JIPK. Volume 15 No 2. November 2023
Sinta 1 (Decree No: 158/E/KPT/2021)
e-ISSN:2528-0759; p-ISSN:2085-5842
Available online at https://e-journal.unair.ac.id/JIPK

JIPK
(JURNAL ILMIAH PERIKANAN DAN KELAUTAN)
Scientific Journal of Fisheries and Marine

Research Article

Modified Media for Fungi Symbiont Sponge *Agelas* sp. (*Fusarium* sp.) Cultivation against Multidrug-Resistant Bacteria

Khoeruddin Wittriansyah[1*], Agus Trianto[2], and Ocky Karna Radjasa[3]

[1]Agroindustrial Product Development, State Polytechnic of Cilacap, Sidakaya, Cilacap, Central Java, 53212. Indonesia
[2]Department of Marine Science, Faculty of Fisheries and Marine Science, Diponegoro University, Semarang, Central Java, 50275. Indonesia.
[3]Research Center for Oceanography, Indonesian Institute of Sciences (PPO-LIPI), East Ancol, North Jakarta, Jakarta, 14430. Indonesia

OPEN ACCESS

ARTICLE INFO

Received: August 22, 2022
Accepted: November 12, 2022
Published: December 13, 2022
Available online: August 15, 2023

*) Corresponding author:
E-mail: khoeruddin@pnc.ac.id

Keywords:
Bioactive Compound
Fungi Symbiont
Media Nutrition

Abstract

Marine symbiont fungi, specifically, are likely to have antibacterial properties. The production of secondary metabolites in cultures is strongly influenced by nutrient availability. This study aimed to study the modified media for the cultivation of *Fusarium* sp. and its antibacterial bioactivity. *Fusarium* sp. was isolated from *Agelas* sp. sponge collected from Riung Sea, East South Nusa, Indonesia. In this study, the modified media was soursop juice (SJ), ginger juice (GJ), and sago (S). The fungal mycelium was cultured and scaled up for 7–14 days until the mycelium achieved maximum growth. A *filtration funnel* and HPLC were used to purify the bioactive compounds. The diffusion agar method was used to test antibacterial activity against the multi-drug resistant (MDR) *Staphylococcus aureus* and *Escherichia coli*. The results indicated that *Fusarium* sp. could grow on soursop juice and ginger juice but could not grow on sago media. The inhibition zone produced by the filtration fraction of *Fusarium* sp. from each media differed. The soursop juice media produced the largest inhibition zone against both *S. aureus* (11.56 mm ± 0.140) and *E. coli* (12.16 mm ± 0.094) at 100 µg/disc. The ginger juice (GJ) and soursop juice (SJ) media are promising as alternative culture media for *Fusarium* sp. The structure of the bioactive compound *Fusarium sp*. from GJ and MJ media culture can be further investigated using NMR.

Cite this as: Wittriansyah, K., Trianto, A., & Radjasa, O. K. (2023). Modified Media for Fungi Symbiont Sponge *Agelas* sp. (*Fusarium* sp.) Cultivation against Multidrug-Resistant Bacteria. *Jurnal Ilmiah Perikanan dan Kelautan*, 15(2):278–289. http://doi.org/10.20473/jipk.v15i2.38453

1. Introduction

The emerging resistance of bacteria to existing drugs is a major problem in the treatment of infectious diseases. *E. coli* in hospitalized patients is resistant to several antibiotics that were previously effective (Lestari *et al*., 2008). Research into obtaining new bioactive compounds against bacteria that have developed a resistance to antibiotics is therefore of a high priority (Rajasekar *et al*., 2012).

Sponges are known as hosts for marine microorganisms including fungi (Li and Wang, 2009). Sponges, being a filter feeder, house various microorganism within its body. These microorganisms then adapt and survive in the sponge's body (Lee *et al*., 2001). Microorganisms interact symbiotically to sponges. Microorganisms use nutrients in the body of the sponge to grow and develop itself while at the same time assist in the sponge's metabolic processes e.g., intracellular digestion or by translocation of metabolism (nitrogen fixation, nitrification, and photosynthesis). In addition, microorganisms also contribute to the sponge's immunity (Lee *et al*., 2001). Symbiont microorganisms have been reported to produce the same compounds as their host sponges (Wittriansyah *et al*., 2016). This can also occur in the symbiont fungi *Fusarium* sp. with *Agelas* as the host, which reported to have antibacterial bioactivity. *Agelas dispar* produces antibacterial bioactive compounds *Aminozooanemonin* and *Pyridinebetaine A* (Lee *et al*., 2001). *Agelas nakamurai* produces *Ageliferine, Debromosceptrin and Nakamuric* acid compounds which possess antibacterial properties. *Agelas* sp. contains the bioactive compound *Agelasine D* which is an antibacterial compound effective against *S. aureus and S. pyogenes* (Lee *et al*., 2001; Hedner, 2007).

Marine symbiont fungi are source of new compounds useful in medicine (Zheng *et al*., 2013; Lee *et al*., 2013). Sponge symbiont fungi have bioactive compounds with anticancer (Lee *et al*., 2010), antifungal (Bugni and Ireland, 2004), and antibacterial properties (Jansen *et al*., 2013). The marine fungus *Fusarium* is known to have a variety of medicinal properties: *Fusarium* isolated from the surface of seagrass *Halodule wrightii* produces *Sansalvamide* compounds with anticancer properties (Belofsky *et al*., 1999), *Fusarium lateritium* 2016F18-1 from sponge *Phyllospongia foliascens* produces *Pyripyropnes* compounds with anticancer properties (Cao *et al*., 2017), and finally, *Fusarium heterosporum* produces tetramic acid compounds with antiviral properties (Moghadamtousi *et al*., 2015). Mangrove symbiont fungi *Rhizophora mucronata* and *Acanthus ilicifolius*, namely the genus *Bacillus* and *Paracoccus*, are able to inhibit pathogenic bacteria *Staphylococcus aureus*, *Escherichia coli*, and *Vibrio harveyi* (Pringgenies *et al*., 2021).

