Pertanika J. Trop. Agric. Sci. 44 (4): 845 - 864 (2021)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Phytochemical Analysis and Antibacterial Activities of Sidr Leaf Extract (*Ziziphus spina-christi*) against Pathogenic Bacteria in Aquaculture

Sarjito[1]*, Lukita Purnamayati[2], Putut Har Riyadi[2], Desrina[1] and Slamet Budi Prayitno[1]

[1]Aquaculture Study Program, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. H. Soedarto, SH Tembalang, Semarang, Central Java, Indonesia
[2]Fish Product Utilization Department, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. H. Soedarto, SH Tembalang, Semarang 50275, Central Java, Indonesia

ABSTRACT

The success rate of aquaculture is highly influenced by several factors, including optimum water quality, feed management, and microorganism control. Several microorganisms interfere with the quality of media and fish culture, *i.e.*, fish growth. *Aeromonas* and *Vibrio* are the main pathogenic bacteria that disrupt fish growth and cause mortality. Sidr leaf (*Ziziphus spina-christi*) extract contains phytochemicals that have antibacterial properties. This study aimed to identify the phytochemical components and analyze the effect of Sidr leaf extract on the growth of aquaculture-based pathogenic bacteria. Sidr leaf extract was obtained using ethanol and tested via phytochemical analysis, chemical analysis, prediction of activity spectra for substances (PASS) examination, and inhibition capability against *Aeromonas hydrophila*, *Aeromonas caviae*, *Aeromonas sobria*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, *Vibrio vulnificus*, *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Vibrio alginolyticus*. The results showed that Sidr leaf extract contained phytochemicals, namely, flavonoids, alkaloids, saponins, tannins, and steroids. Gas chromatography-mass spectrometry analyses showed that the Sidr leaf extract contained 30 compounds with antiseborrheic effects. PASS analysis demonstrated that 15 compounds (64.51% level) have potential as antibacterial, with

ARTICLE INFO

Article history:
Received: 28 May 2021
Accepted: 7 September 2021
Published: 3 November 2021

DOI: https://doi.org/10.47836/pjtas.44.4.08

E-mail addresses:
sarjito@live.undip.ac.id (Sarjito)
lukita_anandito@yahoo.com (Lukita Purnamayati)
putut.riyadi@live.undip.ac.id (Putut Har Riyadi)
rinadesrina@yahoo.com (Desrina)
sbudiprayitno@gmail.com (Slamet Budi Prayitno)
*Corresponding author

ISSN: 1511-3701
e-ISSN: 2231-8542

a probability activity value of more than 0.300. The inhibition test showed that the Sidr leaf extract exhibited moderate-to-strong inhibition against pathogenic bacterial growth, except for *V. vulnificus*, for which it produced a weak inhibition. The results indicate that Sidr leaf extract can be used as a natural herb to control bacterial pathogens in fish cultivation.

Keywords: *Aeromonas*, antibacterial, aquaculture, Sidr leaf extract, *Vibrio*

INTRODUCTION

Aquaculture plays an essential role in the Indonesian economy, and its production increases annually. Fish production from the marine culture in 2016 reached 9,773,055 tons and increased to 9,808,494 tons in 2017, and it is projected to reach 12 million tons in 2023 (Statistics Indonesia [BPS], 2020). This target projection was launched to meet both domestic and export needs. Tran et al. (2017) stated that there is a continuous increase in international market demand. Therefore, considerable effort should be exerted to achieve production and fulfill food safety and food security.

One of the efforts to increase aquaculture fish production is to avoid antibiotics while improving fish culture and survival rate. Feeds with good nutrition can also provide well-maintained fish growth (Prabu et al., 2017). Microorganisms in fish farming grow naturally and, in several cases, are intentionally added to maintain water quality and fish survival. However, several organisms interfere with fish growth through infection and cause mortality to cultured fish (Bentzon-Tilia et al., 2016). The pathogenic bacteria that frequently disrupt fish culture are generally members of *Aeromonas* and *Vibrio*. In *Aeromonas*, fish infected include *A. sobria*, *A. caviae*, and *A. hydrophila*, whereas those from vibrios comprise *V. vulnificus* and *V. harveyi* (Atujona et al., 2018; Monteiro et al., 2018; Pan et al., 2017). Several antibiotics, including tetracycline, oxolinic acid, and florfenicol, have suppressed pathogenic bacteria. However, these antibiotics leave a residue, and certain fish are resistant to antibiotics. The improper utilization of antibiotics in aquaculture can result in multidrug-resistant bacteria in media culture (Igbinosa et al., 2017). *Aeromonas* isolated from fish farming in Denmark has shown 69% resistance to oxytetracycline, 20% resistance to oxolinic acid, and resistance to florfenicol for several isolates. Antibiotic-resistant bacteria can spread and infect humans (Monteiro et al., 2018). Therefore, minimizing the use of antibiotics is needed to prevent the development of resistant bacteria. The addition of microalgae (*Spirulina platensis* and *Chorella vulgaris*) to aquafeed was reported by Joshua and Zulperi (2020) to improve fish immunity. Other natural antibacterial sources have been used in conventional medicine, such as *Ziziphus* (Al-Mutairi et al., 2016), and may prove useful to preventing disease outbreaks and supporting sustainable aquaculture.

The Sidr plant (*Ziziphus spina-christi*) is a tropical plant that has been used as

herbal medicine to treat fever, dandruff, eye diseases, and inflammation. The studies show that Sidr leaves have antibacterial properties as demonstrated by the ability to inhibit the growth of *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Salmonella* sp. (Al-Mutairi et al., 2016). Ethanol- and methanol-derived Sidr leaf extracts can also inhibit the growth of various Gram-positive and Gram-negative bacteria (Khaleel et al., 2019; Temerk et al., 2017). These effects are due to the phytochemical contents of Sidr leaves, such as alkaloids, flavonoids, and saponins (Asgarpanah & Haghighat, 2012). Sidr leaves also contain phenols with a lethal concentration 50 (LC_{50}) concentration of 21.40 µg/mL. These serve as a source of antioxidants for pharmaceuticals (Khaleel et al., 2019). Based on their phytochemical content, Sidr leaves can be a source of natural antibiotics in aquaculture. Such a natural source would be beneficial because it can reduce antibiotic residues in cultured products and bacteria resistance in the aquaculture environment. This study aimed to determine the phytochemical compounds and the performance of Sidr leaf extract to control various bacterial pathogens in aquaculture *in vitro*.

MATERIALS AND METHODS

Materials

Dry Sidr leaves (*Ziziphus spina-christi*) were obtained from Sukoharjo District, Central Java, Indonesia. Aquatic pathogenic bacteria (*A. hydrophila*, *A. caviae*, *A. sobria*, *P. putida*, *P. aeruginosa*, and *S. agalactiae*) were isolated from tilapia from Magelang, Central Java, Indonesia. Additionally, *V. vulnificus*, *V. harveyi*, *V. parahaemolyticus*, and *V. alginolyticus* were obtained from the Center for Brackish Water Cultivation Fisheries Jepara, Central Java, Indonesia. The bacteria were cultured on trypticase soy agar, tryptone soya broth, nutrient broth, and nutrient agar (Merck, Germany).

Preparation of Sidr Leaf Extract

Sidr leaf extract was prepared according to Al-Mutairi et al. (2016) with several modifications. A total of 200 g dried Sidr leaves were sieved using a 60-mesh sieve. The resulting Sidr leaf powder was then immersed in 96% ethanol at a ratio of 1:10 (vol/vol) and then ultrasonicated for 1 hr. The powder was then macerated for 24 h and filtered using Whatman No. 1 filter paper with a vacuum pump to speed up the process. The aqueous filtered part was evaporated in a rotary evaporator at a temperature of 45°C–60°C for 2 hr until the extract thickened. The extract was stored at ±5°C for phytochemical and antibacterial tests.

