooth and then weighed as much as 250.06 grams.

Extraction by maceration method

Extraction using the maceration method was carried out by incorporating 250.06 grams of date palm powder into a 500 mL beaker glass which had been filled with 500 mL technical methanol. The sample is then soaked for 2x24 hours where it is stirred once a day until the solvent is completely mixed. Dates powder that has been soaked in methanol for 2 days, then the liquid filtrate is filtered with Whatman paper No. 1. The liquid extract obtained from maceration is then concentrated using a rotary evaporator at a temperature of 60°C at 93 rpm for 2 days. If there is residual liquid, then it is evaporated with a water bath for 3 hours to produce a solvent-free viscous extract with a yield percentage of 8.82%.

Making the concentration of methanol extract from dates

In this study, variations in the concentration of methanol extract of dates were 50%, 60%, 70%, 80%, and 90% (g/mL). The concentration of the methanol extract of the dates was made by weighing the thick extract of 2.5 g, 3 g, 3.5 g, 4 g, and 4.5 g respectively taken from the thick extract stock, then each concentration was diluted with sterile distilled water as much as 5 mL (Ridha, 2021).

Antibacterial compound test

Sterilization of tools and materials

Sterilized tools and materials include Petri dishes, test tubes, 0.9% NaCl, dark glass bottles, Erlenmeyer containing NA and MHA media, tweezers, and distilled water. All tools are washed first using detergent, then rinsed under running water and dried. The dried tools were wrapped tightly in paper and then sterilized using an autoclave at 121°C with a pressure of 2 atm for 15 minutes. For use needles, it is sterilized by heating over a Bunsen flame.

Preparation of Nutrient Agar (NA) and Mueller-Hinton Agar (MHA) media

Preparation of NA media was carried out by weighing 3.78 grams of NA powder and then

putting it into an Erlenmeyer and then dissolving it by adding 135 mL of distilled water while making MHA media was carried out by weighing 18.9 grams of MHA media then putting it into an Erlenmeyer and then dissolving it by adding 657 mL of distilled water. For complete compaction of NA and MHA media, bacto agar was added to each media as much as 10% of the required weight of NA and MHA media, namely 0.378 g and 1.89 g. All media were then heated to boiling on a hot plate while homogenizing using a magnetic stirrer. The homogeneous media is then sterilized using an autoclave with a temperature of 121 °C with a pressure of 2 atm for 15 minutes (Hasanah & Novian, 2020).

Bacterial rejuvenation

Bacterial rejuvenation was carried out aseptically by inoculating 1 ose each of *P. acnes*, *S. epidermidis* and *S. aureus* bacteria on slanted Nutrient Agar (NA) media in a test tube with streaks then incubated for 24 hours at room temperature 37°C.

Control treatment

The controls in this study consisted of positive controls (standardized chloramphenicol (oxid) antibiotic discs with a dose of 30 µg with a diameter of 6 mm and negative controls (sterile distilled water which was dripped as much as 30 µL on sterile blank discs (oxid) with a diameter of 6 mm.

Preparation of Physiological NaCl 0.9 %

The preparation of 0.9% physiological NaCl was carried out by weighing 2.25 grams of NaCl (Merck) powder and then dissolving it in 250 mL of sterile distilled water and homogenizing it. The finished 0.9% physiological NaCl was then put into 10 15 mL test tubes, each of which was 9 mL of 0.5% physiological NaCl taken from a 0.9% NaCl stock solution. All physiological 0.9% NaCl solution was then sterilized by autoclaving.

Preparation of test bacterial suspension (*P. acnes*, *S. epidermidis* and *S. aureus*)

Preparation of the test bacterial suspension was carried out by taking several oses of pure

cultures of the test bacterial sub-cultures, then putting them in 0.9% NaCl and then vortexing until homogeneous, then the results were compared to the turbidity with McFarland 0.5 solution (equivalent to a bacterial suspension of 1.5×10^8 CFU/mL). after being compared with McFarland it turns out that the bacterial suspension is still too clear, then a few more oses of the test bacteria can be added, whereas if it turns out to be too cloudy, 0.9% NaCl can be added again to obtain a solution of the test bacterial suspension with the same level of turbidity as the standard McFarland 0.5 solution.