Each microbial strain has the possibility to produce a variety of compounds, and only a portion of these compounds are produced under specific growth conditions (Zhao *et al*., 2019). Fungi require the correct amount and proportion of nutrients to grow optimally. Nutrient fluctuation will affect the metabolism and growth of fungi. When one or more nutrients are scarce and fungal growth slows due to an unbalanced primary metabolism, nutrients are diverted to the secondary metabolism with specific goals (Moore, 1998). Secondary metabolites and compound production are directly affected by the nutrients and the media used (Aldred *et al*., 1999; Lawrence, 1999). In addition to nutrient compositions in culture media affecting cellular productivity, the cultural media design is therefore crucial in producing secondary metabolites (Atalla *et al*., 2008). Fungi cultivation is engineered to achieve the production of specific or new compounds by isolating the fungus and cultivating it in modified media (Bills *et al*., 2008). Tweaking microbial culture parameters to increase the diversity of metabolism production is referred to the One Strain, Many Compounds (OSMAC) approach.

For example, *Penicillium* spp. when culture is limited to the element C (carbon) it will produce *penicillin* compounds, while culture with limited element P (phosphorus) will produce *cephalosporins* and *vancomycin*. Likewise, fixed N (Nitrogen) will make *carbapenems* (Lawrence, 1999). The fungus *Phoma* sp. is grown on culture media in the form of Malt Extract Agar (MEA), Wheat Extract Agar(WEA), Oat Extract Agar (OEA), and Seed Oil Agar (OSREA). The results showed that the fungus *Phoma* sp. grows well in MEA and WEA media. The presence of lipids in OSREA media is suspected to cause the fungus *Phoma* sp. cannot to grow to its full potential. Maximum production of *squalestatin* compounds was found in MEA media. This study shows that the influence of media and nutrients greatly affects the secondary metabolites and the compounds produced (Aldred *et al*., 1999). Research on the marine fungus *Varicosporina ramulosa* was grown on five different growth media. This was done in order to obtain the most suitable media and to increase the production of bioactive compounds. Initial screening of *Varicosporina ramulosa* yielded 13 different compounds. Compounds number 3 and number 10 were the most active against test bacteria (gram-positive and gram-negative). Compounds number 3

and 10 were then cultured using Biomalt medium, Malt Extract Broth, Potato Carrot Broth, Cellulose Broth, and Glucose Peptone Yeast Extract Broth. The experimental results showed that the Malt Extract Broth Medium was most suitable for producing compounds number 3 and 10 with the amount of 7,598 mg/L and 17,298 mg/L. The medium that produced the least amount of compound 3 (2.147 mg/L) was Potato Carrot Broth and the one that produced the least amount of compound number 10 (1.603 mg/L) was the Cellulose Broth. Compounds number 3 and 10 in Malt Extract Broth with culture conditions of pH 6 at 24-26 °C, the incubation period of 8-10 days, and the shaker at 65 rpm were identified as *dibutyl phthalate* and *ergosterol*. *Ergosterol* has bioactivity as an antitumor (Atalla *et al*., 2008).

Previous research on the fungus *Aspergillus sydowii* symbiont sponge *Axinella* sp. showed that the fungus can grow on Noni Juice Media (MG), Avocado leaves media (AL), and Soursop leaves media (SR). Media (MG) produced the highest crude extract of 4,244 g compared to other modified media. The extracts of the three modified media have different inhibition against antagonistic bacteria. The largest inhibition zone was found in the extract fraction (MG) media of 0.71 mm (*E. Coli*) and 10.98 mm (*S. aureus*). Based on the results of TLC analysis, *Aspergillus sydowii* contains steroid and terpenoid compounds (Widyaningsih *et al*., 2018). Research in microbiology that uses growth media is challenging, especially in developing countries. This is due to the high cost of laboratory culture media (Karimi *et al*., 2019). The utilization of local resources and alternatives to cheaper modified media needs to be inspected. Utilization of local materials, easy to obtain and more affordable prices needs to be done (Shareef, 2019). Utilizes legume seeds such as cowpea, lentil, split peas, chickpeas, soy protein, and mung beans as a growth medium for *Staphylococcus aureus*, *Escherichia coli*, *B. cereus*, *Pseudomonas aeruginosa*, *Penicillium* sp. and *Aspergillus* sp. Research by Grandes-Blanco *et al*. (2020) utilized vermicompost, vermiwash, pork bone, and fish waste as culture media for *Pleurotus eryngy*, *Lentinula edodes*, and *Flammulina velutipes*.

The media use soursop juice, ginger juice, and sago because these three ingredients are easy to find and widely available in Indonesia. The price comparison for making soursop juice, ginger juice, and sago media per 1 liter is also cheaper than the standard laboratory malt extract broth media. This is because making 1 liter of modified media in liquid medium only takes less than 400 grams of the material. The choice of soursop and ginger is also because both of these ingredients are well-known as traditional medicinal ingredients. Soursop is known to have Antidiarrheal activity and Antiprotozoal activity against *Toxoplasma gondii* (Mutakin *et al*., 2022). Ginger is known as an herbal and traditional drink, especially in Asia, which is useful as an antiemetic, rheumatism, headache, anorexia, and cholera. Ginger contains *shogaol* and *gingerol* which are antioxidant compounds (Harmayani *et al*., 2019). Sago is known as a traditional food in eastern Indonesia. Sago contains carbohydrates and is high in energy (Sidiq *et al*., 2021). The addition of honey to ginger juice and sago is intended to meet the nutrient elements needed for culture that are not present in the two ingredients. Honey contains glucose and sucrose. Good quality honey has a minimum of 65% glucose and a maximum of 5% sucrose (Zuhairah *et al*., 2019). Glucose and sucrose are useful as carbon sources in microorganism culture. Microorganisms utilize carbon in vegetative growth and encourage the production of secondary metabolites (Septiana and Simanjuntak, 2017).

The use of soursop, ginger, and sago media as media culture has never been done before. There is also no information on the effect of bioactive compounds produced using these media compared to standard media. This study aims to determine whether the symbiont fungi *Fusarium* sp. can grow on modified media, specifically soursop juice (SJ), ginger juice (GJ), and sago (S). This is a more economical alternative to traditional media designed for mass production. In addition, this study aims to determine whether compounds produced by SJ and GJ media retained their antibacterial properties against test bacteria from Malt Extract Broth (MEB) standard media.

2. Materials and Methods

2.1 Material

2.1.1 Microbial strain

The 2x2 cm^2 sized sponge was cleaned with sterile seawater. The pieces of sponge were placed on the surface of the Malt Extract Agar (MEA) media and incubated for 24-36 hours. The growing fungus isolates were separated based on their morphology until pure isolates were obtained (Wittriansyah *et al*., 2016).

