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CASE STUDY

The antibacterial and antifungal potential of marine natural ingredients from the symbiont bacteria of mangrove

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ABSTRACT

BACKGROUND AND OBJECTIVES: Mangroves are known to contain tannins, flavonoids, and quinones, which have the potential to be antibacterial, effective even against multidrug-resistant bacteria. Mangroves also have antifungal and antiviral properties. Although, mangroves are known for their use as medicinal ingredients, information regarding symbiont bacteria's antibacterial and antifungal potential is still scarce. Therefore, this study aimed to examine symbiont bacteria in the fruit and leaves of *Xylocarpus* granatum as additional raw materials for anti-acne cosmetic creams and moisturisers.

METHODS: Symbiont bacteria were isolated using the pour plate method through Zobell 2216E and incubated for 2 x 24 hours at 27.5 Celcius degree. Afterwards, 13 isolates were successfully isolated and characterised based on their morphology. Further, everal tests were conducted, including the antibacterial test, antifungal test, molecular identification, and gas chromatography-mass spectrometry. The pathogenic bacteria used in the antibacterial test were *Staphylococcus aureus*, *Vibrio harveyi*, and *Vibrio alginolyticus*

FINDINGS: The antibacterial test results showed that eight isolates were capable of producing an inhibition zone against S. aureus, seven isolates were positive for antibacterial activity against *Vibrio harveyi*, and 10 isolates were positive for antibacterial activity against *Vibrio alginolyticus*. The pathogenic fungi used in the antifungal test were *Malassezia furfur* and *Candida albicans*. The antifungal test results demonstrated that six isolates could produce inhibition zones against *Malassezia furfur* and *Candida albicans*. Furthermore, molecular identification was carried out on six potential isolates based on the antibacterial and antifungal tests, which were X2.52, X1.65, X1.64, X1.53, X1.54, and X1.63. The molecular identification results revealed the occurrence of four species in the *Xylocarpus granatum* mangroves, namely, *Sinomicrobium oceani*, *Proteus mirabilis*, *Pseudomonas khazarica*, and *Alcaligenes aquatilis*.

CONCLUSION: The study found that the mangrove symbiont bacteria had antibacterial and antifungal potential. The compound with the highest concentration in six isolates was 9-octadecenoic acid, methyl ester. This type of content has antibacterial potential and is also predicted to have antifungal potential.

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INTRODUCTION

Indonesia is listed as a mega-biodiverse country with abundant natural wealth in terms of macro as well as microorganisms, which have not been widely studied. Marine and coastal environments have the potential to be the source of natural antioxidants, antibacterial agents, and enzyme inhibitors for the pharmaceutical and cosmetic industries (Simlaiet *et al.,* 2014; Eswaraiah *et al.,* 2020). Mangrove plants that inhabit these environments contain tannins, flavonoids, and quinones (Pringgenies *et al.,* 2018), micro and macro element content (Ariyanto *et al.,* 2019) and amino acid contents (Ningsih *et al.,* 2020). As flavonoid compounds function as antibacterial, antifungal, and antiviral agents, they can be pharmacologically accepted to treat human diseases. In comparison, tannin compounds are biomaterial components with antiviral and antibacterial properties (Kaczmarek, 2020), while quinone derivatives have antifungal and antibacterial properties (Amani, 2014). However, there is a fear that the continuous usage of mangrove plants in large quantities can cause the overexploitation of these species, thereby disrupting the balance of the ecosystem. In order to avoid this issue, many researchers have used microbes associated with marine and coastal natural resources, especially marine facultative moulds, as a source of active ingredients because of their well-known ability to produce potential bioactive compounds (Darmadi *et al.,* 2021). Marine mangrove symbiont bacteria are eukaryotic microorganisms from the microbial kingdom isolated from coastal and marine environments. According to a study, the fruit extract of *X. granatum* has medicinal properties that can be used to heal wounds (Das *et al.,* 2019; Dey *et al.,* 2021, Pringgenies *et al.,* 2021). Moreover, bacterial symbionts in mangroves have the potential as multidrug resistance (MDR) antibacterial agents, along with the potential to decompose organic matter. Furthermore, symbiotic bacteria found in mangroves have demonstrated antibacterial activity against *Pseudomonas aeruginosa*. The bacterial gene contains L-2-amino-4-methoxy-trans-3-butenoic acid l-2-Amino-4-methoxy-trans-3-butenoic acid (AMB), which is a powerful antibiotic and toxin (Pringgenies *et al.,* 2021). The supernatant obtained from the extraction of *P. aeruginosa* in symbiosis with *Cerithidea sp.* in the mangrove area was found to have pyocyanin (PYO) and phenazine-1-carboxylic (PCA) pigments. The emerald colour of the sample culture indicated the presence of the phenazine pigment in the bacterial sample (Pringgenies and Setyati, 2021). Phenazine is a natural pigment that plays an important role in health as an anti-cancer, anti-malarial, anti-tumour, and potentially antibiotic agent. The fruit of *X. granatum*, also known locally as *buah pengantin* (the bride's fruit), is often used as a mask to make the bride's face smooth and look beautiful, which is everyone's desire on their wedding day. All these biological activities can be utilised in the cosmetic production industry. As time goes by and the public's awareness about the importance of natural ingredients for health is increasing, the demand for natural-based products is also expected to increase. This is due to consumer concerns regarding synthetic cosmetics made from artificial ingredients that are widely circulated in the market and can cause skin irritation and, at worst, skin cancer (Panico *et al.,* 2019). Antibacterial and antifungal bacteria are natural ingredients (Kokoska *et al.,* 2019; Qadri *et al.,* 2022) that are rarely utilised for cosmetic purposes, especially for body and skin care products; therefore, it was challenging opportunity to conduct preliminary research using antibacterial and antifungal bacteria as raw material to produce anti-acne cream cosmetic products, with moisturising cosmetics using *X. granatum* mangrove fruit symbiont bacteria, which have potential as antibacterial and antifungal agents. Based on this potential, it is possible that the fruit of this species can be applied as a source of medicine and cosmetics. The research consisted of nanosystems against a wide variety of fungal species (Jangjou *et al.,* 2022), antibacterial and antibiofilm activities against clinical pathogens (Balaraman *et al.,* 2022), antimicrobial activity of flavonoids (Cushnie and Lamb, 2005), and hazardous ingredients in cosmetics (Zulaikha *et al.,* 2015). The present study aimed to investigate symbiont bacteria in the fruit and leaves of *X. granatum* as an additional raw material for cosmetic anti-acne creams and moisturisers. The samples of *X. granatum* mangrove fruit and leaves were collected from the water of the Baturusa River, Merawang District, Bangka Regency, Bangka Belitung Islands, Indonesia in 2022.