Phytochemical Analysis of Sidr Leaf Extract

Phytochemical analysis was conducted to identify the compounds in Sidr leaf extract, including flavonoids, tannins, alkaloids, saponins, and steroids. The phytochemical procedure was performed according to Temerk et al. (2017). The flavonoid test

was conducted by adding 1 mL of 10% sodium hydroxide (NaOH) to a 3 mL extract. A yellow extract color exhibited the presence of flavonoids. The tannin test was conducted using one to two drops of 1% iron (III) chloride ($FeCl_3$) to 1 mL extract. A positive reaction showed a color change to green-black or dark-blue color. The alkaloid test was conducted by adding 1 mL of 1% hydrogen chloride (HCl) to 3 mL extract in a test tube. The mixture was then heated for 20 min, chilled, and filtered. Two drops of Mayer's reagent were then added to 1 mL of extract. A thick residue indicated the presence of alkaloids. The saponin test was conducted by shaking a 2 mL aliquot in a test tube for 2 min. A foam formation indicated a positive reaction. Finally, the steroid test was conducted by adding five drops of concentrated sulfuric acid (H_2SO_4) to 1 mL extract in a test tube. The appearance of a red color indicated the presence of steroids.

Chemical Component Analysis of Sidr Leaf Extract with Gas Chromatography-Mass Spectrometry (GC-MS)

Phytochemical analysis with GC-MS was conducted according to Ads et al. (2017). A total of 1 µL sample was injected into the GC-MS equipment (GC17A MSQP 5000 Shimadzu, Japan) using a TG-SQC column 15 MX (0.25 mm × 0.25 µm) at an injector temperature of 250°C. The oven temperature was set to 50°C and increased to 150°C with an average increase of 7°C/min. Then, heating was continued until 250°C with an average rate of 5°C/min and to 290°C with an average rate of 10°C/min. The extract was injected in split mode. The results were then matched with the mass spectra peaks in the Wiley library (Stein et al., 2011).

Prediction of Activity Spectra for Substances (PASS)

PASS analysis was conducted according to Parasuraman (2011) using the PASS web tool. The PASS web tool interprets active biological spectra with a two-dimensional structure and predicts probability to be active and probability to be inactive (Pa: Pi) ratio. The research was conducted in two phases: (1) accessing the Pub Chem server (https://pubchem.ncbi.nlm.nih.gov/) to obtain the canonical simplified molecular-input line-entry system (SMILES) information and (2) predicting the biological activity via PASS analysis using the website, http://www.way2drug.com/PASSOnline/index.php, by entering the canonical SMILES structure (Riyadi et al., 2020).

Inhibition Test

The inhibition test was conducted according to Motamedi et al. (2014) using the disk method. As much as 0.1 mL cultured bacteria with 10^8 cfu/mL density were obtained and spread onto an agar plate. Sterilized 6-mm-diameter paper disks were immersed in various Sidr leaf extracts prepared using distilled water (100, 300, 500, and 800 mg/L) for 1 hr. Additionally, tetracycline (10 mg/L) was used as the positive control, and distilled water was

used as the negative control. The paper disks were then placed onto inoculated pathogenic bacterial agar and incubated for 24–48 hr. The clear zone produced around the paper disk was measured as the inhibition zone. The test was conducted in triplicate at each extract concentration.

RESULTS AND DISCUSSION

Phytochemistry of Sidr Leaf Extract and Their Properties

The phytochemical screening tests (Figure 1) showed that the Sidr leaf extract contained secondary metabolite compounds, such as flavonoids, alkaloids, saponins, tannins, and steroids.

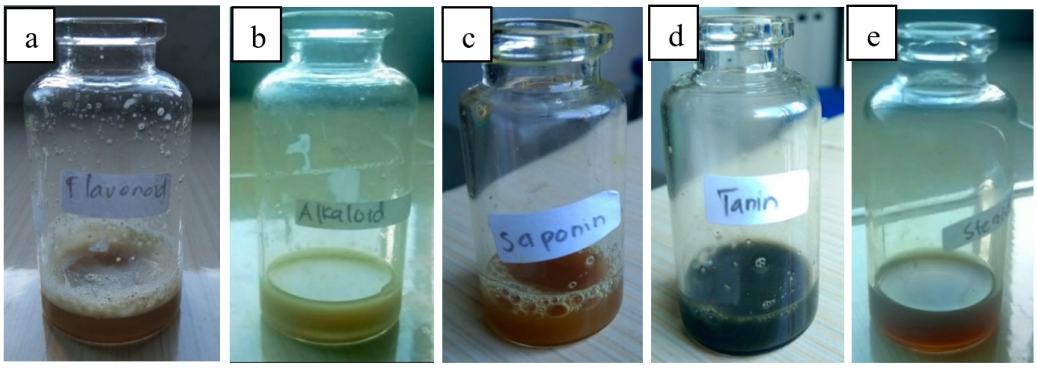

Figure 1. Phytochemicals contained in Sidr (*Ziziphus spina-christi*) leaf extract

Note. a) flavonoid, b) alkaloid, c) saponin, d) tannin, and e) steroid

Table 1

Phytochemicals identified in Sidr (Ziziphus spina-christi) *leaf extract*

No.	Compound	Color	Result analysis
1.	Flavonoid	Yellow	+ (Positive)
2.	Alkaloid	Thick sediment	+ (Positive)
3.	Saponin	Bubble	+ (Positive)
4.	Tannin	Blueish green color	+ (Positive)
5.	Steroid	Red color	+ (Positive)
6.	Triterpenoid	Brownish red	+ (Positive)

Flavonoids, phenolic compounds, triterpenic acids, and polysaccharides have been reported as the major phytoconstituents of *Zizyphus* species (Soni & Malik, 2021). However, various studies have reported different phytochemical contents in *Z. spina-christi* leaf extracts. According to Asgarpanah and Haghighat (2012), Sidr leaf phytochemical content consists of saponins, alkaloids, and flavonoids.

Different results are shown by Ibrahim et al. (2015), who found alkaloids to be the significant phytochemical of Sidr leaf from Nigeria. Ermias et al. (2011) and Alhassan et al. (2019) found that steroid, flavonoid, tannin, lipid, anthraquinone, saponin, and alkaloid are present in *Z. spina-christi* leaf extracts. By contrast, Suliman and Mohammed (2018) found that the phytochemical components of Sidr leaf from Sudan do not contain steroids. Furthermore, Taghipour et al. (2020) stated that the ethanol extract of Iranian Sidr leaf contains alkaloids, tannins, saponins, and flavonoids. This variation in phytochemical content is likely due to differences in climate and environmental conditions where the plant grows. Environmental and climatic conditions, especially temperature, soil type, and age of the plant, affect herbs' chemical content and functional properties (Gull et al., 2012; Inbathamizh & Padmini, 2013). Mbunde et al. (2018) also showed that the areas where herbs grow, such as the coast, highlands, and mountains with different soil types, cause differences in phytochemical content such as phenolics and flavonoids.

The phytochemical components of Sidr leaf extract (Table 1) can have antibacterial properties. For example, flavonoids have antibacterial, antioxidant, and anti-inflammatory properties (Adamczak et al., 2020). Alkaloids possess antibacterial and antifungal properties. Saponins function as barriers against pathogenic bacteria, improve immunity, and have antibacterial, antioxidant, anticancer, and antidiabetic activities. Tannin is a compound that binds proteins and forms water-insoluble compounds. Tannins, as an antibacterial, bind proteins in the bacterial cell wall and coagulate the materials to inhibit bacterial growth (Dangoggo et al., 2012; Ravi et al., 2016). Steroids, consisting of cholesterol and ergosterol, are components of cell membranes. Fusidic acid is a steroid compound that can prevent Gram-positive and Gram-negative bacterial infections (Doğan et al., 2017).