The fungus isolates were screened to determine their bioactivity against *S aureus* and *E. Coli*. The antagonist bacteria were grown in Zobell soft agar medium (1% v/v), then poured into the media that had previously grown fungus isolate. The zone of inhibition will appear after 24 hours (the data were not shown in this paper) (Terkina *et al*., 2006).

Table 1. The weight of sponge symbiont fungus Fusarium sp. crude extract cultured on standard and modified media.

No.	Symbiont	Media	Weight of mycelium layer (g)	Extract weight (g)	Extract (%)
1.	*Fusarium* sp.	Malt Extract Broth	207.72	2,719	1,309
2.		Soursop juice	308.51	4,162	1,349
3.		Ginger juice	166.18	1,569	0,944
4.		Sago	-	-	-

Table 2. The results of the crude extract separation from *Fusarium* sp.

No.	Symbiont	Media	Crude extract weight (g)	Extract weight	
				EtOAc fractions (mg)	H_2O fractions (g)
1.	*Fusarium* sp.	Malt Extract Broth	2,719	214.2	2,404
2.	*Fusarium* sp.	Soursop juice	5,092	205.6	2,664
3.	*Fusarium* sp.	Ginger juice	1,787	284.9	1,281

Table 3. The results of filtration fraction *Fusarium* sp.

Media	Ethyl acetate fraction (g)	Fraction of filtration	Weight (mg)
Malt Extract Broth	0.2142	1	16
		2	46.4
		3	33.6
		4	4.5
		5	4.5
Soursop juice	0.2056	1	11.3
		2	31.7
		3	31
		4	4.8
		5	48.4
Ginger juice	0.2849	1	20.9
		2	94.8
		3	60
		4	41.2

Figure 1. (a) culture of *Fusarium* sp. on Malt Extract Broth (MEB); (b) on Ginger Juice (GJ) media

Fusarium sp. has been identified through molecular analysis and morphological characterization. The molecular analysis involved DNA extraction, 18s rRNA amplification, sequencing, BLAST homology, and phylogenetic analysis (Trianto *et al*., 2017). Amplification of 18S rRNA used specific primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) (Fredricks *et al*., 2005). Sequencing was carried out using Big Dye Terminator v.3.1. The sequencing materials and reagent used were: 2 µl buffer 10x, 2 µl big dye, 4 µl DNA template, 1 µl primer with a concentration of 3.2 pmol, distilled water (ddH2O) until the final volume was 10 µl (Handoyo and Rudiretna, 2000). Phylogenetic analysis was conducted using the Basic Local Alignment Search Tool (BLAST) program (Altschul *et al.*, 1997) with DNA sequence database from the National Center for Biotechnology Information, National Institute for Health, USA (www.ncbi.nlm.nih.gov). The phylogenetic tree was analyzed using MEGA version 5 software. The morphological characterization of fungal isolates was carried out using a microscope to determine their characteristics based on the fungal taxonomic book and taxonomic guidance (Seifert, 1996).

The multidrug-resistant clinical isolates were *Staphylococcus aureus* and *Escherichia coli* obtained from the Laboratory of Microbiology, dr. Kariadi General Hospital, Semarang.

2,2 Method

2.2.1 Cultivating Fusarium sp. *in varying media*

Culture of *Fusarium* sp. was conducted according to the method by Widyaningsih *et al.* (2018) with a slight modification. *Fusarium* sp. was cultivated in four media. The standard was an MEB Himedia medium in seawater. The SJ modified media consisted of soursop fruit (398.4 g) and honey (20 mL) in 1.5 L of seawater, the GJ media consisted of ginger (122.8 g) and honey (20 mL) in 1 L of seawater, and the sago media consisted of sago flour (20 g) and honey (20 mL) in 800 mL of seawater. The honey used was traditional honey commonly found in markets or pharmacies.

The SJ and GJ media were prepared by boiling the material and filtering the liquid and the plants' pulp to create a clear solution. The sago media did not require filtration. All media were autoclaved before use.

The fungal strain was grown in 5 mL of MEB inside two test tubes. The fungal *mycelium* from two of the test tubes was transferred into an Erlenmeyer container. Five Erlenmeyer containers were used for this scale-up process, with each containing 200 ml. Next, the cultures were left for seven days to allow the mycelium to fully grow. It was then harvested through filtration and the mycelium films were soaked in methanol (MeOH) for 2 × 24 hours. Sonication was used to maximize the harvesting process. Finally, the MeOH extract was evaporated in a vacuum.

2.2.2 Extraction and fractionation

Each medium's mycelial crude extract was partitioned with ethyl acetate (EtOAc):H_2O (1:3/v:v) and both layers were concentrated *in vacuo* to give EtOAc and H_2O extracts (Kasitowati *et al*., 2019). Antibacterial test of *Fusarium* sp. against *S. aureus* and *E.coli* strain MDR was performed by overlay method (Radjasa *et al*., 2007). All extracts were tested against *S. aureus* and *E. coli* at 200 µg/disc. For the positive control, chloramphenicol 100 g/disc was used.

Based on the test results, the H_2O fraction was inactive and thus not analyzed further. The ethyl acetate fraction was further separated with the use of a filtration funnel. A total of 214.2 mg MEB, 205.6 mg SJ, and 284.9 mg GJ fractions were prepared for filtration. The weight of silica Si-60 used was a multiplication of 30 times the weight of the individual extracts, namely 6.426, 6.168, and 8.547 g respectively.

The EtOAc fraction from each medium was subjected to silica gel column chromatography with the following eluent: n-hexane, chloroform ($CHCl_3$), $CHCl_3$: EtOAc (1:1, v:v), EtOAc, and EtOAc: MeOH. All fractions were evaporated until dry and analyzed on a TLC plate (Si 60 F254 plate, Merck) developed with $CHCL_3$: EtOAc (4:1) mixtures (Touchstone and Dobbins, 1983). The fractions were then tested for antibacterial activity against *S. aureus* and *E. coli* at 100 µg/disc.

One of the active fractions, namely the 3rd fraction from MEB, was further analyzed using HPLC. The method is based on Sabdaningsih with modification. The 33.6 g of 3rd fraction MEB was analyzed using RP-18/250-10 (5 µm)/Cica reagent column; the mobile phase was ethyl acetate (EtOAc) and dichloromethane (Ch_2CL_2) (1:2) (Sabdaningsih *et al*., 2020). The results of the HPLC fractions were then tested for antibacterial activity against *S. aureus* and *E. coli* at concentrations of 15 µg/disc.