MATERIALS AND METHODS

Sample collection

Samples of *X. granatum* mangrove were taken from the river flow area (RFA) of Baturusa, Merawang, Bangka, Bangka Belitung Islands, Indonesia. In this

process, leaves and fruits were picked using a knife, put in a dark-coloured plastic ziplock, and stored in a coolbox filled with ice at 4 ˚C on the way to the laboratory. Subsequently, the samples were washed thoroughly with distilled water to remove the attached epiphytes and dried until no water was left.

Bacterial procedure

Bacterial isolation was performed using the pour plate method. For this, the incubation process was carried out at 27.5 ˚C for 2 x 24 hours (Pringgenies *et al.,* 2021), while the separation and purification of bacterial isolates that managed to grow were performed using the streak method on a petri dish with incubation at 27.5 ˚C for 48 h. The antibacterial activity was measured using the disc diffusion method (Balouiri *et al.,* 2016). For this, 100 microliter (µL) of pathogenic bacteria were spread on agar media, and several paper discs (8 mm, Advantec, Toyo Roshi, Ltd, Japan) containing 10 microliter of bacterial isolates were placed on each surface of the paper discs. The Petri dishes were incubated at room temperature for 48 h. Antibacterial activity was defined as the formation of an inhibition zone greater than 9 milimeter around the paper disc. The positive control test was conducted using the antibiotic amoxicillin with a concentration of 20 µg/disk. This aimed to determine the existence of an inhibition zone formed by antibiotics that are available on the market so that it can be used for the comparison of antibacterial abilities. The negative control test was carried out using a sample of the symbiont bacteria tested against the test bacteria. The pathogens used in testing antibacterial activity were *Staphylococcus aureus*, *Vibrio harveyi*, and *Vibrio alginolyticus*, while the pathogenic fungi used were *Malassezia furfur* and *Candida albicans*. When isolating pathogenic bacterial and fungal species, the protective measure is to mix antibiotics with as much as 2% of the culture medium used. Therefore, nystatin-type antibiotics were mixed for the bacterial test media, while penicillin-type antibiotics were mixed for the anti-fungal test media.

DNA extraction, PCR, and sequencing

First, a bacterial colony isolate was taken and dissolved in 1 mililitre (mL) of sterile water in a micro-centrifuge tube with a volume of 1.5 mL before being centrifuged for 1 minute at 10,000–12,000 rotation per meter. Then, the supernatant obtained was discarded, and 200 µL of instance matrix pellet (bacterial precipitate) was added before being vortexed and incubated at 56 °C using a heat block for 30 minute. It was vortexed again at high speed for 10 second. The tube was placed at 100 °C on the heat block for 8 min, then vortexed at high speed for 10 s and centrifuged at 12,000 rpm for 3 min. Later, 20 µL of the resulting supernatant (genome DNA solution) was used per 50 µL of the PCR reaction. PCR analysis and electrophoresis were conducted afterwards (Pringgenies *et al.,* 2021; Yuan *et al.,* 2021). The amplification results were sent to PT Genetika Science Indonesia to identify the nucleotide base sequence based on the Sanger dideoxy method.

Data analysis

Molecular data processing

The sequencing results of each sample were aligned and analysed using the molecular evolutionary genetics analysis (MEGA) 11 software. Then, the FASTA (nucleotide base sequences matched against data from GenBank) file from the analysis results was matched with the data in the National Center for Biotechnology Information (NCBI)'s GenBank database to determine the species. Several comparison species from the BLAST results were downloaded, and their phylogenetic trees were compiled using MEGA 11 in order to identify the kinship between the sample species, control species, and species from the other samples.

Gas chromatography—mass spectrometry (GC—MS) analysis

The bacteria were cultured in liquid using the marine broth, after which the bacteria were extracted with methanol using the soxhletation method. The products of soxhletation were treated with a rotary evaporator in order to obtain the extraction products. The gas chromatography—mass spectrometer (GC—MS) analysis was conducted using a GC—MS QP2010S (column: Rtx-5MS 30 m, diameter: 0.25 mm), programmed at 80–300 °C with a temperature rise of 10 °C/min and helium carrier gas while the pressure was 22 kilopascal. The number of compounds present in the extract was indicated by the number of peaks on the chromatogram, while the names/types of compounds present were interpreted based on the data spectra of each of these peaks using the approximation method library on the GC—MS database (Fox 1999; Hsouna and Alayed, 2012).

RESULTS AND DISCUSSION

Isolate morphology

13 isolates were successfully obtained from the leaf

and fruit extract of *X. granatum* from Bangka Regency. Morphologically, each isolated sample was mostly circular and white with an entire margin, convex elevation, and moderate, small, large, and punctiform. Leaf bacterial isolates were coded as X1, while flower bacterial isolates were coded as X2.

Antibacterial and antifungal test

Antibacterial and antifungal tests were carried out qualitatively. Pure isolates were tested on three pathogenic bacteria, namely, *V. harveyi*, *V. alginolyticus*, and *S. aureus*. The qualitative test results demonstrated that eight isolates were capable of producing an inhibition zone against *S. aureus*, seven isolates were tested positive for antibacterial activity against *V. harveyi*, and ten isolates were tested positive for antibacterial activity against *V. alginolyticus*. The antifungal test was conducted on two pathogenic fungi, namely, *M. furfur* and *C. albicans*. The qualitative test results demonstrated that six isolates could produce zones of inhibition against *M. furfur* and *C. albicans*. The results of the qualitative antibacterial and antifungal tests are presented in Table 1.

The antibacterial qualitative test is a preliminary test that aims to ensure the ability of bacterial secondary metabolites against pathogenic bacteria. According to Table 1, there was more antibacterial activity against the pathogen *V. alginolyticus* than against the other pathogens. This could be because *V. alginolyticus* is a gram-negative bacterium with thinner peptidoglycan than that in *S. aureus*, which is a gram-positive bacterium. Quantitative tests were conducted on the obtained pure isolates, and the pure isolates with positive results in the qualitative test were proceeded by the quantitative test to observe the diameter of the inhibition zone. The antibacterial activity was declared positive if a clear zone was formed. The quantitative test results of the mangrove bacterial isolate *X. granatum* against pathogenic bacteria *S. aureus*, *V. harveyi*, and *V. alginolyticus* are presented in Table 2.