Antibacterial compound test of methanol extract of dates using the Kirby Bauer method

The antibacterial compound test of methanol extract of dates was carried out using the Kirby-Bauer method using a 4-quadrant streak plate technique on the surface of MHA media with a sterile cotton swab and left for ± 5 minutes. Then prepare a blank disc that has been dripped with methanol extract of dates with graded concentrations (50%, 60%, 70%, 80%, and 90%) as much as 30 μ L using a micropipette and left for ± 15 minutes, positive control of chloramphenicol antibiotics, and negative control of distilled water sterile and blank discs. All discs (discs) are placed on the agar plate using sterile tweezers. All the Petri dishes were then incubated for 24 hours at 37°C.

The diameter of the inhibition zone was measured after 1x24 hours of incubation by measuring the presence/absence of a clear zone formed around the treatment disc using a ruler. The results of measuring the diameter of the inhibition zone were then compared with [CLSI guidelines \(2016\)](#) to see the sensitivity category of the test bacteria in response to each treatment disc.

Data analysis

Data analysis was carried out using a quantitative descriptive test by looking at the sensitivity category of the test bacterial response

in response to treatment discs that had been processed in tabular form. Data in tabular form contains the mean of the diameter of the inhibition area of the test bacteria. These results are then interpreted based on the category of bacterial sensitivity response that has been adjusted to the [CLSI \(2016\)](#).

RESULTS AND DISCUSSION

Test results of methanol extract of dates with concentrations of 50%, 60%, 70%, 80%, and 90% against test bacteria *P. acnes*, *S. epidermidis*, and *S. aureus* showed a clear area around the disc which had been saturated with methanol extract of dates. with various concentrations.

The clear area indicated that there was inhibition of the growth of the test bacteria by the methanol extract of dates as an antimicrobial agent while at the same time proving that diffusion of the methanol extract of dates occurred on the surface of the NA media. To see the effectiveness of the methanol extract of dates as an antibacterial agent, was then observed from the results of measuring the diameter of the inhibition zone (clear area) for the growth of the test bacteria compared to the [CLSI standard \(2016\)](#).

The results of measuring the diameter of the growth inhibition zone of *P. acnes* after being treated with methanol extract of dates are shown in Table 1. It shows that the methanol extract of dates with concentrations of 50%, 60%, 70%, 80%, and 90% was able to inhibit the growth of *P. acnes* bacteria with an average diameter of the inhibition zone respectively of 8.6 mm, 8.1 mm, 7.0 mm, 7.6mm and 8.0mm. The highest inhibition zone diameter results were shown at a concentration of 50% (8.6 mm) while the lowest was shown at a concentration of 70% (7.0 mm). The population response of *P. acnes* bacteria growth to the methanol extract of dates for all concentrations showed the resistant category. Other test results from the date fruit methanol extract to *S. epidermidis* can be shown in Table 2.

Table 1. Test results of date fruit methanol extract against *P. acnes*.

Treatment	Inhibition zone diameter (mm)			Mean	Category
	Repeat 1	Repeat 2	Repeat 3		
Control (+)	20.5	21.5	21	21	Sensitive
Control (-)	0	0	0	0	Resistant
Concentration 50 %	5.5	11	9.5	8.6	Resistant
Concentration 60 %	5.5	9.5	9.5	8.1	Resistant
Concentration 70 %	5.0	9.0	9.0	7.0	Resistant
Concentration 80 %	5.0	9.0	9.0	7.6	Resistant
Concentration 90 %	6.0	9.0	9.0	8.0	Resistant

Standard CLSI (2016) : ≥ 18 mm : Sensitive ; 13-17 mm : Intermediet ; ≤ 12 mm : Resistant

The results in Table 2 can be seen that the treatment with methanol extract of dates with concentrations of 50%, 60%, 70%, 80%, and 90% of *S. epidermidis* bacteria showed an inhibition zone diameter of 8.8 mm, 9.6 mm, 9.3 mm, 9.6 mm

and 10mm. The highest inhibition zone diameter was found at a concentration of 90% (10.0 mm) while the lowest was found at a concentration of 50% (8.8 mm).