3. Results and Discussion

3.1 Results

The result showed that mycelium layers produced by ginger juice media were thicker than those

by soursop juice media and MEB media, in that order (Figure 1). This research showed soursop juice media produced a thicker mycelium layer than MEB media, and ginger juice produced an even thinner layer. The weight of the extracts produced from the three media; MEB, soursop juice, and ginger juice were different. SJ produced more extracts than MEB and GJ. This shows that the growth medium used affects the growth of mycelium and also the number of compounds produced (Table 1).

Table 4. The total weight of the HPLC fractions

Code	Weight (mg)	Fraction HPLC	Weight (mg)
MEB-3	33.6	MEB-3-1	1.4
		MEB-3-2	2.4
		MEB-3-3	6
		MEB-3-4	5.7
		MEB-3-5	7.2

Crude extract of *Fusarium* sp. was successfully separated with a separatory funnel into polar (H_2O) and nonpolar (EtOAc) fractions from each medium. The weight of the EtOAc fraction of the three media was less than the H_2O fraction. This shows that most compounds originated from *Fusarium* sp. are soluble in polar solvents (Table 2).

The results of the antibacterial test against *S. aureus* and *E. coli* at a concentration of 200 µg/disc, showed that the ethyl acetate fraction of the three media managed to inhibit the bacteria test unlike the H_2O fraction. The largest inhibition zone against *S. aureus* was found on the MEB (9.28 ± 0.028 mm) and against *E. coli* on GJ (9.43 ± 0.141 mm). Meanwhile, SJ produced inhibition zones of 6.28 ± 0.311 mm against *S. aureus* and 4.27 ± 0.205 mm against *E. coli* (Figure 2).

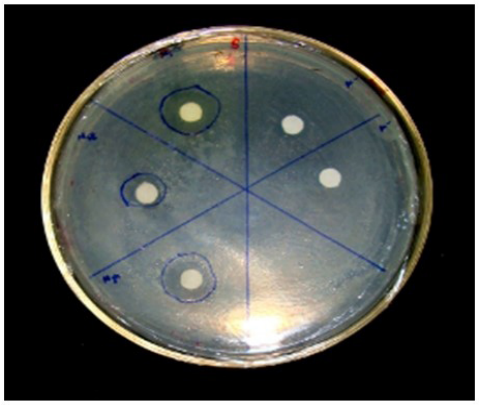

Figure 2. Inhibition zone of *Fusarium* sp. extract against *S. aureus*

3.1.1 Filtration fraction

The results of filtration fraction (FF) *Fusarium* sp. obtained five fractions in MEB, five fractions from SJ media, and four fractions from GJ media. The difference in weight fraction of the three media indicated that there was an influence of nutrients on the number of compounds and bioactivity produced (Table 3).

The spots formed or the Rf value indicates that there are differences in each extract. This shows that the use of modified media (SJ and GJ) produces compounds that are different from the standard media (MEB) even though there are identical compounds as in standard media (MEB) (there are the same Rf values in all extracts media). The colored spots that appeared on the TLC of the MEB, SJ, and GJ media were blue, yellow, and pink respectively (Figure 3).

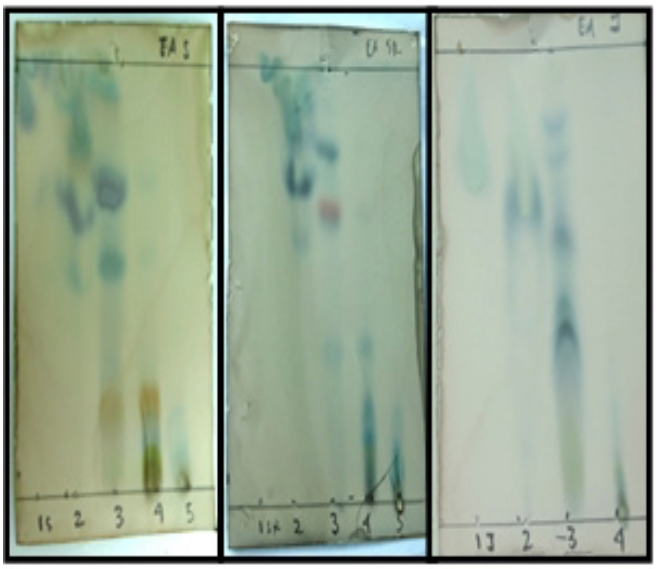

Figure 3. Results of TLC filtration fraction from (a) malt extract broth; (b) soursop juice; and (c) ginger juice

The results of the antibacterial activity test at 100 µg/disc showed that there were five fractions with activity against the bacteria. The largest inhibition zones against *S. aureus* and *E. coli* were 11.56 ± 0.140 mm and 12.16 ± 0.094 mm in size, found in the SJ-5 fraction of the SJ media. In comparison, the positive control used chloramphenicol 100 g/disc and had 8.94 mm (*S. aureus)* and 8.32 mm *(E. coli*) inhibition zones. Meanwhile, the largest inhibition zone of the GJ media was 6.32 ± 0.311 mm, found in the GJ-1 fraction against *E. coli.* The largest inhibition zone of the MEB media was 5.24 ± 0.108 mm, found in the MEB-3 fraction against *S. aureus* (Figure 4).

The results of purification using HPLC of the MEB-3 fraction resulted in five fractions (Figure 5) (Table 4). The HPLC results for the five fractions against

S. aureus and *E. coli* at 15 g/disc, showed two active fractions, namely MEB-3-3 and MEB-3-5. The largest inhibition zones were in the MEB-3-5 fraction with 7.15 ± 0.117 mm and 8.1 ± 0.047 mm against *S. aureus and E. coli* respectively. The MEB-3-3 had inhibition zones of 6.18 ± 0.070 mm and 4.1 ± 0.047 mm against *S. aureus* and *E. coli* respectively.