According to the test results, 8 out of 13 isolates showed antibacterial activity against *S. aureus*, and isolates X1.62 and X1.131 did not show any inhibition zones at 24 h of observation. The highest isolates at 24 h were X1.53 (3.31 ± 0.12 mm), X1.65 (2.99 ± 0.01 mm), and X1.54 (2.52 ± 1.30 mm). The isolates with the largest inhibition zone area at 48 h of observation were X1.53 (3.39 ± 0.42 mm), X1.54 (3.1 ± 1.13 mm), and X1.62 (2.8 ± 5.66 mm), while the isolates with the largest inhibition zones at 72 h of observation were X1.53 (3.09 ± 0.55 mm), X1.631 (1.82 ± 0.45 mm), and X1.131 (1.52 ± 0.45 mm). Table 2 presents results related to the mangrove symbiont bacterial isolates *Xylocorpus sp*, which is known for its antibacterial activity against pathogenic bacteria *S. aureus*, as it is known to exist in other mangrove species (Pringgenies *et al.,* 2021). *S. aureus* pathogenic bacteria are gram-positive bacteria that are round, golden in colour, and anaerobic. Although these bacteria can secrete endotoxin, they are unable to form spores. Moreover, *S. aureus* die when kept at 60 ˚C for 60 min (Tam and Torres, 2019). The antibacterial test result regarding the pathogenic bacteria *V. harveyi* showed that 8 out of 13 isolates had positive results. *V. harveyi* is a gram-negative bacterium that has a positive oxidase, can ferment glucose, and produces lysine decarboxylase. These bacteria can also produce catalase, degrade gelatin, are motile, and have flagella (Yuan *et al.,*

Table 1: Antibacterial and antifungal qualitative test

Isolate	Pathogenic bacteria			Pathogenic fungi	
	S. aureus	*V. harveyii*	*V. alginolyticus*	*M. furfur*	*C. albicans*
X1.212	-	+	+	-	-
X1.63	+	+	+	-	+
X1.54	+	+	+	-	+
X1.62	+	+	+	-	-
X1.631	+	+	+	-	-
X1.632	-	+	-	-	-
X1.131	+	-	-	-	-
X1.64	+	-	-	+	+
X1.53	+	-	+	+	-
X1.65	+	-	+	+	-
X2.54	-	-	+	+	+
X2.52	-	-	+	+	+
X2.55	-	-	+	+	-

Table 2: Antibacterial quantitative test on *staphylococcus aureus, vibrio harveyi, vibrio alginolyticus* pathogen

Isolate	Pathogenic bacteria (mm)								
	Staphylococcus aureus			*Vibrio harveyi*			*Vibrio alginolyticus*		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
X1.212	0±0	0±0	0±0	2.15±3.04	2.87±0.61	2.4± 0.14	1.43±2.02	1.43±0.66	1.6± 0.42
X1.63	2.15±0.09	2.25±0.07	1.5± 0.42	0±0	0,48±0	1.55±0.77	1.51±1.25	0.14±0.19	4.64±1.66
X1.54	2.52±1.30	3.1±1.13	1.34±0.50	1.21±1.71	0.85±1.2	1.1±0.84	2.4±0	1±1.41	2.25±0.35
X1.62	0±0	2.8±5.66	1.26±1.78	1.71±2.42	0.9± 1.27	1.2± 0.71	0.3± 0.42	1.24±1.07	1.53±0.32
X1.631	0±0	1.67±0.81	1.82±0.45	2.37±0.49	1.29±1.15	1.8± 1.15	0.7± 2.08	0.71±0.99	2.08±0.37
X1.632	0±0	0±0	0±0	3.05±2.59	1± 1.15	2± 1.15	0±0	0±0	0±0
X1.131	2.45±3.46	2.25±1.62	1.52±0.51	0±0	0±0	0±0	0±0	0±0	0±0
X1.64	1.75±2.47	2.10±1.41	1.50±1.13	0±0	0±0	0±0	0±0	0±0	0±0
X1.53	3.31±0.12	3.39±0.42	3.09±0.55	0±0	0±0	0±0	1.33±0.24	148±0.68	1.72±0.11
X2.54	0±0	0±0	0±0	0±0	0±0	0±0	0.63±0.89	0.66±0.91	0.24±0.34
X1.65	2.99±0.01	1,11±1.56	0.75±1.06	11±15.5	3.25±4.59	6.35±2.33	0.5±0.7	0.66±0.9	1± 1.35
X2.52	0±0	0±0	0±0	0±0	0±0	0±0	1.23±0.89	0.91±1.29	2.17±0.13
X2.55	0±0	0±0	0±0	0±0	0±0	0±0	0.7± 0.99	0.86±0.48	1.22±0.17

2021). Isolate X1.63 did not form any inhibition zone at 24 h of observation. The highest isolates at 24 h were X1.65 (11 ± 15.5mm), X1.632 (3.05 ± 2.59 mm), and X1.631 (2.37 ± 0.49 mm). Isolates with the largest inhibition zone area at 48 h of observation were X1.65 (3.25 ± 4.59 mm), X1.212 (2.87 ± 0.61 mm), and X1.31 (1.29 ± 1.15mm), while the isolates with the largest inhibition zone at 72 h of observation were obtained by X1.65 (6.35 ± 2.33 mm), X1.212 (2.4 ± 0.14 mm), and X1.632 (2 ± 1.15 mm). Furthermore, according to the antibacterial test conducted on the pathogenic bacteria *V. alginolyticus*, 10 isolates showed positive results. *V. alginolyticus* is a gram-negative bacterium that is halophilic and has a crooked (i.e., comma) shape with a length of about 1.4–5.0 micrometer and a width of 0.3–1.3 µm, while, *V. alginolyticus* is motile and has a flagellum with a protective sheath. The highest isolates at 24 h were X1.54 (2.4 ± 0 mm), X1.632 (1.51 ± 1.25 mm), and X1.53 (1.33 ± 0.24 mm). The isolates with the largest inhibition zone area at 48 hours (h) of observation were X1.53 (1.48 ± 0.68 mm), X1.212 (1.43 ± 0.66 mm), and X1.62 (1.24 ± 1.07mm), while the isolates with the largest inhibition zone at 72 hours of observation were obtained by X1.63 (4.64 ± 1.66 mm), X1.54 (2.25 ± 0.35 mm), and X2.52 (2.17 ± 0.13mm). According to the test results, there were isolates that showed bacteriostatic and bactericidal antibacterial activity. One such example of bacteriostatic antibacterial activity is X1.63 against the pathogen *S. aureus*. As the growth of the inhibition zone on isolate X1.63 tended to increase, it can be said that X1.63 showed bacteriostatic ability. Additionally, the isolates X1.54, X1.62, X1.131, X1.64, X1.53, and X1.65 showed bacteriocidal activity against *the S. aureus* pathogen in the antibacterial test, while X1.63, X1.54, and X2.54 showed bacteriocidal activity against *V. alginolyticus pathogen*. Only isolate X1.63 showed bacteriostatic activity against the *V. harveyi* pathogen in the antibacterial test. Thus, based on the results, mangrove symbiont bacteria *Xylocorpus sp* has potential as an antibacterial agent, especially against the test bacteria *S. aureus*, *V. alginolyticus*, and *V. harveyi*. Moreover, mangrove plants contain alkaloids, flavonoids, saponins, and tannins (Pringgenies *et al.,* 2021). As this compound has potential as an antibacterial agent, the bacteria found were also suspected to have potential as antibacterial agents.