Sidr Leaf Compound Components and PASS Results

Figure 2 and Table 2 describe the GC-MS analysis results on Sidr leaf compounds.

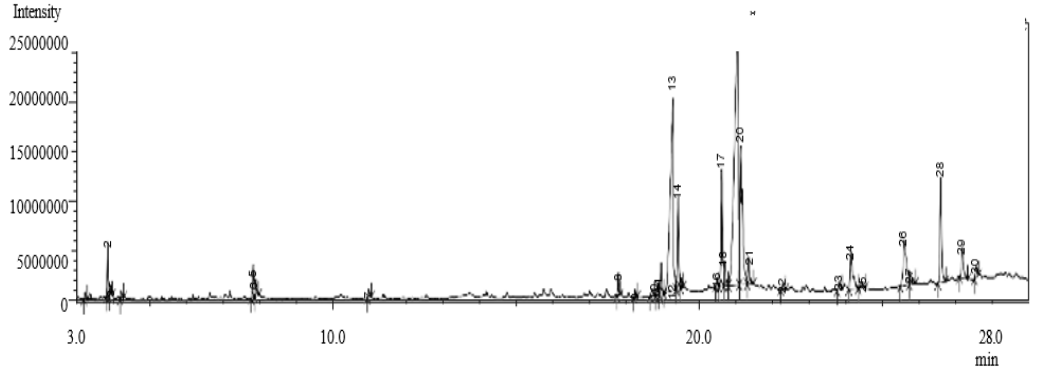

Figure 2. GC-MS chromatogram of the Sidr (*Ziziphus spina-christi*) leaf extract

Table 2

*Phytochemical compounds identified in Sidr (*Ziziphus spina-*christi) leaf extract obtained using chemical component analysis of Sidr leaves via GC-MS*

Peak#	Retention time	Area	Area (%)	Name
1	3.245	1426541	0.23	1-butanol, 3-methyl-
2	3.860	11016330	1.79	Pyrrolidine-.alpha.,.alpha.,.alpha.',.alpha.'-d4
3	3.940	3423281	0.56	(O-D) ethanol ethen-2-d-ol(E) CAS
4	4.226	2301742	0.37	N-methoxy formamide
5	7.826	9238879	1.50	1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane-6,7-endo,endo-diol
6	7.895	5171975	0.84	1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6,7-endo,endo,diol
7	10.979	2836928	0.46	Methanamine, N-[3-methyl-2-butenylidene]
8	17.792	4892173	0.79	Neophytadiene
9	18.250	1847442	0.30	3,7,11,15-tetramethyl-2-hexadecen-1-ol
10	18.716	2585580	0.42	Hexadecanoic acid, methyl ester
11	18.869	2749748	0.45	n-hexadecanoic acid
12	18.954	8944771	1.45	n-hexadecanoic acid
13	19.276	112224191	18.23	n-hexadecanoic acid
14	19.422	24469703	3.98	Hexadecanoic acid, ethyl ester (CAS)
15	19.510	2191255	0.36	Farnesyl acetate 2
16	20.474	1807268	0.29	11-octadecenoic acid, methyl ester
17	20.611	31375266	5.10	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-(CAS)
18	20.690	7944588	1.29	14-pentadecenoic acid
19	21.033	194401927	31.58	cis-9-hexadecenal
20	21.119	69774864	11.34	Ethyl oleate

Table 2 *(Continued)*

Peak#	Retention time	Area	Area (%)	Name
21	21.345	8269672	1.34	Heptadecanoic acid, ethyl ester (CAS)
22	22.287	2009621	0.33	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (CAS)
23	23.822	2855504	0.46	Di-(9-octadecenoyl)-glycerol
24	24.133	18521526	3.01	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (CAS)
25	24.440	1983710	0.32	1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester
26	25.586	28747798	4.67	9-tetradecenal, (Z)-
27	25.760	5323334	0.86	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (CAS)
28	26.583	31995999	5.20	Squalene
29	27.172	11436638	1.86	Behenic alcohol
30	27.553	3734209	0.61	Solanesol
		615502463	100.00	

Based on the GC-MS analysis, the Sidr leaf extract contained 30 chemical compounds (Table 2). These 30 chemical compounds comprised the following: 1-butanol, 3-methyl; optical density (OD) ethanol; pyrrolidine; n-methoxy formamide; two kinds of 1,3,3-trimethyl-2-oxabicyclo [2.2.2] octane-6,7-endo, endo-diol; methenamine, N-[3-methyl-2-butenylidene]; neophytadiene; 3,7,11,15-tetramethyl-2-hexadecen-1-ol; hexadecanoic acid, methyl ester; three kinds of n-hexadecanoic acid; farnesyl acetate 0.4780.4370.807; 11-octadecenoic acid, methyl ester; 2-hexadecen 3,7,11,15-tetramethyl-[R-[R*,R*-(E)]]-0.5490.4170.736; 14-pentadecenoic acid; cis-9-hexadecenal; ethyl oleate; heptadecanoic acid, ethyl ester; hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethaned iyl ester; di-(9-octadecenoyl)-glycerol; hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester; 1,2-benzene dicarboxylic acid, mono (2-ethylhexyl) ester; 9-tetradecenal, (Z)-; octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester; squalene; behenic alcohol; and solanesol. Based on chemical analysis, two major components were contained in large quantities, *i.e*., n-hexadecanoic acid (31.58%) and cis-9 hexadecenal (18.23%). By contrast, Moustafa et al. (2016) found that the main chemical compounds of *Z. spina-christi* leaf extract are n-hexadecanoic acid, tetradecanoic acid, and cis-vaccenic acid, and Abu-Raghif et al. (2017) identified considerable amounts of n-hexadecanoic acid and the flavonoid 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-. These different results are likely due to differences in the growing environment (Inbathamizh & Padmini, 2013). However, these studies have something in common. They contain n-hexadecanoic acid in the chemical composition of Sidr leaf, and n-hexadecanoic acid has antioxidant, anticancer, and antiinflammatory properties (Aparna et al., 2012; Mazumder et al., 2020).

The chemical compounds in the Sidr leaf extract can act as antibacterial agents. The mechanism for inhibiting microbial growth involves the destruction of the bacterial cytoplasm cell membrane's integrity and triggering intracellular leakage (Bouyahya et al., 2019). This phenomenon is achieved by accumulating hydrophobic groups on phospholipids, causing cell death (Huang et al., 2019; Kinnunen et al., 2012). Phenolic components can inhibit cell DNA and RNA synthesis (Ulanowska et al., 2006). Additionally, the terpenoid group can damage cell membrane efficacy and interfere with microbial growth (Wu et al., 2016). The most significant antibacterial compound detected in the Sidr leaf extract was cis-9 hexadecenal (31.58%). Pyrrolidine, neophytadiene, farnesyl acetate, and squalene were also detected. Cis-9 hexadecenal is a family of agrochemicals used to prevent and eradicate pests (Mensah-Agyei et al., 2020). Pyrrolidine is an alkaloid class compound known to function as an antifungal and antibacterial agent (Thawabteh et al., 2019). Neophytadiene and farnesyl acetate are members of the terpenoid group. Neophytadiene is an

antimicrobial, antioxidant, antipyretic, and anti-inflammatory agent (Swamy et al., 2017). Neophytadiene, together with sitosterol components, can inhibit the growth of *Vibrio parahaemolyticus* (Raman et al., 2012).

Meanwhile, farnesyl acetate is a volatile component that can inhibit the growth of Gram-positive bacteria, namely, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Micrococcus luteus* (Boussaada et al., 2008). Squalene, a steroid class member, detected in the Sidr leaf extract, is also an antioxidant and antibacterial agent that can inhibit *Pseuduomonas aeruginosa* (Awang-Jamil et al., 2021).