Table 2. Test results of date fruit methanol extract against *S. epidermidis*.

Treatment	Inhibition zone diameter (mm)			Mean	Category
	Repeat 1	Repeat 2	Repeat 3		
Control (+)	20.1	20.5	20	20.2	Sensitive
Control (-)	0	0	0	0	Resistant
Concentration 50 %	8.5	9.0	9.0	8.8	Resistant
Concentration 60 %	9.0	10.0	10.0	9.6	Resistant
Concentration 70 %	9.0	9.5	9.5	9.3	Resistant
Concentration 80 %	10.0	9.5	9.5	9.6	Resistant
Concentration 90 %	10.0	10.0	10.0	10.0	Resistant

Standard CLSI (2016) : ≥ 18 mm : Sensitive ; 13-17 mm : Intermediet ; ≤ 12 mm : Resistant

The population response of *S. epidermidis* bacteria growth for all concentrations showed the resistant category. The third bacterium tested with methanol extract of dates in this study

was *S. aureus*. The results of the date fruit methanol extract test against *S. aureus* can be seen in Table 3.

Table 3. Test results of date fruit methanol extract against *S. aureus*.

Treatment	Inhibition zone diameter (mm)			Mean	Category
	Repeat 1	Repeat 2	Repeat 3		
Control (+)	21.5	22.5	23	22.3	Sensitive
Control (-)	0	0	0	0	Resistant
Concentration 50 %	8.5	8.5	8.5	8.5	Resistant
Concentration 60 %	7.5	7.5	7.5	7.5	Resistant
Concentration 70 %	9.0	9.0	9.0	9.0	Resistant
Concentration 80 %	9.0	9.0	8.5	8.8	Resistant
Concentration 90 %	9.5	9.5	9.5	9.5	Resistant

Standard CLSI (2016): ≥ 18 mm: Sensitive ; 13-17 mm : Intermediet ; ≤ 12 mm : Resistant

The results in Table 3 report that the methanol extract of dates with concentrations of 50%, 60%, 70%, 80%, and 90% against *S. aureus* bacteria showed an inhibition zone diameter of 8.5 mm, 7.5 mm, 9.0 mm, 8.8 mm and 9.5 mm. The highest inhibition zone diameter was found at a

concentration of 90% (9.5 mm) while the lowest was found at a concentration of 60% (7.5 mm). The population response of *S. aureus* bacteria growth for all concentrations showed the resistant category.

Overall the results of the methanol extract of the date palm against the three test bacteria namely *P. acnes*, *S. epidermidis*, and *S. aureus* can be shown in Table 4.

Table 4. Differences in the results of date fruit methanol extract against *P. acnes*, *S. epidermidis*, and *S. aureus*.

Treatment	Inhibition zone diameter (mm)		
	<i>P. acnes</i>	<i>S. epidermidis</i>	<i>S. aureus</i>
Concentration 50 %	8.6 ^R	8.8 ^R	8.5 ^R
Concentration 60 %	8.1 ^R	9.6 ^R	7.5 ^R
Concentration 70 %	7.0 ^R	9.3 ^R	9.0 ^R
Concentration 80 %	7.6 ^R	9.6 ^R	8.8 ^R
Concentration 90 %	8.0 ^R	10.0 ^R	9.5 ^R
Control (+)	21.00 ^S	20.20 ^S	22.30 ^S
Control (-)	0 ^R	0 ^R	0 ^R

Standard CLSI (2016): ≥ 18 mm: Sensiitive ; 13-17 mm : Intermediet ; ≤ 12 mm : Resistant

The results in Table 4 show that the population growth response for all bacteria to the methanol extract of dates with concentrations of 50%, 60%, 70%, 80%, and 90% showed resistance to both *P. acnes*, *S. epidermidis*, and *S. aureus*. Table 4 also shows that all the test bacteria are given the methanol extract treatment had an average diameter of ≤ 12 mm, while those treated with chloramphenicol had an average diameter of ≥ 18 mm. The average interval of the diameter of the bacterial inhibition zone treated with methanol extract in this study was between 7.0 – 10.0 mm. These results prove that the treatment of date fruit extract has not been able to match the ability of chloramphenicol as a positive control. This is because the methanol extract of the Sukkari variant of dates in this study was included in the very ripe category. This category level causes dates to contain more glucose and fructose as primary metabolites compared to secondary metabolites which function as antibacterials.