Antifungal test

The antifungal quantitative tests were conducted on the obtained pure isolates. The antifungal activity was tested on pathogenic fungi *M. furfur* and *C. albicans*. The results of the quantitative tests conducted on *M. furfur* and *C. albicans* can be observed in Table 3.

According to the results of the antifungal test, six isolates exhibited antifungal activity against the two types of fungi tested, namely, *M. furfur* and *C. albicans*, while the remaining seven isolates out of thirteen only showed activity on one of the tested fungi. *M. furfur* is a pathogenic fungal species and is a constituent of the human microflora (Rojas *et al.*, 2014). Moreover, *M. furfur* is a lipophilic dimorphic fungus belonging to the flora group. Isolates X1.54, X1.53, and X2.52 did not show any inhibition zones

at 72 h of observation. This was predicted due to the inability of pure isolates to fight pathogenic bacteria. The highest isolates at 24 h were X1.53 (3.65 ± 5.16 mm), X1.64 (2.05 ± 2.33 mm), and X1.63 (1.65 ± 0.78 mm). The isolates with the largest inhibition zone area at 48 h of observation were X1.64 (2.8 ± 3.95 mm), X1.53 (2.55 ± 3.60 mm), and X1.63 (2.32 ± 0.45mm), while the isolates with the largest inhibition zone at 72 hours of observation were obtained by X1.64 (4 ± 0 mm) and X2.54 (2.73 ± 1.03 mm). According to the antifungal test results, 6 out of 13 isolates exhibited antifungal activity against *C. albicans*. *C. albicans* is a fungal pathogen that belongs to the Ascomycota group and has opportunistic characteristics. Isolate X1.53 did not show any inhibition zone at 24 h of observation. The highest isolates at 24 h were X2.55 (4.8 ± 3.68 mm), X2.52 (3.81 ± 0.26 mm), and X2.54 (2.85 ± 4.03 mm). The isolates with the largest inhibition zone area at 48 h of observation were X2.52 (3.75 ± 0.35 mm), X1.53 (3.5 ± 0.70 mm), and X2.55 (3.25 ± 1.06 mm), while the isolates with the largest inhibition zone at 72 h of observation were obtained by X2.52 (3.6 ± 0.56 mm), X1.64 (3 ± 0.56 mm), and X2.55 (2.6 ± 0.85 mm). The bacterial isolates tested were assumed to produce clear zone areas that inhibited the growth of pathogens in the test medium due to secondary metabolite processes. Secondary metabolites that have bioactive compounds are produced by bacteria as a self-defence response. These bioactive compounds inhibit the growth of fungal cells by damaging the cell walls, causing the fungal cells to either experience inhibited growth or even die. Based on their activity, antifungal drugs are grouped into two types: fungicidal and fungistatic. While fungicide antifungals kill fungal growth, fungistatic antifungals inhibit fungal growth without killing the fungal population (Cowen *et al.,* 2015; Revie *et al.,* 2018; Hossain *et al.,* 2022). All the isolates that were tested against *M. furfur* and *C. albicans* produced an inhibition zone of <5 mm, indicating that the three isolates possessed weak antibacterial property.

Molecular identification

The analysis of the qualitative and quantitative bacterial tests conducted on pathogenic bacteria and pathogenic fungi selected the three best isolates had antibacterial properties and the best isolates had antifungal properties. However, each of these isolates has the potential to be an antibacterial and antifungal agent. In other words, the six selected bacteria simultaneously have antibacterial and antifungal potential. The conditions of these selected bacteria are specific because of their potential against bacterial and fungal pathogens. The discovery of bacteria that have antibacterial and antifungal potential can lead to their use as ingredients for moisturising cosmetic formulas, which can be expected to prevent acne growth, although this hypothesis needs to be tested by further studies.

Through the methods of molecular identification, we identified six potential isolates in mangrove *X. granatum,* including *Sinomicrobium oceani* (99%), *Proteus mirabilis* (100%), *Pseudomonas khazarica* (100%), *Sinomicrobium oceani* (85%), *Sinomicrobium oceani* (99%), and *Alcaligenes aquatilis* (98%), as presented in Table 4. Based on the data from

Table 3: Antifungal Quantitative Test on *Malassezia furfur* and *Candida albicans* Pathogens

Isolate Code	Pathogenic bacterial (mm)					
	Malassezia furfur			*Candida albicans*		
	24 h	48 h	72 h	24 h	48 h	72 h
X1.212	0±0	0±0	0±0	0±0	0±0	0±0
X1.63	0±0	0±0	0±0	1.65±0.78	2.32±0.45	0.3±0.42
X1.54	0±0	0±0	0±0	1.7±0.57	2.15±0.92	0±0
X1.62	0±0	0±0	0±0	0±0	0±0	0±0
X1.631	0±0	0±0	0±0	0±0	0±0	0±0
X1.632	0±0	0±0	0±0	0±0	0±0	0±0
X1.131	0±0	0±0	0±0	0±0	0±0	0±0
X1.64	1.32±1.86	3.2±0.28	3±0.56	2.05±2.33	2.8±3.95	4±0
X1.53	0±0	3.5±0.70	1.85±0.21	3.65±5.16	2.55±3.60	0±0
X2.54	2.85±4.03	2.8±3.96	1.9±2.69	1.38±1.95	1.5±2.12	2.73±1.03
X1.65	1.91±2.70	1.6±2.26	1.75±2.47	0±0	0±0	0±0
X2.52	3.81±0.26	3.75±0.35	3.6±0.56	0.59±0.014	0.5±0.70	0±0
X2.55	4.8±3.68	3.25±1.06	2.6±0.85	0±0	0±0	0±0