3.2 Discussion

The mycelium layers on the modified media SJ and GJ was different in appearance and weight. The SJ media produced a mycelium layer that was thicker and more elastic, whereas the GJ layer was thinner and finer than the others. According to Sharma and Pandey (2010),

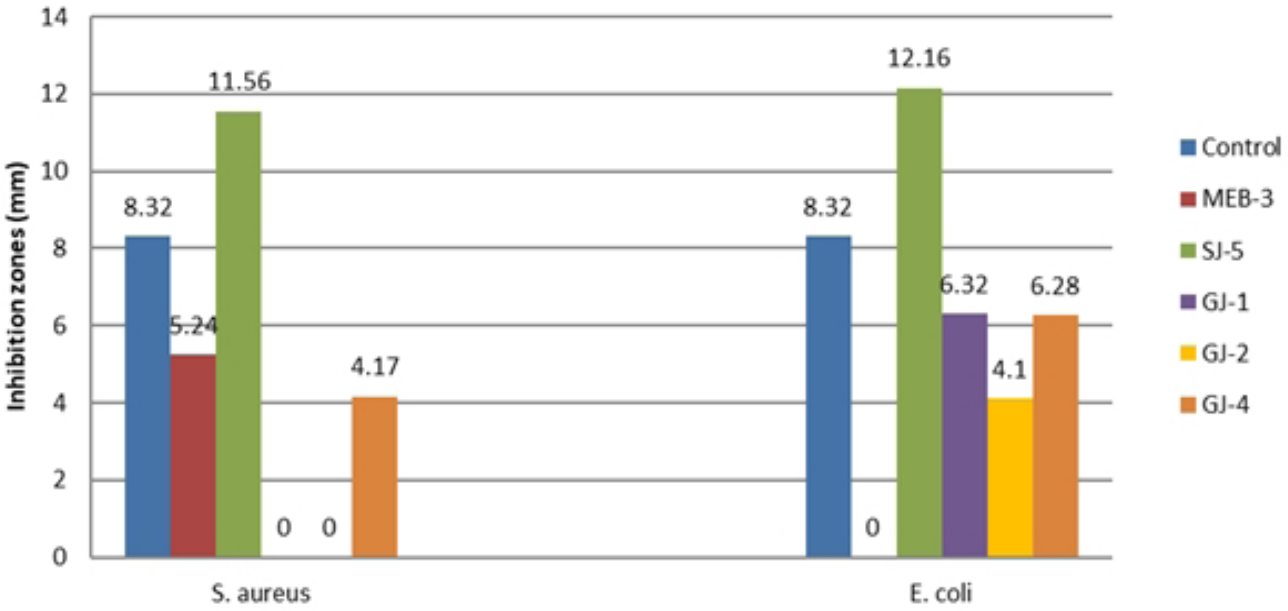

Figure 4. Inhibition zones of the filtration fractions of *Fusarium* sp. against *S. aureus* and *E. coli* (concentration 100 µg/disc)

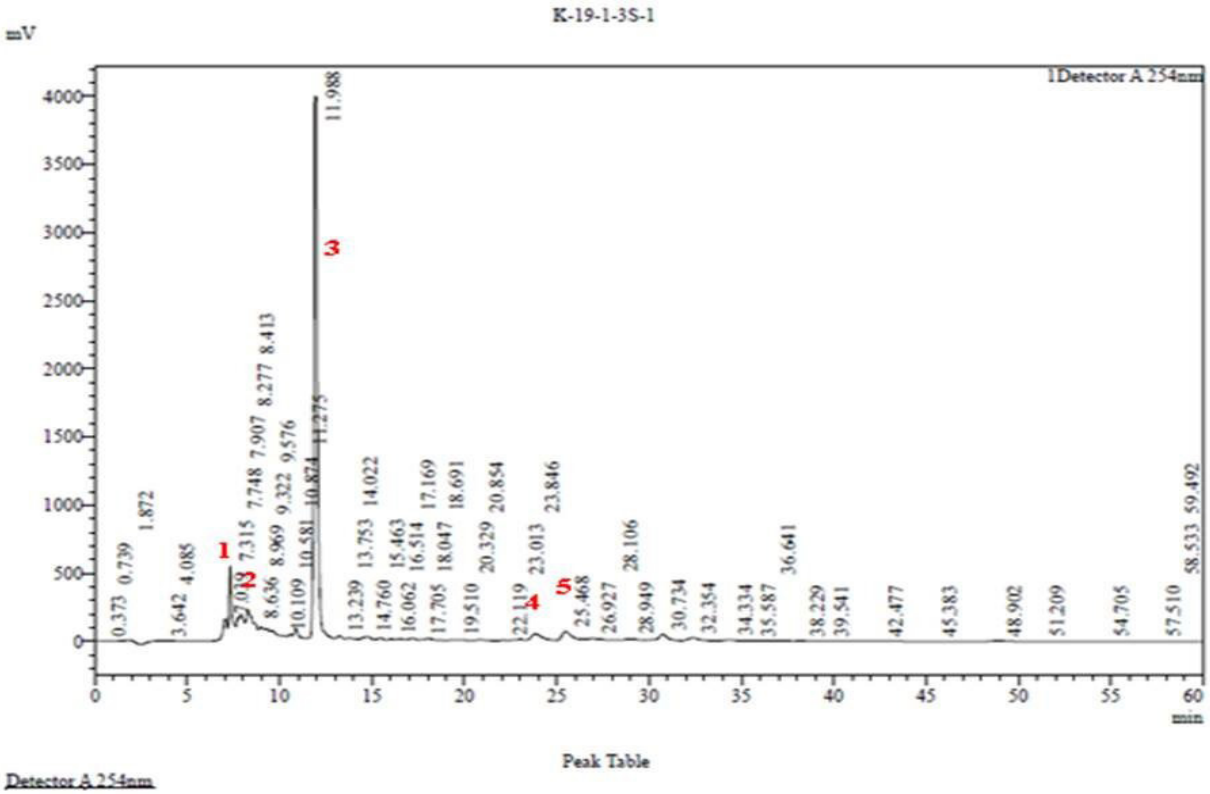

Figure 5. HPLC results of the MEB-3 fraction from *Fusarium* sp.

fungal culture media affect colony diameters and characteristics (texture and color), pigmentation, and sporulation. The composition of the culture media is very decisive as a medium for the growth of microorganisms (Shareef, 2019).

The percentage extracts weighed differently based on the media, namely 2.9059 g (3.85%), 1.0692 g (2.62%), and 1.5682 g (2.98%) for SJ, GJ, and MEB respectively. The *Fusarium* sp. crude extract of the SJ culture had a water fraction of 98.38% and an ethyl fraction of 1.61%. The GJ crude extract had a water fraction of 92.80% and an ethyl fraction of 7.19%.