The fatty acids detected in the Sidr leaf extract were saturated fatty acids (25.39%) consisting of hexadecenoic acid and octadecanoic acid, whereas the unsaturated fatty acids (11.63%) included ethyl oleic and 11-octadecanoic acids. Fatty acids can also have antimicrobial activity. Fatty acids with an atomic C length less than or equal to 6 can inhibit the growth of Gram-negative bacteria (Yoon et al., 2018). Fatty acids with C atoms between 10 and 12 can inhibit yeast growth, whereas those with a C atomic length of more than 12 can inhibit the growth of Gram-positive bacteria (Potocki et al., 2021; Yoon et al., 2018). Additionally, oleic fatty acid acts as a bactericidal against the development of mycobacteria and *S. aureus* (Orhan et al., 2011). Stearic acid and palmitic acid also have an inhibitory effect but are not as significant as oleic acid (Ababutain et al., 2019).

PASS analysis was applied to understand further the potential antimicrobial role of the compounds mentioned above in combating bacterial pathogens in aquaculture (Table 3).

Table 3

PASS analysis of compounds identified in Sidr leaf (Ziziphus spina-christi) *extract*

No.	Compound	Amount (%)	Antibacterial	
			Pa	Pi
1	1-butanol, 3-methyl-	0.23	0.298	0.061
2	Pyrrolidine	1.79	0.379	0.003
3	(OD) ethanol	0.56	ND	ND
4	N-methoxy formamide	0.37	0.424	0.025
5	1,3,3-trimethyl-2-oxabicyclo [2.2.2] octane-6,7-endo, endo-diol	1.50	0.470	0.019
6	1,3,3-trimethyl-2-oxabicyclo [2.2.2] octane-6,7-endo, endo-diol	0.84	0.470	0.019
7	Methanamine, N- [3-methyl-2-butenylidene]	0.46	0.372	0.037
8	Neophytadiene	0.79	0.363	0.040

Table 3 *(Continued)*

No.	Compound	Amount (%)	Antibacterial	
			Pa	Pi
9	3,7,11,15-tetramethyl-2-hexadecen- 1-ol	0.30	0.417	0.026
10	Hexadecanoic acid, methyl ester	0.42	0.263	0.076
11	n-hexadecanoic acid	0.45	0.300	0.060
12	n-hexadecanoic acid	1.45	0.300	0.060
13	n-hexadecanoic acid	18.23	0.300	0.060
14	Acid	3.98	0.186	0.022
15	Farnesyl acetate0.4780.4370.807	0.36	0.437	0.023
16	11-octadecenoic acid, methyl ester	0.29	0.298	0.061
17	2-hexadecen 3,7,11, 15-tetramethyl- [R- [R *, R * - (E)]] - 0.5490.4170.736	5.10	0.417	0.026
18	14-pentadecenoicacid	1.29	0.356	0.042
19	cis-9-hexadecenal	31.58	0.400	0.030
20	Ethyl oleate	11.34	0.270	0.073
21	Heptadecanoic acid, ethyl ester	1.34	0.186	0.022
22	Hexadecanoic acid, 1- (hydroxymethyl) -1.2-ethaned iyl ester	0.33	0.277	0.069
23	Di- (9-octadecenoyl)–glycerol	0.46	0.340	0.046
24	Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl) ethyl ester	3.01	0.295	0.062
25	1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester	0.32	0.309	0.057
26	9-tetradecenal, (Z) -	4.67	0.400	0.030
27	Octadecanoic acid, 2- hydroxy-1- (hydroxymethyl) ethyl ester	0.86	0.295	0.062
28	Squalene	5.20	0.295	0.062
29	Behenic alcohol	1.86	0.225	0.008
30	Solanesol	0.61	0.424	0.025

Note. Pa = Probability to be active ; Pi = Probability to be inactive

Fifteen compounds (64.51% levels) with a probability to be active (Pa) value of more than 0.300 were found, indicating that they possess antibacterial activity. The bioactive compounds detected were as follows: 1,3,3-trimethyl-2-oxabicyclo [2.2.2] octane-6,7-endo, endo-diol (Pa: 0.470); farnesyl acetate 0.4780.4370.807 (Pa: 0.437); N-methoxy formamide (Pa: 0.424); and solanesol (Pa: 0.424). Interestingly, cis-9-hexadecenal (palmitic acid), a main chemical compound in Sidr leaf, also has antimicrobial activity (Pa: 0.400). Previously, Kumar and Rajakumar (2016) found cis-9-hexadecenal to have anti-inflammatory, nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic 5-alpha reductase inhibitor, antioxidant, and hypocholesterolemic properties.

Pseudo-peptide pyrrolidinedione natural products, namely moiramide B and andrimid, represent a new class of antibiotics that target bacterial fatty acid biosynthesis (Pohlmann et al., 2005). N-methyl formamide isolated from the red algae *Portieria hornemannii* (Lyngbye) P.C.Silva is effective against two plant pathogenic bacteria (Sivakumar et al., 2017). Additionally, Chen et al. (2007) showed that solanesol has significant inhibitory effects against *E. coli*, *Mycobacterium phlei*, *P. aeruginosa*, and *S. aureus* but poor inhibitory effects against *Bacillus circulans* and *Bacillus subtilis*. The PASS forecasting tool was used to estimate Pa:Pi (active, inactive ratio) at the forecast thresholds of Pa > 30%, Pa > 50%, and Pa > 70% according to Parasuraman (2011). The forecast's average accuracy was approximately 95% based on the leave-out cross-validation calculation. The PASS forecast accuracy depends on detailed knowledge of the spectrum of biological activity for each compound available in the PASS training package. Thus, the estimation of biological activity is precise (Filimonov et al., 2014).

Inhibition Test of Sidr Leaf Extract against Several Aquaculture Pathogenic Bacteria

In vitro testing showed that the Sidr leaf extract can be used to control several aquaculture-based pathogenic bacteria. Table 4 and Figure 3 show that the Sidr leaf extract at a concentration of 500 ppm was almost as effective as tetracycline, especially for *A. hydrophila*, *A. sobria*, *S. agalactiae*, and *V. parahaemolyticus*. By contrast, the extract was less effective at controlling *A. caviae*, *P. aeruginosa*, and *V. vulnificus*.

Table 4

*Average inhibition zone size caused by Sidr leaf (*Ziziphus spina-christi*) extract when exposed to pathogenic bacteria found in aquaculture settings*

Pathogenic bacteria	Average inhibition zone (mm)				
	Tetracycline (mg/L)	100 (mg/L)	300 (mg/L)	500 (mg/L)	800 (mg/L)
Aeromonas hydrophila	15.20	2.87	9.50	13.17	6.87

Table 4 *(Continued)*

Pathogenic bacteria	Average inhibition zone (mm)				
	Tetracycline (mg/L)	100 (mg/L)	300 (mg/L)	500 (mg/L)	800 (mg/L)
Aeromonas caviae	8.67	6.57	6.20	4.50	6.20
Aeromonas sobria	5.40	13.90	10.43	12.67	4.53
Pseudomonas putida	15.17	4.83	5.87	9.23	9.33
Pseudomonas aeruginosa	12.10	4.83	5.87	5.80	6.07
Streptococcus agalactiae	8.73	11.00	10.97	8.47	7.90
Vibrio vulnificus	12.70	2.87	3.80	2.50	0.00
Vibrio harveyi	18.67	12.20	9.23	0.00	3.43
Vibrio parahaemolyticus	13.00	3.40	7.23	12.37	6.17
Vibrio alginolyticus	12.03	10.67	6.93	3.83	00.00