Table 4: Identification Results of Species Isolate Bacteria of the mangrove symbiont *X. granatum*

Code	Relative similarity	Scientific name	Query cover	E-value	Percent identify	Acc number
X2.52	*Sinomicrobium oceani* strain SCSIO 03483 16S ribosomal RNA, partial sequence	*Sinomicrobium oceani*	99%	0	89.84%	NR_109592.1
X1.65	*Proteus mirabilis* strain JCM 1669 16S ribosomal RNA, partial sequence	*Proteus mirabilis*	100%	0	98.52%	NR_113344.1
X1.64	*Pseudomonas khazarica* strain TBZ2 16S ribosomal RNA, partial sequence	*Pseudomonas khazarica*	100%	0	99.53%	NR_169334.1
X1.53	*Sinomicrobium oceani* strain SCSIO 03483 16S ribosomal RNA, partial sequence	*Sinomicrobium oceani*	85%	1.00E-153	84.15%	NR_109592.1
X1.54	*Sinomicrobium oceani* strain SCSIO 03483 16S ribosomal RNA, partial sequence	*Sinomicrobium oceani*	99%	3.00E-165	83.60%	NR_109592.1
X1.63	*Alcaligenes aquatilis* strain LMG 22996 16S ribosomal RNA, partial sequence	*Alcaligenes aquatilis*	98%	3.00E-155	83.47%	NR_104977.1

NCBI, *Proteus mirabilis* is classified as follows: Bacteria (kingdom), Proteobacteria (phylum), Gammaproteobacteria (class), Enterobacterales (order), Morganellaceae (family), and *Proteus* (genus). *Sinomicrobium pectinilyticum* is classified as follows: Bacteria (kingdom), Bacteroidota (phylum), Flavobacteriia (class), Flavobacteriales (order), Flavobacteriaceae, (family) and *Sinomicrobium* (genus). *Alcaligenes aquatilis* is classified as follows: Bacteria (kingdom), Pseudomonadota (phylum), Betaproteobacteria (class), Burkholderiales (order), Alcaligenaceae (family), and *Alcaligenes (genus)*. *Sinomicrobium oceani* is classified as follows: Bacteria (kingdom), Bacteroidota (phylum), Flavobacteria (class), Flavobacteriaceae (order), and *Sinomicrobium* (genus). *Pseudomonas khazarica* is classified as follows: Bacteria (kingdom), Proteobacteria (phylum), Gammaproteobacteria (class), Pseudomonas (order), Pseudomonas (family), and *Pseudomonas* (genus). Similarly, based on the data from NCBI, *Proteus mirabilis* is classified as follows: Bacteria (kingdom), Proteobacteria (phylum), Gammaproteobacteria (class), Enterobacterales (order), Morganellaceae (family), and *Proteus* (genus). *Sinomicrobium pectinilyticum* is classified as follows: Bacteria (kingdom), Bacteroidota (phylum), Flavobacteriia (class), Flavobacteriales (order), Flavobacteriaceae (family), and *Sinomicrobium* (genus). *Alcaligenes aquatilis* is classified as follows: Bacteria (kingdom), Pseudomonadota (phylum), Betaproteobacteria (class), Burkholderiales (order), Alcaligenaceae (family), and *Alcaligenes* (genus). The *Sinomicrobium oceani* species is classified as follows: Bacteria (kingdom), Bacteroidota (phylum), Flavobacteria (class), Flavobacteriaceae (order), and *Sinomicrobium* (genus). *Pseudomonas khazarica* is classified as follows: Bacteria (kingdom), Proteobacteria (phylum), Gammaproteobacteria (class), Pseudomonas (order), Pseudomonas (family), and *Pseudomonas* (genus). Based on the compilation results of a phylogenetic tree using MEGA 11, *Sinomicrobium oceani* (isolate X1.52; bootstrap value: 90) had the closest kinship with *S. pectinilyticum* (isolate X1.54; bootstrap value: 90) and *P. mirabilis* (isolate X1.53; bootstrap value: 83), while *Proteus mirabilis* (isolate X1.65; bootstrap value: 99) was related to *Sinomicrobium oceani* (isolate X1.64; bootstrap value: 99). These five isolates had a kinship that was still in a large clade. Additionally, *Alcaligenes aquatilis* (isolate X1.63; bootstrap value: 55) had a very different kinship with other isolates. A high bootstrap value (i.e. over 70%) indicates that the kinship shown by the phylogenetic tree in figure x has a relatively good level of trust (Park *et al.*, 2010). There are six types of mangrove symbiont bacteria *X. granatum* that have potential as antibacterial and antifungal agents. These are *Sinomicrobium oceani* (99%), *Proteus mirabilis* (100%), *Pseudomonas khazarica* (100%), *Sinomicrobium oceani* (85%), *Sinomicrobium oceani* (99%), and *Alcaligenes aquatilis* (98%), all of which have been registered at the NCBI with the submission number SUB12685225.

GC—MS analysis was conducted using bacterial extracts with methanol solvent. The GC—MS results from a total of six identified isolates showed 20 types