According to Lay (1994), culture media affect the growth rate of microorganisms. Culture media are the only source of nutrients for energy and growth for microorganisms and must therefore contain sufficient amounts (Pitt and Hocking, 1997). According to Waluyo (2005), the main role of nutrients is to act as an energy source, to build materials for cells, and act as electron acceptors in bioenergetic reactions (reactions that produce energy). A lack of nutrients, therefore, affects the growth of microorganisms. The basic elements (major elements) that must be available are carbon (C), nitrogen (N), hydrogen (H), oxygen (O), sulfur (S), phosphorus (P), iron (Fe), and small amounts of trace elements. Research conducted by Grandes-Blanco *et al.* (2020) showed that using modified vermicompost and fish waste media for *P. eryngii* had a higher radial growth rate than standard media (PDA). This is influenced by the comparison of the C: N ratio, mineral content, and pH in the media.

Soursop is rich in carbohydrates (68%) and 81.9–93.6% of the sugar content is glucose and fructose. Soursop, aside from containing phosphorus (27/1.000 mg) and calcium (14/1.000 mg), is rich in potassium (278/1.000 mg) and poor in sodium (14/1.000 mg) (Indriaty, 2014). The contents of 100 g of ginger are 17.86 g carbohydrates, 3.57 g protein, 14 mg sodium, and 33 mg potassium (Aryanta, 2019). In this study, honey was added to the ginger juice with a ratio of 2:100 ml for use in the ginger juice media. This allowed the ginger media to contain necessary elements other than those found in ginger. Honey contains the minerals calcium (6 mg), magnesium (2 mg), phosphorus (4 mg), sodium (4 mg), and potassium (52 mg). A 100 g of honey contains carbohydrates (82.4 g), sugar (82.12 g), and protein (0.3 g) (Sakri, 2015). With these compositions, the SJ and GJ media meet the nutrients requirements for microorganism growth and, specifically, those of *Fusarium* sp. The *Fusarium* sp. cannot grow on Sago plus Honey media. Sago is rich in carbohydrates but lacking in other nutritional components (Sidiq *et al.*, 2021). This was overcome by adding honey to complement the nutritional components, especially glucose in Sago. Analysis of *Fusarium* sp. showed that it cannot grow on sago media, this needs to be investigated further, such as the pH of the media and the temperature of the culture, because in terms of nutrient, it is sufficient.

According to Manitto (1981), primary metabolism in microorganisms synthesizes macromolecules for growth, namely proteins, carbohydrates, nucleic acids, and fats. Apart from being a carbon source, protein can also be converted by microorganisms and utilized as nitrogen and phosphorus. The remaining carbon sources also come from carbohydrates. The high content of carbohydrates, energy, and water in soursop juice is thought to cause the mycelium to be thicker than that in ginger juice. Sufficient availability of carbon, nitrogen, and polysaccharides supports the formation of a better cell wall (Paturau, 1969). The enrichment of carbon sources in culture media will increase the growth of *Fusarium solani* F7 and the production of enzymes (xylanase enzymes) (Gupta *et al.*, 2009). In addition, the culture period in this study (after which mycelium reached its maximum growth and was ready for extraction) was eight days for the soursop juice media, faster than that of the ginger juice media which reached 14 days. The overhaul of macromolecules (carbohydrates and proteins) through primary metabolic processes for cell formation and growth, usually occurs in the exponential phase (Stanbury *et al.*, 2017).

Antibacterial tests of *Fusarium* sp. cultured on MEB, SJ, and GJ media against *S. aureus* and *E. coli* showed bioactivity. The largest inhibition zone against *S. aureus* was produced by the crude extract of MEB (9.28 mm ± 0.02), while that against *E. coli* was produced by the GJ extract (9.43 mm ± 0.14) at 200 g/disc. The soursop media culture extract resulted in inhibition zones of 6.28 mm ± 0.31 against *S. aureus* and 4.27 mm ± 0.20 against *E. coli* at 200 g/disc. As the TLC results demonstrate, the differences in inhibition zones on each extract indicate the influence each culture media has. The spots formed, or the *Rf* values differed between each extract despite there being some identical spots. This shows that the modified media (SJ and GJ) produced compounds different from the standard media (MEB). The Blue colored spots on the TLC visualization of the filtration fraction indicate steroid compounds (Widyaningsih *et al.*, 2018).

The quantity and diversity of bioactive compound production differ based on the media's nutrient compositions and culture conditions (Bills *et al.*, 2008). The modified cultivation parameters such as medium composition affect chemical diversity and improve the

yields of new microbial bioactive compounds produced by microorganisms (Romano *et al*., 2018; Gao *et al*., 2020). The compounds contained in the standard media culture (MEB) extract can also be produced by soursop juice and ginger juice media extracts as they all had the same Rf values. Bioactive compounds from secondary metabolites isolated on a laboratory scale can be modified by the addition of precursors such as amino acids and carbohydrates (Nofiani, 2008). For example, the optimized media will produce maximum *mevinolin* compounds from *Aspergillus terreus*. The media affects the ratio of carbon and nitrogen (Atalla *et al*., 2008). According to Crueger *et al*. (1989), the production of secondary metabolites is strongly influenced by environmental conditions.

4. Conclusion

Fusarium sp. can be grown on soursop juice (SJ) and ginger juice (GJ) modified media. The ability of the bioactive compound *Fusarium* sp. of the two modified media were different against the bacteria test. The largest inhibition zone was produced by soursop juice (SJ) media against *S. aureus* and *E. coli* at 100 100 µg/disc.

Acknowledgement

The authors thank Mrs. Desy Triningsih from Toyama University for assisting in writing reviews and the help from Mrs. Sekar Widyaningsih in research activities in the laboratory.

Authors' Contributions

The contribution of each author is as follows, KW; played a role in the entire process from the laboratory to the preparation of the manuscript. AT; supervised the manuscript and collected sponge samples in NTT. OKR; supervised the research implementation. All authors discussed the ideas and concepts of research and contributed to the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

Funding Information

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

Aldred, D., Magan, N., & Lane, B. S. (1999). Influence of water activity and nutrients on growth and production of squalestatin S1 by a *Phoma* sp. *Journal of Applied Microbiology*, 87(6):842-848.

Atalla, M. M., Hamed, E. R., El-Shami, A. R. (2008). Optimization of a culture medium for increased mevinolin production by *Aspergillus terreus* strain. *Malaysian Journal of Microbiology*, 4(2):6-10.

Altschul, S. F., Madden, T. L, Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, 25(17):3389-3402.

Aryanta, I. W. R. (2019). Manfaat jahe untuk kesehatan. *Widya Kesehatan*, 1(2):39-43.