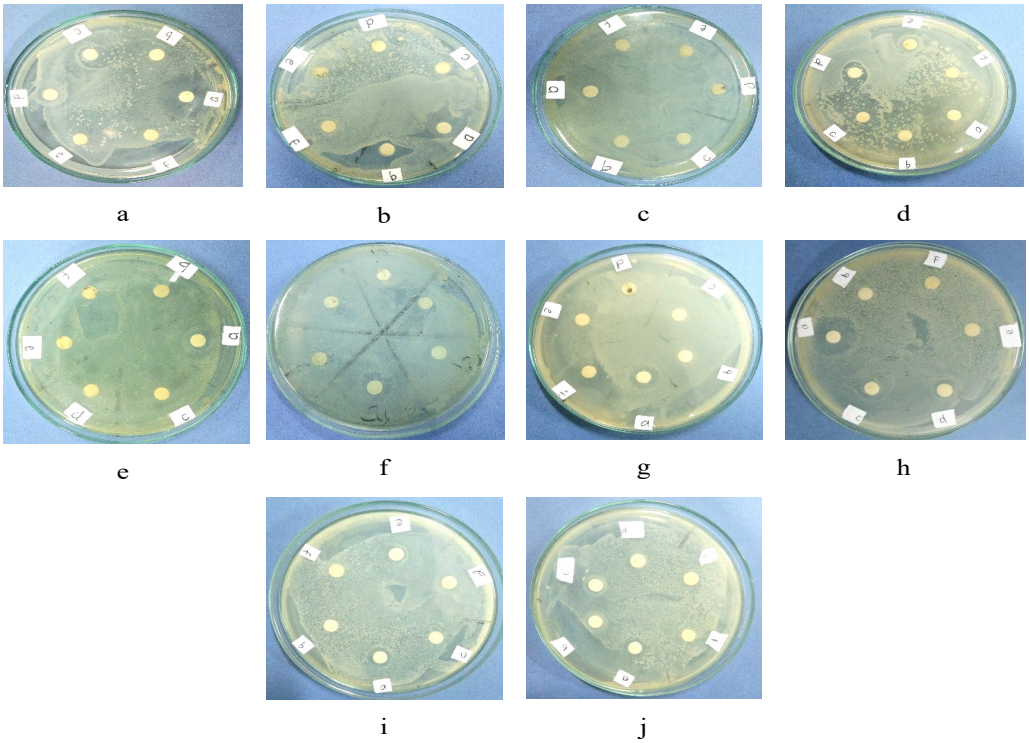

Figure 3. Visual examples of the inhibition zone caused by the Sidr leaf (*Ziziphus spina-christi*) extract when exposed to pathogenic bacteria from aquaculture settings

Note. a) *Aeromonas hydrophila*, b) *Aeromonas caviae*, c) *Aeromonas sobria*, d) *Aeromonas putida*, e) *Pseudomonas aeruginosa*, f) *Streptococcus agalactiae*, g) *Vibrio vulnificus*, h) *Vibrio harveyi*, i) *Vibrio parahaemolyticus*, and j) *Vibrio alginolyticus*

Based on the *in vitro* results, the Sidr leaf extract can inhibit the growth of 10 pathogenic bacteria, with the degree of inhibition ranging from moderate to strong, except for *V. vulnificus* (Table 4 and Figure 3). The 10 bacteria tested were pathogenic to farmed fish and shrimp. *Aeromonas* are pathogenic bacteria that cause diseases in freshwater fish, such as common carp (Baba et al., 2016), catfish (Sarjito et al., 2018a), tilapias, eel, and goldfish (Algammal et al., 2020). Meanwhile, members of *Vibrio* cause a common disease among groupers, such as marine fish (Sarjito et al., 2009), mud crabs (Sarjito et al., 2018b), shrimp (Novriadi, 2016; Sarjito et al., 2018c), and cultured shellfish (Novriadi, 2016). In addition, these pathogenic bacteria can be transmitted to humans through fresh seafood (Praja & Safnurbaiti, 2018).

For inhibition tests, the potency of inhibition is indicated by the zone diameter (strong: 10–20 mm, moderate: 5–10 mm, and weak: less than 5 mm) (Pargaputri et al., 2016). The zone diameter observed from the Sidr leaf extract strongly showed inhibition of *V. parahaemolyticus* and *Aeromonas* bacteria at a dose of 500 mg/L, except for *A. caviae*. Similarly, the Sidr leaf extract strongly inhibited vibrios (*V. harveyi* and *V. alginolyticus*), *A. sobria*, and *S. agalactiae* at 100 mg/L. The Sidr leaf extract produced weak and moderate inhibitions for *V. vulnificus* and *P. aeruginosa*, respectively. Therefore, the inhibition test results indicated that the Sidr leaf extract could be used as a natural antibiotic in fish cultivation.

The antibacterial properties of the Sidr leaf extract are consistent with a previous research report that methanolic extracts from Apocynaceae and Lamiaceae moderately inhibit *A. hydrophila* with an inhibition zone of 9.67 mm (Haniffa & Kavitha, 2012). Additionally, Sidr leaf extract is antibacterial agent that is both bacteriostatic, *i.e.*, able to inhibit the growth of bacteria, and bactericidal, *i.e.*, able to kill bacteria (Abdel-Fatah et al., 2016; Mulyani et al., 2021). For example, Motamedi et al. (2014) showed that Sidr leaf extract is effective against *S. aureus* with the same value of minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of 8 mg/mL. Al-Mutairi et al. (2016) reported that Sidr leaf extract could inhibit the growth of *P. aeruginosa* with a MIC of 100 mg/L. Mulyani et al. (2021) demonstrated that MIC and MBC of Sidr leaf extract impede *E. coli* with a zone diameter of 11.1 mm were at 50 % and 75 %, respectively. Moreover, Brito et al. (2015) demonstrated that Sidr leaf extract could inhibit several antibiotic-resistant bacterial strains and acts against rhabdomyosarcoma cells (Abu-Raghif et al., 2017). Table 2 shows that the lowest concentration of Sidr leaf extract used in the present study (100 mg/L) could inhibit tested aquatic pathogenic bacteria. Based on these data, it seems likely that Sidr extract is potentially a natural antibiotic in aquaculture. The results in this study are in line with those of Alhassan et al. (2019), Al-Mutairi et al. (2016), Khaleel et al. (2019), and Temerk et al. (2017). The results indicate

that the Sidr leaf extract has the potential as an antibacterial agent. Thus, Sidr leaf extract should be tested as an alternative antibacterial agent to inhibit pathogenic bacterial growth in aquaculture.

CONCLUSION

The Sidr leaf extract contains phytochemicals, namely, flavonoids, alkaloids, saponins, tannins, and steroids, with antibacterial properties. GC-MS analysis showed that the Sidr leaf extract has 30 compounds that function as an antiseborrheic. These compounds include the following: 1-butanol, 3-methyl; (OD) ethanol; pyrrolidine; n-methoxy formamide; two molecules of 1,3,3-trimethyl-2-oxabicyclo [2.2.2] octane-6,7-endo, endo-diol; methenamine, n-[3-methyl-2-butenylidene]; neophytadiene; 3,7,11,15-tetramethyl-2-hexadecen-1-ol; hexadecanoic acid, methyl ester; three kinds of n-hexadecanoic acid; acid; farnesyl acetate 0.4780.4370.807; 11-octadecenoic acid, methyl ester; 2-hexadecen-3,7,11, 15-tetramethyl-[R-[R*, R*-(E)]]-0.5490.4170.736; 14-pentadecenoic acid; cis-9-hexadecenal; cis-9-hexadecenal; ethyl oleate; heptadecanoic acid, ethyl ester; hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester; di-(9-octadecenoyl)-glycerol; hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester; 1,2 benzene dicarboxylic acid, mono (2-ethylhexyl) ester; 9-tetradecenal, (Z); octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester; squalene; behenic alcohol; and solanesol. PASS analysis demonstrated 15 compounds (64.51% level) having antibacterial activity and a Pa value of more than 0.300. The inhibition test demonstrated that the Sidr leaf extract exhibits moderate-to-strong inhibition to aquaculture pathogenic bacteria, except for *V. vulnificus*, which produced a weak inhibition. These results indicate that the Sidr leaf extract can be used as a natural herb to control bacterial pathogens in fish cultivation.