of compounds for each tested bacterium. The compounds with the highest concentration as revealed by analysis of the content in the isolates are as follows: *Sinomicrobium oceani* (isolate X1.53) containing Hexadecanoic acid, methyl ester (CAS) 27,52%; *Sinomicrobium pectinilyticum* (isolate X1.54) containing 9-octadecenoic acid (Z)-, methyl ester (CAS) 35,61%; *Alcaligenes aquatilis* (Isolate X1.63) containing 9-octadecenoic acid, methyl ester, (E)- (CAS) 44,02%; *Pseudomonas khazarica* (isolate X1.64) containing 9-octadecenoic acid, methyl ester, (E)- (35,64% area); Proteus mirabilis (isolate X1.65) with the highest content of 9-octadecenoic acid, methyl ester, (E)- 33,28%; and *Sinomicrobium oceani* (isolate X2.52) has the highest content of 9-octadecenoic acid, methyl ester, (E)- 46,58%. The biggest and highest compound based on the six isolates was 9-octadecenoic acid, methyl ester, (E)-. 9-Octadecenoic acid, methyl ester, (E)- is a compound derived from unsaturated fatty acids that has potential as an antifungal and antibacterial agent (Tahir *et al.,* 2018). The biosynthesis process of 9-octadecenoic acid, methyl ester, (E)- generally involves the conversion of stearic acid into unsaturated fatty derivatives due to the dehydrogenation process of the 9-desaturase stearoyl-CoA enzyme (Zahara *et al.,* 2022). *Sinomicrobium oceani* is a bacterium commonly found in marine sediments (Xu *et al*., 2013). It is known that this type of *S. oceani* bacteria has a wide distribution, ranging from the waters of China to Indonesia. Moreover, these bacteria have the potential to degrade alginate as well as seaweed; therefore, they can be used for the degradation of seaweed biomass using non-toxic and environmentally safe biological methods (Jegatheesan *et al.,* 2017). However, in the present study, the bacterium *S. oceani* has potential as an antifungal agent, while the bacterium *Proteus mirabilis* is a gram-negative, facultative anaerobic, rod-shaped bacterium. These bacteria are known to have urease activity, which causes all infections in human proteus. The present review focused on the mechanism that forms *Proteus mirabilis* biofilms, along with a state-of-the-art update regarding the prevention of biofilm formation and reduction of mature biofilms. These treatment approaches include natural and synthetic compounds targeting virulence factors and quorum sensing, along with other strategies that include carrier-mediated diffusion of antimicrobials into biofilm (Wasfi *et al.,* 2020). Moreover, the bacterium *Pseudomonas khazarica* is also found in the Khazar (Caspian) sea, which is probably how it got its name. These bacteria have the potential to degrade polycyclic aromatic hydrocarbons (Tarhriz *et al.,* 2020). *Alcaligenes aquatilis* is a motile Gram-negative, catalase-positive, and cytochrome oxidase-positive bacterium, with peritrichous flagella and of the genus *Alcaligenes*. These bacteria are found in sediments in the waters of the United Kingdom and even Japan, meaning that this type of bacteria has a wide distribution in the waters (Durán *et al.,* 2019). Furthermore, *Alcaligenes aquatilis* are also bacteria that are commonly found in sediments. These bacteria show lignin peroxidase activity, which is responsible for the bronze green decolourisation that was detected. The dominant ingredients found in bacterial isolates that have antibacterial and antifungal potential are hexadecanoic acid, methyl ester (CAS), and 9-octadecenoic acid (Z). These types of content have potential as both antibacterial and antifungal agents. Skin infections are caused by pathogenic bacteria (Chiller *et al.,* 2001), which cause a red rash filled with fluid; if the red rash breaks out, it can leave sores on the skin. Moreover, if not treated properly, skin infections can cause further complications, resulting in skin and fat tissue infections. For instance, pathogenic fungal infection is one of the most challenging diseases to treat in humans today. The increase in the cases of pathogenic fungal infections has been caused by the need for existing antifungal compounds which can result in unwanted side effects and are no longer effective against new pathogenic fungal strains. Thus, the present study is a comprehensive exploration of the natural resources of Indonesian microorganisms for microbes producing bioactive compounds as raw materials for anti-acne cosmetics, skin lightening, and skin moisturisers. The study found bacteria with potential anti-bacterial and antifungal properties, namely, *Sinomicrobium oceani, Proteus mirabilis*, *Pseudomonas Khazaria*, and *Alcaligenes aquatilis*. Bacteria contain 9-octadecenoic acid compounds, namely, the C18 straight-chain saturated fatty acid component. Additionally, the compound 9-octadecenoic acid, methyl ester, contains compounds with potential anti-bacterial and anti-cancer properties (Krishnamoorthy and Subramaniam, 2014). The high levels of nutrients in mangrove areas are caused by mangrove waste, which turns into mangrove litter and is decomposed by bacteria so that it can be used as a nutrient. Mangrove extract has

Tabel 5: GC-MS Results of Species Isolate Bacteria of the mangrove symbiont *X. granatum*

S. oceani (99%) (X2.52)		*P. mirabilis* (100%) (X1.65)	
46.58	9-Octadecenoic acid, methyl ester, (E)-	33.28	9-Octadecenoic acid, methyl ester, (E)-
30.78	Hexadecanoic acid, methyl ester (CAS)	31.49	Hexadecanoic acid, methyl ester (CAS)
7.99	Octadecanoic acid, methyl ester	14.91	9-Octadecenoic acid (Z)-, methyl ester (CAS)
3.21	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)	9.34	Octadecanoic acid, methyl ester
1.76	Eicosanoic acid, methyl ester (CAS)	1.95	Eicosanoic acid, methyl ester (CAS)
1.54	9,12-Octadecadienoic acid, methyl ester, (E,E)- (CAS)	1.14	9,12-Octadecadienoic acid, methyl ester, (E,E)- (CAS)
1.31	9-Octadecenal, (Z)- (CAS)	0.89	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)
1.26	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS)	0.88	Tridecanedial
0.93	Tetradecanoic acid, methyl ester (CAS)	0.83	Tetradecanoic acid, methyl ester (CAS)
0.88	Octadecanoic acid, 10-oxo-, methyl ester	0.72	Octadecanoic acid, 10-oxo-, methyl ester (CAS)
0.78	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester (CAS)	0.68	9-Octadecenoic acid (Z)-, methyl ester (CAS)
0.76	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	0.66	DI-(9-OCTADECENOYL)-GLYCEROL
0.49	Dodecanoic acid, methyl ester	0.65	7,7-dimethyl-3-vinyl-bicyclo[4.1.0]hept-3-ene
0.35	3,9-Dodecadiyne	0.60	Dodecanoic acid, methyl ester
0.31	METHYL 3-acetylhydroxypalmitate	0.51	1,2-Benzenedicarboxylic acid, 3-nitro- (CAS)
0.30	1H-Indene, 1-(1,5-dimethyl-2-hexenyl)octahydro-7a-methyl-, [1R-[1.alpha.(1R*,2Z),3a.beta.,7a.alpha.]	0.43	9,12-Octadecadienoic acid, methyl ester
0.24	Docosanoic acid, methyl ester (CAS)	0.31	Cyclopentadecanone, 2-hydroxy-
0.23	methyl dihydromalvalate	0.24	RANS-2-TRIDECENAL
0.18	Tetracosanoic acid, methyl ester (CAS)	0.24	DECANOIC ACID, METHYL ESTER
0.12	01297107001 TETRANEURIN - A - DIOL	0.24	Octadecanoic acid, 3-oxo-, methyl ester (CAS)
Pseudomonas khazarica (100%) (X1.64)		*Sinomicrobium oceani* (85%) (X1.53)	
35.64	9 -Octadecenoic acid, methyl ester, (E)-	27.52	9-Octadecenoic acid, methyl ester, (E)-
27.68	Hexadecanoic acid, methyl ester (CAS)	25.06	Hexadecanoic acid, methyl ester (CAS)
13.34	9-Octadecenoic acid, methyl ester, (E)-	10.12	9-Octadecenoic acid, methyl ester, (E)-
8.62	Octadecanoic acid, methyl ester	7.26	Octadecanoic acid, methyl ester
2.54	9,12-Octadecadienoic acid, methyl ester, (E,E)- (CAS)	4.68	Oleic Acid
2.17	Eicosanoic acid, methyl ester (CAS)	3.70	9,12-Octadecadienoic acid, methyl ester, (E,E)- (CAS)
1.32	13-Docosenoic acid, methyl ester, (Z)-	3.09	9-Octadecenal, (Z)- (CAS)
1.20	9,12-Octadecadienoic acid, methyl ester, (E,E)- (CAS)	2.79	2-NORPINENE-2-ETHANOL, 6,6-DIMETHYL-, ACETATE
1.16	Docosanoic acid (CAS)	2.53	9,12-Octadecadienoic acid, methyl ester
1.01	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	2.32	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS)
0.93	9-Octadecenal, (Z)- (CAS)	2.00	Ethyl linoleate
0.88	Heptadecanoic acid, 8-oxo-, methyl ester (CAS)	1.66	Dodecanoic acid, methyl ester
0.72	Tetradecanoic acid, methyl ester (CAS)	1.56	Eicosanoic acid, methyl ester (CAS)
0.59	(Z)14-Tricosenyl formate	1.41	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester (CAS)
0.46	Nopyl acetate	1.40	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)
0.43	Dodecanoic acid, methyl ester	1.19	Tetradecanoic acid, methyl ester (CAS)
0.41	Tricosanoic acid, methyl ester (CAS)	0.67	Octadecanoic acid, 10-oxo-, methyl ester (CAS)
0.40	Cyclohexadecanone	0.61	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS)
0.27	9-Octadecenoic acid (Z)-, methyl ester (CAS)	0.34	7-Octylidenebicyclo[4.1.0]heptane
0.24	1H-Indene, 1-(1,5-dimethyl-2-hexenyl)octahydro-7a-methyl-, [1R-[1.alpha.(1R*,2Z),3a.beta.,7a.alpha.]	0.09	LIMONENE DIOXIDE 1