Belofsky, G. N., Jensen, P. R., & Fenical, W. (1999). Sansalvamide: A new cytotoxic cyclic depsipeptide produced by a marine fungus of the genus *Fusarium*. *Tetrahedron Letters*, 40(15):2913-2916.

Bills, G. F., Platas, G., Fillola, a., Jiménez, M. R., Collado, J., Vicente, F., Martín, J., González, A., Bur-Zimmermann, J., Tormo, J. R., & Peláez, F. (2008). Enhancement of antibiotic and secondary metabolite detection from filamentous fungi by growth on nutritional arrays. *Journal of Applied Microbiology*, 104(6):1644-1658.

Bugni, T. S., & Ireland, C. M. (2004). Marine-derived fungi: a chemically and biologically diverse group of microorganisms. *Natural Product Reports*, 21(1):143-163.

Cao, Q. X., Wei, J. H., Deng, R., Feng, G. K., Zhu, X. F., Lan, W. J., & Li, H. J. (2017). Two new Pyripyropenes from the Marine Fungus *Fusarium lateritium* 2016F18-1. *Chemistry & Biodiversity*, 14(3):e1600298.

Crueger, W., Crueger, A., Brock, T. D. (1989). Biotechnology: a textbook of industrial microbiology (2nd ed.). Sunderland, USA:

Sinauer Associates.

Fredricks, D. N., Smith, C., & Meier, A. (2005). Comparison of six DNA extraction methods for recovery of fungal DNA as assessed by quantitative PCR. *Journal of Clinical Microbiology*, 43(10):5122-5128.

Gao, Y., Stuhldreier, F., Schmitt, L., Wesselborg, S., Guo, Z., Zou, K., Mándi, A., Kurtán, T., Liu, Z., & Proksch, P. (2020). Induction of New lactam derivatives from the endophytic fungus *Aplosporella javeedii* through an OSMAC approach. *Frontiers in Microbiology*, 11(600983):1-13.

Grandes-Blanco, A. I., Antonio-Flores, A. L., Garcia-Barrientos, R., Tlecuitl-Beristain, S., Diaz-Godinez, G., & Sanchez-Minutti, L. (2020). Agro-food waste employed to design and develop culture media for fungal growth. *Journal of Environmental Biology*, 41(2):195-201.

Gupta, V. K., Gaur, R., Gautam, N., Kumar, P., Yadav, I. J., & Darmwal, N. S. (2009). Optimization of xylanase production from *Fusarium solani* F7. *American Journal of Food Technology*, 4(1):20-29.

Handoyo, D., & Rudiretna, A. (2000). Prinsip umum dan pelaksanaan Polymerase Chain Reaction (PCR) [General Principles and Implementation of Polymerase Chain Reaction]. *Unitas*, 9(1):17-29.

Harmayani, E., Anal, A. K., Wichienchot, S., Bhat, R., Gardjito, M., Santoso, U., Siripongvutikorn, S., Puripaatanavong, J., & Payyappallimana, U. (2019). Healthy food traditions of Asia: Exploratory case studies from Indonesia, Thailand, Malaysia, and Nepal. *Journal of Ethnic Foods*, 6(1):1-18.

Hedner, E. (2007). Bioactive compounds in the chemical defence of marine sponges. Dissertation. Uppsala, Sweden: Uppsala University.

Indriaty, F. (2014). Pengaruh variasi penambahan sari buah sirsak terhadap mutu kembang gula keras (Effect of the addition of soursop extract on the quality of hard candy). *Jurnal Penelitian Teknologi Industri*, 6(2):71-82.

Jansen, N., Ohlendorf, B., Erhard, A., Bruhn, T., Bringmann, G., & Imhoff, J. F. (2013). Helicusin E, Isochromophilone X and Isochromophilone XI: New chloroazaphilones produced by the fungus *Bartalinia robillardoides* strain LF550. *Marine Drugs*, 11(3):800-816.

Karimi, S., Soofiani, N. M., Lundh, T., Mahboubi, A., Kiessling, A., & Taherzadeh, M. J. (2019). Evaluation of filamentous fungal biomass cultivated on vinasse as an alternative nutrient source of fish feed: Protein, lipid, and mineral composition. *Fermentation*, 5(99):1-17.

Kasitowati, R. D., Wittriansyah, K., Trianto, A., Pratiwi, D. C., & Panjaitan, M. A. P. (2019). Antifungal activity of marine sponges (Class Demospongiae) collected from Biak, Indonesia. IOP Conference Series: *Earth and Environmental Science*, 236(012102):1-7.

Lawrence, R. N. (1999). Rediscovering natural product biodiversity. *Drug Discovery Today*, 4(10):449-451.

Lay, B. W. (1994). Analisis mikroba di laboratorium. Jakarta: Raja Grafindo Persada.

Lee, Y. K., Lee, J. H., & Lee, H. K. (2001). Microbial symbiosis in marine sponges. *Journal of Microbiology*, 39(4):254-264.

Lee, Y. M., Li, H., Hong, J., Cho, H. Y., Bae, K. S., Kim, M. A., Kim, D.-K., & Jung, J. H. (2010). Bioactive metabolites from the sponge-derived fungus *Aspergillus versicolor*. *Archives of Pharmacal Research*, 33(2):231-235.

Lee, D. S., Jang, J. H., Ko, W., Kim, K. S., Sohn, J. H., Kang, M. S., Ahn, J. S., Kim, Y. C., & Oh, H. (2013). PTP1B inhibitory and anti-inflammatory effects of secondary metabolites isolated from the marine-derived fungus *Penicillium* sp. JF-55. *Marine Drugs*, 11(4):1409-1426.

Li, Q., & Wang, G. (2009). Diversity of fungal isolates from three Hawaiian marine sponges. *Microbiological Research*, 164(2):233-241.

Lestari, E. S., Severin, J. A., Filius, P. M. G., Kuntaman, K., Duerink, D. O., Hadi, U., Wahjono, H., & Verbrugh, H. A. (2008). Antimicrobial resistance among commensal isolates of *Escherichia coli* and *Staphylococcus aureus* in the Indonesian population inside and outside hospitals. *European Journal of Clinical Microbiology &*

Infectious Diseases, 27(1):45-51.

Manitto, P. (1981). Biosynthesis of natural products. New York: Ellis Horwood Publishing Limited.