This is to the results of research by Masfiah & Rahayu (2019) which states that the fifth stage of the maturity level of dates is tamr or dates in the ripe phase with the texture of the dates drying and browning. At this stage, there is a decrease in the levels of phytochemical compounds followed by an increase in high sugar levels. The younger the dates, the higher the levels of secondary metabolites such as tannins and flavonoids, whereas the more mature the dates, the lower the levels of secondary

metabolites. Sequentially the best inhibition ability of the methanol extract of dates was shown for *S. epidermidis* bacteria, then *S. aureus*, and finally *P. acnes*.

Overall the results of the research shown in tables 1,2,3 and 4 are almost the same as the study of Albab et al. (2020) who reported that the distilled water extract of the ajwa date fruit was able to prevent the reproduction of *S. aureus* bacteria as indicated by the diameter of the inhibition zone between 4.55 – 7.67 mm. The difference is in the study by Albab et al. (2020) using dates of the Ajwa variety extracted with distilled water and concentrations of 12.5%, 25%, 50%, and 100% given to *S. aureus* whereas in this study using dates of the extracted Sukkari variety with methanol and concentrations of 50%, 60%, 70%, 80%, 90% given to *P. acnes*, *S. epidermidis* and *S. aureus* bacteria. The diameter interval of the inhibition zone which was almost the same as this study was shown in the study of Saleh & Otaibi (2013) which proved that the Khulase, Shesi, and Rezaz varieties of dates with different maturity levels (Biser, Rutab, and Tamer) were extracted with water, ethanol and ether were able to inhibit the growth of *S. aureus* bacteria with an interval of inhibition zone diameter between 8.5 -17.5 mm.

This research continues research conducted by Utami et al. (2021) who reported that the ethanol extract of the Sukkari variety of dates with concentrations of 5%, 10%, 15%, and 20% was able to inhibit the growth of

Methicillin-resistant S. aureus (MRSA). with an inhibition zone diameter of ≤ 6 mm. By the CLSI standard (2016) if the diameter of the bacterial inhibition zone is ≥ 18 mm then it can be categorized as Sensitive, 13-17 mm is Intermediate and ≤ 12 mm is Resistance. The results of the research in Tables 1, 2, and 3 for all bacteria show a sensitivity response with the resistant category. The term resistant does not mean that the test bacteria used in this study are bacteria that are resistant to antibiotics but indicates that the methanol extract of the Sukkari variety of dates with concentrations of 50%, 60%, 70%, 80%, and 90% is not yet effective or very weak in inhibiting population growth of test bacteria, namely *P. acnes*, *S. epidermidis*, and *S. aureus*.

According to Al-Zoreky & Al-Taher (2015) and Abdillah et al. (2018) in general the use of methanol can attract secondary metabolites contained in dates such as triterpenoids, phenolics, flavonoids, tannins, and saponins. Zahrah et al. (2019) and Wardania et al. (2020) stated that the presence of triterpenoids, tannins, flavonoids, phenolics, and saponins contained in dates can cause damage to the cell membrane of the test bacteria. Yonanda et al. (2016) added that the simple mechanism of triterpenoids in blocking the reproduction of the test bacteria is by closing the porins found in the bacterial plasma membrane so that it has implications for bacteria that lack nutrition. Meanwhile, according to Mihoub et al. (2019), the presence of saponins can reduce the surface tension of the bacterial plasma membrane resulting in cell leakage which results in the release of compounds within the bacterial cell, while tannins can cause cytoplasmic damage and deactivate reverse transcriptase enzymes and DNA topoisomerase thereby inhibiting the reproduction of the test bacteria.