Continued Tabel 5: GC-MS Results of Species Isolate Bacteria of the mangrove symbiont *X. granatum*

Sinomicrobium oceani (99%) (X1.54)		*Alcaligenes aquatilis* (98%) (X1.63)	
35.61	9-Octadecenoic acid (Z)-, methyl ester-(CAS	44.02	9-Octadecenoic acid, methyl ester, (E)- (CAS
26.72	Hexadecanoic acid, methyl ester-(CAS)	29.98	Hexadecanoic acid, methyl ester (CAS)
11.50	9-Octadecenoic acid, methyl ester, (E)-	6.98	Octadecanoic acid, methyl ester
8.14	Octadecanoic acid, methyl ester (CAS)	4.01	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)
2.94	9,12-Octadecadienoic acid, methyl ester, (E,E)- CAS	2.94	9-Octadecenal, (Z)- (CAS)
1.83	12-Octadecadienoic acid (Z,Z)-, methyl ester-(CAS)	2.35	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS)
1.95	Eicosanoic acid, methyl ester	1.41	Tetradecanoic acid, methyl ester (CAS)
1.54	13-Octadecenal,(Z)-	1.26	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)
1.53	Cyclopropaneoctanoic acid, 2-hexyl-, methyl-ester	1.10	Eicosanoic acid, methyl ester (CAS)
1.53	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	0.90	Dodecanoic acid, methyl ester
1.02	Tetradecanoic acid, methyl ester- (CAS)	0.81	Octadecanoic acid, 10-oxo-, methyl ester (CAS)
0.88	Dodecanoic acid, methyl ester	0.75	Ethyl linoleate
0.84	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	0.67	Benzoic acid, methyl ester
0.75	Heptadecanoic acid, 8-oxo-, methyl ester CAS	0.50	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS)
0.69	9-Octadecenal, (Z)-	0.50	Cyclopropaneoctanoic acid, 2-octyl-, methyl ester (CAS)
0.63	Docosanoic acid(CAS)	0.49	9-Hexadecenoic acid, methyl ester, (Z)- (CAS)
0.59	Tricosanoic acid, methyl ester	0.41	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (CAS
0.46	2-Pentadecanone, 6,10,14-trimethyl-(CAS)	0.36	Cyclohexasiloxane, dodecamethyl
0.43	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	0.33	1H-Indene, 1-(1,5-dimethyl-2-hexenyl)octahydro-7a-methyl-, [1R-[1.alpha.(1R*,2Z),3a.beta.,7a.alpha
0.41	2-NORPINENE-2-ETHANOL, 6,6-DIMETHYL-, ACETATE	0.23	14-.BETA.-H-PREGNA

been shown to have potential as an antibacterial agent that is resistant to various drugs, and mangrove symbiont bacteria are thought to have potential as anti-bacterial agents because the active compounds in mangrove symbionts are similar to their host. The mangrove symbiont's antimicrobial properties can fight against MDR bacteria, namely, *Staphylococcus aureus, Escherichia coli*, and *Vibrio haryeyi* (Pringgenies *et al.,* 2021). Additionally, dead mangrove leaves decompose on the ground and become a source of nutrition, contributing to their immediate vicinity (Ariyanto *et al.,* 2018). The decomposition process by microbes is found in the mangrove ecosystem. Symbiont microbes of marine life can act as antimicrobial agents even against MDR strains. The MDR microbes are pathogenic strains that have mutated and become immune to various antibiotics. Several studies have been conducted on mangrove symbiont bacteria, and the results have demonstrated that the symbiont bacteria could show antibacterial activity against pathogenic bacteria. Even actinomycetes from mangrove sediments have been discovered. The present study concluded that the products were estimated to be in the NRPS, thiopeptide, RiPP-like, siderophore, beta lactone, terpene, Type III PKS, CDPS, and lasso peptide groups. Moreover, DNA identification of the isolates found three species of actinomycetes with antibacterial potential: *Virgibacillus salaries*, *Bacillus licheniformis*, and *Priestia flexa* (Setyati *et al.,* 2021), while molecular identification found that the bacteria were similar to *Brachybacterium paraconglomeratum* (99.92%), *Streptomyces pluripotent* (100%), and *Micromonospora chersina* (99.08%). Therefore, the study concluded that the three bacterial isolates with bacterial activity have similar genes with known antibiotic-producing genes and can potentially provide new antibiotic candidates (Anggelina *et al.,* 2021). Furthermore, the findings of this study prove that mangrove symbiont bacteria are more potent than synthetic antibiotics because of their ability to fight MDR bacteria. Bacteria contain 9-octadecenoic acid (oleic acid), which is in the group of fatty acids, i.e., unsaturated fatty acids— the most widely distributed and abundant in nature. Fatty acids