ACKNOWLEDGMENTS

The authors thank the Dean of Faculty of Fisheries and Marine Science of Diponegoro University for financial support (91/UN7.5.10.2/PP/2021). The authors also thank Dr. Lestari Lakshmi Widowati, the Head of the Aquaculture Laboratory at the Faculty of Fisheries and Marine Science, Diponegoro University, for facilities support.

REFERENCES

Ababutain, I. M. (2019). Antimicrobial activity and gas chromatography-mass spectrometry (GC-MS) analysis of Saudi Arabian *Ocimum basilicum* leaves extracts. *Journal of Pure and Applied Microbiology*, *13*(2), 823–833. https://doi.org/10.22207/JPAM.13.2.17

Abdel-Fatah, M. A., Hussein, N. H., Hawash, S. I., & Shaarawy, H. H. (2016). Investigation of using Sidr leave extracts in nano-silver preparation. *ARPN Journal of Engineering and Applied Sciences*, *11*(19), 11649–11654.

Abu-raghif, A. R., Jasim, G. A., & Hanoon, M. M. (2017). Anti-proliferative activity of *Zizyphus spina-christi* leaves methanol extract against rhabdomyosarcoma (RD) cell line. *International Journal of Pharmacy and Pharmaceutical Sciences*, *9*(2), 279–282. https://doi.org/10.22159/ijpps.2017v9i2.15013

Adamczak, A., Ożarowski, M., & Karpiński, T. M. (2020). Antibacterial activity of some flavonoids and organic acids widely distributed in plants. *Journal of Clinical Medicine*, *9*(1), 109. https://doi.org/10.3390/jcm9010109

Ads, E. N., Rajendrasozhan, S., Hassan, S. I., Sharawy, S. M. S., & Humaidi, J. R. (2017). Phytochemical, antimicrobial and cytotoxic evaluation of *Ziziphus spina-christi* (L.) stem bark. *Biomedical Research*, *28*(15), 6646–6653.

Algammal, A. M., Mohamed, M. F., Tawfiek, B. A., Hozzein, W. N., Kazzaz, W. M. E., & Mabrok, M. (2020). Molecular typing, antibiogram and PCR-RFLP based detection of *Aeromonas hydrophila* complex isolated from *Oreochromis niloticus*. *Pathogens*, *9*(3), 238. https://doi.org/10.3390/pathogens9030238

Alhassan, K. A., Indabawa, A. S., & Shah, M. M. (2019). Phytochemical analysis, proximate composition and antibacterial activities of *Ziziphus* species (*Z. jujube* and *Z. spina christi*). *Journal of Applied and Advanced Research*, *4*(1), 42–46. https://doi.org/10.21839/jaar.2019.v4i1.262

Al-Mutairi, M. H., Ali, S., Aly, S. M., & Aldebasi, Y. (2016). Antibacterial activity of Sidr (*Ziziphus spina-christi*), leaves extract against selected pathogenic bacteria. *European Journal of Pharmaceutical and Medical Research*, *3*(5), 138–144.

Aparna, V., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C., & Haridas, M. (2012). Anti-inflammatory property of n-hexadecanoic acid: Structural evidence and kinetic assessment. *Chemical Biology and Drug Design*, *80*(3), 434–439. https://doi.org/10.1111/j.1747-0285.2012.01418.x

Asgarpanah, J., & Haghighat, E. (2012). Phytochemistry and pharmacologic properties of *Ziziphus spina christi* (L.) Willd. *African Journal of Pharmacy and Pharmacology*, *6*(31), 2332–2339. https://doi.org/10.5897/ajpp12.509

Atujona, D., Cai, S., & Amenyogbe, E. (2018). Mini review on *Vibrio* infection - A case study on *Vibrio harveyi* clade. *Fisheries and Aquaculture Journal*, *9*(4), 1000258. https://doi.org/10.4172/2150-3508.1000258

Awang-Jamil, Z., Aminuddin, M. F., Zaidi, B. Q., Basri, A. M., Ahmad, N., & Taha, H. (2021). Phytochemicals and antimicrobial analysis of selected medicinal plants from Brunei Darussalam. *Biodiversitas*, *22*(2), 601–606. https://doi.org/10.13057/biodiv/d220211

Baba, E., Acar, Ü., Öntaş, C., Kesbiç, O. S., & Yilmaz, S. (2016). The use of *Avena sativa* extract against *Aeromonas hydrophila* and its effect on growth performance, hematological and immunological parameters in common carp (*Cyprinus carpio*). *Italian Journal of Animal Science*, *15*(2), 325–333. https://doi.org/10.1080/1828051X.2016.1185977

Bentzon-Tilia, M., Sonnenschein, E. C., & Gram, L. (2016). Monitoring and managing microbes in aquaculture – Towards a sustainable industry. *Microbial Biotechnology*, *9*(5), 576–584. https://doi.org/10.1111/1751-7915.12392

Boussaada, O., Ammar, S., Saidana, D., Chriaa, J., Chraif, I., Daami, M., Helal, A. N., & Mighri, Z. (2008). Chemical composition and antimicrobial activity of volatile components from capitula and aerial parts of *Rhaponticum acaule* DC growing wild in Tunisia. *Microbiological Research*, *163*(1), 87–95. https://doi.org/10.1016/j.micres.2007.02.010

Bouyahya, A., Abrini, J., Dakka, N., & Bakri, Y. (2019). Essential oils of *Origanum compactum* increase membrane permeability, disturb cell membrane integrity, and suppress quorum-sensing phenotype in bacteria. *Journal of Pharmaceutical Analysis*, *9*(5), 301-311. https://doi.org/10.1016/j.jpha.2019.03.001

Brito, S. M. O., Coutinho, H. D. M., Talvani, A., Coronel, C., Barbosa, A. G. R., Vega, C., Figueredo, F. G., Tintino, S. R., Lima, L. F., Boligon, A. A., Athayde, M. L., & Menezes, I. R. A. (2015). Analysis of bioactivities and chemical composition of *Ziziphus joazeiro* Mart. using HPLC-DAD. *Food Chemistry*, *186*, 185–191. https://doi.org/10.1016/j.foodchem.2014.10.031

Chen, H. B., Zhang, J., Yu, H. X., & Hu, Q. L. (2007). *In vitro* study on the antibacterial of a medicinal intermediate, solanesol. *Qilu Pharmaceutical Affairs*, *26*, 558–559.

Dangoggo, S. M., Hassan, L. G., Sadiq, I. S., & Manga, S. B. (2012). Phytochemical analysis and antibacterial screening of leaves of *Diospyros mespiliformis* and *Ziziphus spina-christi*. *Chemical Engineering Journal*, *1*(1), 31–37.

Doğan, A., Otlu, S., Çelebİ, Ö, Aksu Kiliçle, P., Gülmez Sağlam, A., Doğan, A. N. C., & Mutlu, N. (2017). An investigation of antibacterial effects of steroids. *Turkish Journal of Veterinary and Animal Sciences*, *41*(2), 302–305. https://doi.org/10.3906/vet-1510-24

Ermias, R., Tukue, M. & Bahlbi, A. (2011). Phytochemical screening of *Ziziphus spina-christi* and *Piper nigrum*. *Journal of Atoms and Molecules*, *1*(1), 41–47.