In this study (based on Tables 1, 2, 3 and 4), the sterile distilled water (negative control) to *P. acnes*, *S. epidermidis*, and *S. aureus* did not show any clear areas around the discs. This indicates that the sterile aqua dest does not contain any content that acts as an antibacterial. The

chloramphenicol as a positive control was able to inhibit the growth of *P. acnes*, *S. epidermidis*, and *S. aureus* bacteria with an inhibition zone diameter of 21.0 – 22.3 mm (≥ 18 mm) with a sensitive category. This means that chloramphenicol is effective or very strong in inhibiting the growth of the bacterial population of *P. acnes*, *S. epidermidis*, and *S. aureus*. According to Cahyono (2013) chloramphenicol belongs to the category of broad-spectrum bacteriostatic antibiotics that are active against aerobic and anaerobic microorganisms, Gram-positive and negative bacteria. This antibiotic works by binding to the 50S ribosome which stops the process of protein synthesis so that both structural and functional proteins in bacteria are not formed.

The advantage of this study was using 3 test bacteria that cause acne, namely *P. acne*, *S. epidermidis*, and *S. aureus*. However, this study has limitations, including the use of an antibacterial test method which is still simple and cannot be used as a reference for clinicians, the structure of damaged bacteria has not been seen with an electron microscope and most importantly the phytochemical screening test has not been carried out both qualitatively and quantitatively, especially quantitatively. to find out the reason for the weak antibacterial effect of the Sukkari variety dates, bearing in mind that several more studies have proven that dates contain more antioxidants than antibacterials.

CONCLUSION

This study concluded that the Sukkari variety of dates was not effective in inhibiting the growth of *P. acne*, *S. epidermidis* and *S. aureus* bacteria as causes of acne disease so they were not the main choice to be developed into skin care products.

REFERENSI

Abdillah, M. M., Nazilah, N. R. K. & Agustina, E. (2018). Identification of active substance in ajwa date (*Phoenix dactylifera* L.) fruit flesh methanol extract (retracted). *Biotropic: The Journal of Tropical Biology*, 1(1), 23–31.

<https://doi.org/10.29080/biotropic.2017.1.1.23-31>

- Adedayo, M., Ajiboye, A. & Adetula, F. (2020). Antibacterial screening of *Phoenix dactylifera* L. (date palm) seed extracts on some bacterial isolates associated with dental caries. *Journal of Biological Research & Biotechnology*, 18(2), 1127–1134.
- Al-Zoreky, N. S. & Al-Taher, A. Y. (2015). Antibacterial activity of spathe from *Phoenix dactylifera* L. against some food-borne pathogens. *Industrial Crops and Products*, 65, 241–246.
<https://doi.org/10.1016/j.indcrop.2014.12.014>
- Albab, L. U., Husin, A.U., Azhali, A. B., Respati, T., & Astuti, I.D.R. (2020). Efek antibakteri ekstrak aquades buah kurma (*Phoenix dactylifera* L.) varietas ajwa terhadap *Staphylococcus aureus* secara *in vitro*. *Jurnal Integrasi Kesehatan & Sains*, 2(2), 135–139.
<https://doi.org/10.29313/jiks.v2i2.5769>
- Ammar, N. M., Abou El-Kassem, T. L., El-Sayed, H.N., Calabria, M.L., & Mabry, J.T. (2009). Flavonoid constituents and antimicrobial activity of date (*Phoenix dactylifera* L.) seeds growing in Egypt. *Medicinal and Aromatic Plant Science and Biotechnology*, 3(1), 1–5.
- Cahyono, W. (2013) *Aktivitas antibakteri kombinasi ekstrak etanol daun sirih merah (Piper crocatum Ruiz and Pav) dan kloramfenikol terhadap bakteri Salmonella typhi, Shigella dysenteriae, dan Staphylococcus aureus beserta bioautografinya.* [Skripsi tidak dipublikasikan]. Universitas Muhammadiyah Surakarta.
- Clinical and Laboratory Standard Institute (CLSI) (2016). *M100S: Performance standards for antimicrobial susceptibility testing 26th edition*. 950 West Valley Road, Suite 2500 Wayne, PA 19087 USA.
- Hasanah, N. & Novian, D. R. (2020). Daya hambat ekstrak daun belimbing wuluh (*Averrhoa bilimbi* L.) terhadap bakteri penyebab jerawat (*Propionibacterium acnes*), *Journal Ilmiah Farmasi*, 9(1), 46–53.
- Hastjarjo, T. D. (2019). Rancangan Eksperimen-Kuasi, *Buletin Psikologi*, 27(2), 187-253. 10.22146/buletinpsikologi.38619.
- Maged, N. Q. A. & Abbas, N. A. (2013) Antibacterial activity of *Phoenix dactylifera* L. leaf extracts against several isolates of bacteria. *Kufa Journal for Veterinary Medical Sciences*, 4, 45–50.
- Masfiah, M. & Rahayu, R. (2019). Perbedaan zona hambat ciprofloksasin dengan ekstrak kurma (*Phoenix dactylifera*) terhadap bakteri gram negatif secara *in vitro*. *Media Farmasi Indonesia*, 14(2), 1517–1521.
- Meer, S., Akhtar, N., Mahmood, T. & Igielska-Kalwat, J. (2017). Efficacy of *Phoenix dactylifera* L. (Date Palm) creams on healthy skin, *Cosmetics*, 4(2), 1-8.
<https://doi.org/10.3390/cosmetics4020013>
- Mihoub, F. Gourchala, F. & Lakhdar-Toumi, S. (2019). Bioactivity of algerian palm dates *Phoenix dactylifera* L. *Ukrainian Food Journal*, 8(2), 249–259.
<https://doi.org/10.24263/2304-974x-2019-8-2-5>
- Raja, S. K., Taip, S.F., Azmi, Z.M.M. & Shishir, I.R.M. (2019). Effect of pre-treatment and different drying methods on the physicochemical properties of *Carica papaya* L. leaf powder. *Journal of the Saudi Society of Agricultural Sciences*, 18(2), 150–156.
<https://doi.org/10.1016/j.jssas.2017.04.001>