and their derivatives have potential as antifungal agents. Notably, several types of fatty acids and their derivatives—oxylipins—have been found to have an inhibitory effect towards fungal growth and the production of mycotoxins. The use of fatty acids as antifungals could fulfil consumers' requests for more natural and environmentally friendly compounds while being less likely to promote fungal resistance. In addition, fatty acids as food additives due to their nature (Guimarães and Venâncio, 2022). Although, the findings related to the studies conducted on mangrove symbiont bacteria have not been used in treating bacterial or fungal infections, consortium symbiont bacteria have been used for composter production and deodorising, while reuse composter liquid bio-activator has been produced for wastewater treatment, which consists of four probiotic bacteria that function as pathogenic antibacterial agents (Pringgenies *et al.,* 2021). Additionally, natural products continue to be an excellent choice, especially those extracted from actinobacteria as they are a major source of metabolites with multiple biological activities, especially marine actinobacteria, which are less studied than their terrestrial counterparts. As regards this connection, studies have been conducted on marine organisms, which have the potential to fight skin diseases and inflammation (Chen *et al.,* 2020). Moreover, studies have also been conducted on marine bacteria, which have the potential to become skin pathogenic bacteria, such as anti-infection against bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* (De La Hoz-Romo MC *et al.,* 2022). Thus, the results of research conducted on the mangrove symbiont bacteria *X. granatum*, which has antibacterial and antifungal potential, are promising for use as cosmetic ingredients for natural products because they are usually safe and do not cause pollution. To continue the research in this area, we will use references from existing references despite the difficulties in cultivating symbiont bacteria and then use the metagenomic technology of marine species to make products that are greener and more environmentally friendly through biotransformation into things that are need by cosmetic manufacturers. In this case, natural products from marine microorganisms are reviewed and evaluated for various cosmetic applications (Ding *et al.,* 2022). Thus, the existing research became a trigger for further research into the use of mangrove symbiont bacteria *X. granatum* as a cosmetic ingredient. Therefore, this study aimed to obtain materials that can be used in further research into natural cosmetic formulas made from marine nature. Thus, this study is a reference material for further research into the bacterial material found as a reference for obtaining cosmetic formulations, moisturising tests, and the characterisation of cosmetic preparations. The study found that the mangrove symbiont bacteria of the *X. granatum* type have potential as antibacterial and antifungal agents. These findings will be used as a reference for further research on cosmetics.

CONCLUSIONS

The results of bacterial isolation from the leaf and fruit symbiont bacteria of the mangrove *X. granatum* collected from the Baturusa River, Merawang District, Bangka Belitung Islands, Indonesia, demonstrated 13 bacterial isolates originated from the leaf and fruit extract. It was found that circular bacterial isolates dominated. The morphological features of the isolated samples were as follows: white in colour, whole margin, convex elevation, and medium, small, large, and punctiform sizes. It was observed that six isolates showed potential as antibacterial agents against pathogenic bacteria *S. aureus, V. Harvey,* and *V. alginolyticus,* while also showing potential as an antifungal agent against pathogenic fungi *M. furfur* and *C. albicans*. The six isolates of symbiont bacteria collected from mangrove leaf and fruit extracts of *X. granatum* that have antibacterial and antifungal potential are *Sinomicrobium oceani* (99%). *Proteus mirabilis* (100%), *Pseudomonas khazarica* (100%), *Sinomicrobium oceanis* (85%), *Sinomicrobium oceanis* (99%), and *Alcaligenes aquatilis* (98%). The Gas chromatography-mass spectrometry (GC-MS) analysis of six identified isolates showed 20 types of compounds for each of the bacteria tested. The analysis of the content of bacteria that have antibacterial and antifungal potential in the fruit and leaf extract of the mangrove *X. granatum* revealed several compounds, namely, hexadecanoic acid, methyl ester (CAS) (27.52%) in *S. oceani*; 9-octadecenoic acid (Z)-, 35.61% methyl ester (CAS) in *S. pectinilyticum*; 9- octadecenoic acid compound, methyl ester, (E)- (CAS) 44.02% in *A. aquatilis*; Octadecenoic acid, methyl ester, (E) – (35.64 % area) in *P. khazarica*; 9-octadecenoic acid compound, methyl ester, (E)- 33.28% in *P. mirabilis*, and the highest compound content in *S. oceani*, i.e. 9-octadecenoic acid compound, methyl ester, (E)- (46.58%). These

bacteria have the highest content of 9-octadecenoic acid compound, methyl ester, which is a straight-chain saturated fatty acid component of C18. 9-octadecenoic acid compound, methyl ester, contains compounds that have antibacterial and anticancer properties.

AUTHOR CONTRIBUTIONS

D. Pringgenies contributed to original draft preparation, conceptualization, compiled the data and manuscript preparation literature review, validation, review and editing. W. Ari Setyati contributed to original draft preparation, conceptualization, compiled the data and manuscript preparation. F. Feliatra collected the resources. D. Ariyanto contributed to original draft preparation, conceptualization, compiled the data and manuscript preparation literature review, validation, review and editing. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

ABBREVIATIONS

%	Percent
AMB	l-2-Amino-4-methoxy-trans- 3-butenoic acid
BLAST	The Basic Local Alignment Search Tool
^0C	Celsius degree
CAS	Hexadecanoic acid, methyl ester
DNA	deoxyribonucleic acid
GC-MS	Gas Cromatography and Mass Spectroscopy
h	Hours
kPa	Kilopascal
MDR	Multi-Drug Resistance
MEGA	Molecular evolutionary genetics analysis
µg	Micrograms
µg/disk	Microgram per disk
µL	Microliter
mL	Mililiter
mm	Milimeter
min	Minute
NCBI	National Center for Biotechnology Information
PCA	Phenazin I-carboxylic
PCR	polymerase chain reaction
pyo	Pyocyanin
rpm	rotation per minute
RNA	Ribonucleic acid
s	Second

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