Filimonov, D. A., Lagunin, A. A., Gloriozova, T. A., Rudik, A. V., Druzhilovskii, D. S., Pogodin, P. V., & Poroikov, V. V. (2014). Prediction of the biological activity spectra of organic compounds using the pass online web resource. *Chemistry of Heterocyclic Compounds*, *50*(3), 444–457. https://doi.org/10.1007/s10593-014-1496-1

Gull, J., Sultana, B., Anwar, F., Naseer, R., Ashraf, M., & Ashrafuzzaman, M. (2012). Variation in antioxidant attributes at three ripening stages of guava (*Psidium guajava* L.) fruit from different geographical regions of Pakistan. *Molecules*, *17*(3), 3165–3180. https://doi.org/10.3390/molecules17033165

Haniffa, M. A., & Kavitha, K. (2012). Antibacterial activity of medicinal herbs against the fish pathogen *Aeromonas hydrophila*. *Journal of Agricultural Technology*, *8*(1), 205–211.

Huang, F., Kong, J., Ju, J., Zhang, Y., Guo, Y., Cheng, Y., Qian, H., Xie, Y., & Yao, W. (2019). Membrane damage mechanism contributes to inhibition of trans-cinnamaldehyde on *Penicillium italicum* using Surface-Enhanced Raman Spectroscopy (SERS). *Scientific Reports*, *9*(1), 490. https://doi.org/10.1038/s41598-018-36989-7

Ibrahim, J. A., Egharevba, H. O., Nnamdi, R. A., & Kunle, O. F. (2015). Comparative pharmacognostic and phytochemical analysis of *Ziziphus spina*-christi (L.) Desf. and *Ziziphus abyssinica* Hochst. ex A. Rich. *International Journal of Pharmacognosy and Phytochemical Research*, *7*(6), 1160–1166.

Igbinosa, I. H., Beshiru, A., & Igbinosa, E. O. (2017). Antibiotic resistance profile of *Pseudomonas aeruginosa* isolated from aquaculture and abattoir environments in urban communities. *Asian Pacific Journal of Tropical Disease*, *7*(1), 47–52. https://doi.org/10.12980/apjtd.7.2017D6-363

Inbathamizh, L., & Padmini, E. (2013). Effect of geographical properties on the phytochemical composition and antioxidant potential of *Moringa oleifera* flowers. *BioMed Research International*, *1*(3), 239–247.

Joshua, W. J., & Zulperi, Z. (2020). Effects of *Spirulina platensis* and *Chlorella vulgaris* on the immune system and reproduction of fish. *Pertanika Journal of Tropical Agricultural Science*, *43*(4), 429–444. https://doi.org/10.47836/pjtas.43.4.01

Khaleel, S. M., Jaran, A. S., & Al-Deeb, T. M. F. (2019). Antimicrobial and lipid peroxidation inhibition potential of *Ziziphus spina-christi* (Sedr), a Jordanian medicinal plant. *Journal of Biological Sciences*, *19*(2), 131–136. https://doi.org/10.3923/jbs.2019.131.136

Kinnunen, P. K., Kaarniranta, K., & Mahalka, A. K. (2012). Protein-oxidized phospholipid interactions in cellular signaling for cell death: From biophysics to clinical correlations. *Biochimica et Biophysica Acta-Biomembranes*, *1818*(10), 2446-2455. https://doi.org/10.1016/j.bbamem.2012.04.008

Kumar, D. G., & Rajakumar R. (2016). Gas chromatography mass spectrometry analysis of bioactive components from the ethanol extract of *Avicennia marina* leaves. *Innovare Journal of Science*, *4*(4), 9-12.

Mazumder, K., Nabila, A., Aktar, A., & Farahnaky, A. (2020). Bioactive variability and *in vitro* and *in vivo* antioxidant activity of unprocessed and processed flour of nine cultivars of Australian *lupin* species: A comprehensive substantiation. *Antioxidants*, *9*(4), 282. https://doi.org/10.3390/antiox9040282

Mbunde, M., Mdegela, R. H., Laswai, H. S., & Mabiki, F. P. (2018). Quantification of phenolics, flavonoids and antioxidant activity of *Tamarindus indica* from selected areas in Tanzania. *Biofarmasi Journal of Natural Product Biochemistry*, *16*(1), 22–28. https://doi.org/10.13057/biofar/f160103

Mensah-Agyei, G. O., Ayeni, K. I., & Ezeamagu, C. O. (2020). GC-MS analysis of bioactive compounds and evaluation of antimicrobial activity of the extracts of *Daedalea elegans*: A Nigerian mushroom. *African Journal of Microbiology Research*, *14*(6), 204–210. https://doi.org/10.5897/ajmr2019.9120

Monteiro, S. H., Andrade, G. M., Garcia, F., & Pilarski, F. (2018). Antibiotic residues and resistant bacteria in aquaculture. *The Pharmaceutical and Chemical Journal*, *5*(4), 127–147.

Motamedi, H., Seyyednejad, S. M., Hasannejad, Z., & Fariba Dehghani, F. (2014). A comparative study on the effects of *Ziziphus spina-christi* alcoholic extracts on growth and structural integrity of bacterial pathogens. *Iranian Journal of Pharmaceutical Sciences*, *10*(2), 1- 10.

Moustafa, M. F., Hesham, A. E. L., Quraishi, M. S., & Alrumman, S. A. (2016). Variations in genetic and chemical constituents of *Ziziphus spina-christi* L. populations grown at various altitudinal zonation up to 2227 m height. *Journal of Genetic Engineering and Biotechnology*, *14*(2), 349–362. https://doi.org/10.1016/j.jgeb.2016.09.001

Mulyani, S., Adriani, M., & Wirjatmadi, B. (2021). Antibacterial activity of extract ethanol bidara leaves (*Ziziphus spina-christi* L.) on *Enteropathogenic coli*. *Indian Journal of Forensic Medicine and Toxicology*, *15*(1), 1589–1595. https://doi.org/10.37506/ijfmt.v15i1.13638

Novriadi, R. (2016). Vibriosis in aquaculture. *Omni-Akuatika*, *12*(1), 24. https://doi.org/10.20884/1.oa.2016.12.1.24

Orhan, I., Özçelik, B., & Şener, B. (2011). Evaluation of antibacterial, antifungal, antiviral, and antioxidant potentials of some edible oils and their fatty acid profiles. *Turkish Journal of Biology*, *35*(2), 251–258. https://doi.org/10.3906/biy-0907-107

Pan, C. Y., Tsai, T. Y., Su, B. C., Hui, C. F., & Chen, J. Y. (2017). Study of the antimicrobial activity of tilapia piscidin 3 (TP3) and TP4 and their effects on immune functions in hybrid tilapia (*Oreochromis* spp.). *PLOS One*, *12*(1), e0169678. https://doi.org/10.1371/journal.pone.0169678

Parasuraman, S. (2011). Prediction of activity spectra for substances. *Journal of Pharmacology and Pharmacotherapeutics*, *2*(1), 52–53. https://doi.org/10.4103/0976-500X.77119

Pargaputri, A. F., Munadziroh, E., & Indrawati, R. (2016). Antibacterial effects of *Pluchea indica* Less. leaf extract on *E. faecalis* and *Fusobacterium nucleatum* (*in vitro*). *Dental Journal*, *49*(2), 93–98. https://doi.org/10.20473/j.djmkg.v49.i2.p93-98

Pohlmann, J., Lampe, T., Shimada, M., Nell, P. G., Pernerstorfer, J., Svenstrup, N., Brunner, N. A., Schiffer, G., & Freiberg, C. (2005). Pyrrolidinedione derivatives as antibacterial agents with a novel mode of action. *Bioorganic and Medicinal Chemistry Letters*, *15*(4), 1189–1192. https:/doi.org/10.1016/j.bmcl.2004.12.002

Potocki, L., Oklejewicz, B., Kuna, E , Szpyrka, E., Duda, M., & Zuczek, J. (2021). Application of green algal *Planktochlorella nurekis* biomasses to modulate growth of selected microbial species. *Molecules*, *26*(13), 4038. https://doi.org/10.3390/molecules26134038

Prabu, E., Felix, S., Felix, N., Ahilan, B., & Ruby, P. (2017). An overview on significance of fish nutrition in aquaculture industry. *International Journal of Fisheries and Aquatic Studies*, *5*(6), 349–355.