Ridha, A. (2021). Uji aktivitas antibakteri ekstrak etanol daun mangga kasturi (*Mangifera casturi*) terhadap pertumbuhan bakteri *Escherichia coli* dan *Methicillin-resistant Staphylococcus aureus* (MRSA), *Indonesian Journal of Pharmacy and Natural Product*, 5(1), 1-8.

Saleh, F. & Otaibi, M. (2013). Antibacterial activity of date palm (*Phoenix dactylifera* L.) fruit at different ripening stages, *Journal of Food Processing & Technology*, 04(12), 1-6.
<http://doi.org/10.4172/2157-7110.1000285>

Utami, P. R., Indrayati, S. & Hayatang, N. (2021). Ability of ethanol extract from ajwa and sukkari dates (*Phoenix dactylifera* L.) in inhibiting the growth of *Methicillin-Resistant Staphylococcus aureus* (MRSA). *Indonesian Journal of Medical Laboratory Science and Technology*, 3(1), 1-8.
<https://doi.org/10.33086/ijmlst.v3i1.1848>

Wardania, A. K. Malfadinata, S. & Fitriana, Y. (2020). Uji aktivitas antibakteri penyebab jerawat *Staphylococcus epidermidis* menggunakan ekstrak daun ashitaba (*Angelica keiskei*). *Lambung Farmasi: Jurnal Ilmu Kefarmasian*, 1(1), 14-19.
<https://doi.org/10.31764/lf.v1i1.1206>

Yonanda, C. R., Wahyuni, D. & Murdiah, S. (2016). *Pengaruh Ekstrak Etanol Daun Belimbing Wuluh (Averrhoa bilimbi L.) Terhadap Daya Hambat Staphylococcus epidermidis*. Prosiding Seminar Nasional Pendidikan Biologi II.

Zahrah, H., Mustika, A. & Debora, K. (2018). Aktivitas antibakteri dan perubahan morfologi dari *Propionibacterium acnes* setelah pemberian ekstrak *Curcuma xanthorrhiza*. *Jurnal Biosains Pascasarjana*, 20(3), 1-3.
<https://doi.org/10.20473/jbp.v20i3.2018.160-169>