Moghadamtousi, S. Z., Nikzad, S., Kadir, H. A., Abubakar, S., & Zandi, K. (2015). Potential antiviral agents from marine fungi: An overview. *Marine Drugs*, 13(7):4520-4538.

Moore, D. (1998). Fungal morphogenesis. Cambridge: Cambridge University Press.

Mutakin, M., Fauziati, R., Fadhilah, F. N., Zuhrotun, A., Amalia, R., & Hadisaputri, Y. E. (2022). Pharmacological activities of soursop (*Annona muricata* Lin.). *Molecules*, 27(4):1201.

Nofiani, R. (2008). Urgensi dan mekanisme biosintesis metabolit sekunder mikroba laut. *Jurnal Natur Indonesia*, 10(2):120-125.

Paturau, J. M. (1969). By-products of the cane sugar industry. An introduction to their industrial utilization. Amsterdam: Elsevier Science Publishers B. V.

Pitt, J., & Hocking, D. (1997). Fungi and food spoilage (2nd ed.). New York: Springer.

Pringgenies, D., Setyati, W. A., Djunaedi, A., Pramesti, R., Rudiyanti, S., & Ariyanto, D. (2021). Exploration of antimicrobial potency of mangrove symbiont against multi-drug resistant bacteria. *Jurnal Ilmiah Perikanan Dan Kelautan*, 13(2):102-112.

Radjasa, O. K., Salasia, S. I. O., Sabdono, A., Weise, J., Imhoff, J. F., Lämmler, C., & Risk, M. J. (2007). Antibacterial activity of marine bacterium *Pseudomonas* sp. associated with soft coral *Sinularia polydactyla* against *Streptococcus equi* subsp. *zooepidemicus*. *International Journal of Pharmacology*, 3(2):170-174.

Rajasekar, T., Balaji, S., Kumaran, S., Deivasigamani, B., & Pugzhavendhan, S. R. (2012). Isolation and characterization of Marine fungal metabolites against clinical pathogens. *Asian Pacific Journal of Tropical Disease*, 2(Supplement 1):S387-S392.

Romano, S., Jackson, S. A., Patry, S., & Dobson, A. D. W. (2018). Extending the "one strain many compounds" (OSMAC) principle to marine microorganisms. *Marine Drugs*, 16(7):1-29.

Sabdaningsih, A., Liu, Y., Mettal, U., Heep, J., Riyanti, Wang, L., Cristianawati, O., Nuryadi, H., Sibero, M. T., Marner, M., Radjasa, O. K., Sabdono, A., Trianto, A., & Schäberle, T. F. (2020). A new citrinin derivative from the Indonesian marine sponge-associated fungus *Penicillium citrinum*. *Marine Drugs*, 18(4):227.

Sakri, F. M. (2015). Madu dan khasiatnya. Yogyakarta: Diandra Pustaka Indonesia.

Seifert K. A. (1996). FusKey: fusarium interactive key. Canada: Agriculture & Agri-Food Canada.

Septiana, E., & Simanjuntak, P. (2017). Pengaruh kondisi kultur yang berbeda terhadap aktivitas antioksidan metabolit sekunder kapang endofit asal akar kunyit. *Majalah Obat Tradisional*, 22(1):31-36.

Shareef, S. A. (2019). Formulation of alternative culture media from natural plant protein sources for cultivation of different bacteria and fungi. *Zanco Journal of Pure and Applied Sciences*, 31(4):61-69.

Sharma, G., & Pandey, R. R. (2010). Influence of culture media on growth, colony character and sporulation of fungi isolated from decaying vegetable wastes. *Journal of Yeast and Fungi Research*, 1(8):157-164.

Sidiq, F. F., Coles, D., Hubbard, C., Clark, B., & Frewer, L. J. (2021). Sago and the indigenous peoples of Papua, Indonesia: A review. *Journal of Agriculture and Applied Biology*, 2(2):138-149.

Stanbury, P. F., Whitaker, A., & Hall, S. J. (2017). Principles of fermentation technology (3rd ed.). Amsterdam: Elsevier Ltd.

Terkina, I. A., Parfenova, V. V., & Ahn, T. S. (2006). Antagonistic activity of actinomycetes of Lake Baikal. *Applied Biochemistry and Microbiology*, 42:173-176.

Trianto, A., Radjasa, O. K., Pribadi, R., Widyaningsih, S., Wittriansyah, K., Yusidharta, I., Wiratno, & Riniatsih, I. (2017). Isolation and identification of sponge-associated fungus producing Anti Multidrug-resistant (MDR) bacterial substances. Research *Journal of Pharmaceutical Biological and Chemical Sciences*, 8(6):63-71.

Touchstone, J. C., & Dobbins, M. F. (1983). Practice of

thin layer chromatography (2nd ed.). New York: J. Wiley & Sons.

Waluyo, L. (2005). Mikrobiologi umum. Malang: Universitas Muhammadiyah Malang Press.

Wittriansyah, K., Trianto, A., Widyaningsih, S., Radjasa, O. K., & Pribadi, R. (2016). Screening of antibacterial MDR derived from sponge associated fungus of Riung Water, Nusa Tenggara Timur. *Ilmu Kelautan*, 21(4):197-202.

Widyaningsih, S., Trianto, A., Radjasa, O. K., & Wittriansyah, K. (2018). Antibacterial activity symbiotic fungi of marine sponge *Axinella* sp., *Aspergillus Sydowii* on four growth medium. IOP Conference Series: *Earth and Environmental Science*, 116(012084):1-8.

Zheng, C. J., Shao, C. L., Wu, L. Y., Chen, M., Wang, K. L., Zhao, D. L., Sun, X. P., Chen, G. Y., & Wang, C. Y. (2013). Bioactive phenylalanine derivatives and cytochalasins from the soft coral-derived fungus, *Aspergillus elegans*. *Marine Drugs*, 11(6):2054-2068.

Zhao, D., Han, X., Wang, D., Liu, M., Gou, J., Peng, Y., Liu, J., Li, Y., Cao, F., & Zhang, C. (2019). Bioactive 3-decalinoyltetramic acids derivatives from a marine-derived strain of the fungus Fusarium equiseti D39. *Frontiers in Microbiology*, 10(1285):1-10.

Zuhairah, N. S. T., Ginting, E. B. R., Romatua D. G., & Fahdi, F. (2019). Identifikasi kadar glukosa dan sukrosa pada madu hutan. *Jurnal Penelitian Farmasi & Herbal*, 1(2):5-10.