Praja, R. K., & Safnurbaiti, D. P. (2018). The infection of *Vibrio parahaemolythicus* in shrimp and human. *Oceana Biomedicina Journal*, *1*(1), 44–58. http://doi.org/10.30649/obj.v1i1.6

Raman, V., Samuel, L., Saradhi, P., Rao, N., Krishna, N. V., Sudhakar, M., & Radhakrishnan, T. (2012). Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum*. *Asian Journal of Pharmaceutical and Clinical Research*, *5*(2), 99–106.

Ravi, L., Manasvi V., & Praveena Lakshmi, B. (2016). Antibacterial and antioxidant activity of saponin from *Abutilon indicum* leaves. *Asian Journal of Pharmaceutical and Clinical Research*, *9*(3), 344–347. https://doi.org/10.22159/ajpcr.2016.v9s3.15064

Riyadi, P. H., Romadhon R., Anggo A. D., Herawati V. E., & Setyastuti A. I. (2020). PASS and ADMET analyses for eight compounds from Nile tilapia (*Oreochromis niloticus*) viscera waste hydrolysate as anti-inflammatory nutraceutical. *AACL Bioflux*, *13*(5), 2630–2638.

Sarjito, Haditomo, A. H. C., Desrina, Ariyati R. W., & Prayitno, S. B. (2018a). The diversity of causative agent associated with bacterial diseases on catfish (*Clarias gariepinus*) with molecular based from Demak, Indonesia. *Omni-Akuatika*, *14*(2), 100–106.

Sarjito, Haditomo, A. H. C., Desrina, Djunaedi, A., & Prayitno, S. B. (2018b). The bacterial diversity associated with bacterial diseases on mud crab (*Scylla serrata* Fab.) from Pemalang Coast, Indonesia. In *Journal of Physics: Conference Series* (Vol. 1025, No. 1, p. 012076). IOP Publishing. https:/doi.org/10.1088/1742-6596/1025/1/012076

Sarjito, Radjasa O. K, Prayitno, S. B., & Hutabarat, S. (2009). Phylogenetic diversity of the causative agents of vibriosis associated with groupers fish from Karimunjawa Islands, Indonesia. *Current Research in Bacteriology*, *2*(1), 14-21. https://doi.org/10.3923/crb.2009.14.21

Sarjito, Haditomo, A. H. C, Desrina, Djunaedi, A., & Prayitno S. B. (2018c). The diversity of vibrios associated with vibriosis in pacific white shrimp (*Litopenaeus vannamei*) from extensive shrimp pond in Kendal District, Indonesia. In *IOP Conference Series: Earth and Environmental Science* (Vol. 116, No. 1, p. 012011). IOP Publishing. https://doi.org/10.1088/1755-1315/116/1/012011

Sivakumar, S. R., Radhakrishnan, S., & Kulangiappar, K. (2017). Isolation of N-methyl formamide from red algae *Portieria hornemannii* (Lyngbye) P.C. Silva active against two plant pathogenic bacteria. *American-Eurasian Journal of Agricultural and Environmental Sciences*, *17*(1), 99–106.

Soni, H. & Malik, J.K. (2021). Phyto-pharmacological potential of *Zizyphus jujube*: A review. *Scholars International Journal of Biochemistry*, *4*(2), 1–5. https://doi.org/10.36348/sijb.2021.v04i01.001

Statistics Indonesia. (2020). *Produksi perikanan budidaya menurut provinsi dan jenis budidaya,*

2000-2018 [Aquaculture production by province and type of cultivation, 2000-2018]. https://www.bps.go.id/statictable/2009/10/05/1706/produksi-perikanan-budidaya-menurut-provinsi-dan-jenis-budidaya-2000-2017.html

Stein, S. E., Mikaia, A., White, E., Zaikin, V., Zhu, D., Sparkman, O. D., Neta, P., & Zenkevich, I. (2014). *NIST Standard Reference Database 1A*. https://www.nist.gov/document/nist1aver22manpdf

Suliman, M. B., & Mohammed, A. A. (2018). Preliminary phytochemical screening and antibacterial activity of ethanolic and aqueous extracts of Sudanese medicinal plant *Ziziphus spina-christi* L. leaves. *Arabian Journal of Medicinal and Aromatic Plants*, *4*(1), 35–44.

Swamy, M. K., Arumugam, G., Kaur, R., Ghasemzadeh, A., Yusoff, M. M., & Sinniah, U. R. (2017). GC-MS based metabolite profiling, antioxidant and antimicrobial properties of different solvent extracts of Malaysian *Plectranthus amboinicus* leaves. *Evidence-Based Complementary and Alternative Medicine*, *2017*, 1517683. https://doi.org/10.1155/2017/1517683

Taghipour, M. T., Nameni, R., Taghipour, M., & Ghorat, F. (2020). Phytochemical analysis and antimicrobial activity of *Ziziphus spina*-christi and *Tamarix aphylla* leaves' extracts as effective treatment for Coronavirus Disease 2019 (COVID-19). *Thrita*, *9*(2), e107776. https://doi.org/10.5812/thrita.107776

Temerk, H. A., Salem, W. M., Sayed, W. F., & Hassan, F. S. (2017). Antibacterial effect of phytochemial extracts from *Ziziphus spina-christi* against some pathogenic bacteria. *Egyptian Journal of Botany*, *57*(3), 595–604. https://doi.org/10.21608/ejbo.2017.665.1035

Thawabteh, A., Juma, S., Bader, M., Karaman, D., Scrano, L., Bufo, S. A., & Karaman, R. (2019). The biological activity of natural alkaloids against herbivores, cancerous cells and pathogens. *Toxins*, *11*(11), 656. https://doi.org/10.3390/toxins11110656

Tran, N., Rodriguez, U. P., Chan, C. Y., Phillips, M. J., Mohan, C. V., Henriksson, P. J. G., Koeshendrajana, S., Suri, S., & Hall, S. (2017). Indonesian aquaculture futures: An analysis of fish supply and demand in Indonesia to 2030 and role of aquaculture using the AsiaFish model. *Marine Policy*, *79*, 25–32. https://doi.org/10.1016/j.marpol.2017.02.002

Ulanowska, K., Tkaczyk, A., Konopa, G., & Węgrzyn, G. (2006). Differential antibacterial activity of genistein arising from global inhibition of DNA, RNA and protein synthesis in some bacterial strains. *Archives of Microbiology*, *184*(5), 271-278. https://doi.org/10.1007/s00203-005-0063-7

Wu, Y., Bai, J., Zhong, K., Huang, Y., Qi, H., Jiang, Y., & Gao, H. (2016). Antibacterial activity and membrane-disruptive mechanism of 3-*p-trans*-coumaroyl-2-hydroxyquinic acid, a novel phenolic compound from pine needles of *Cedrus deodara*, against *Staphylococcus aureus*. *Molecules*, *21*(8), 1084. https://doi.org/10.3390/molecules21081084

Yoon, B. K., Jackman, J. A., Valle-Gonzalez, E. R., & Cho, N. J. (2018). Antibacterial free fatty acids and monoglycerides: Biological activities, experimental testing, and therapeutic applications. *International Journal of Molecular Sciences*, *19*(4), 1114. https://doi.org/10.3390/ijms19041114