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An antibacterial compound purified from a tropical coastal plant, *Diospyros maritima*

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Abstract. *Isnansetyo A, Handayani DP, Istiqomah I, Arif A, Kaneko T. 2021. An antibacterial compound purified from a tropical coastal plant,* Diospyros maritima. *Biodiversitas 23: 135-142.* This study aimed to isolate, purify, and characterize an antibacterial compound from *Diospyros maritima* and evaluate its antibacterial activity. *Diospyros maritima* bark was collected from Kei Kecil Island, extracted with ethanol, separated with hexane and chloroform, and purified by column chromatography. The isolated compound was tested for antibacterial activity by paper disk diffusion on the double-layer agar medium. The minimum inhibitory concentration (MIC) was determined by the microdilution method in a 96-well microplate. The pure compound inhibited *Staphylococcus aureus* and *Aeromonas hydrophila* at MICs of 0.625 and 5 µg/mL, respectively. The molecular weight of the compound was 188 m/z based on GC-MS analysis. The ^{1}H-NMR spectrum indicated two protons which appeared as a multiplet at 7,687.59 ppm, one proton appeared as a singlet at 6.87 ppm and one proton appeared as a doublet at 7.26 ppm. The ^{13}C-NMR spectrum showed eleven carbon signals, eight signals for the aromatic ring (186.06, 162.38, 151.17, 137.41, 134.49, 133.76, 17.75, and 16.51 ppm) and two signals at 191.91 and 191.05 ppm. The spectrometric data showed that the isolated compound was plumbagin (1,4-naphthalenedione-5-hydroxy-2-methyl). This finding opens up a new perspective for *D. maritima* as a source of lead compounds for the development of antibacterial substances.

Keywords: *Aeromonas hydrophila*, bark, Minimum Inhibitory Concentration, plumbagin, *Staphylococcus aureus*

INTRODUCTION

Antibacterial compounds are widely used to fight not only human diseases but also veterinary and aquaculture diseases. However, antibiotics that are used continuously lead to environmental contamination and microbial resistance (Santos and Ramos 2018). Research into bioactive compounds has been conducted several decades ago and continues to this day. Most commercial antibiotics are isolated from microorganisms but are rarely from plants, especially tropical coastal plants. One of the potential tropical coastal plants that produce different types of natural products is *Diospyros maritima*. This plant species can be easily found in Eastern Indonesian coastal areas, particularly the Kei Kecil Islands in southeast Maluku. The local name of this wild plant is ainum. They do not use the plant for daily needs, as the sap from the bark of *D. maritima* is very concentrated and causes severe itching and inflammation.

Research on antibacterial compounds produced by coastal plants, especially *D. maritima* from Kei Islands, is still very limited. Hamdillah et al. (2019) screened the antibacterial potency of twigs, fruits, leaves, and the bark of coastal plants, and several sponges from Kei Kecil Island, and the results showed that the ethanol extract of bark of *D. maritima* had anti-tuberculosis activity and the ethyl acetate extract of the sponge inhibit *Aeromonas hydrophila* [(Chester 1901) Stanier 1943], *Staphylococcus aureus* (Rosenbach 1884), *Streptococcus* sp., and *Vibrio parahaemolyticus*. Some researchers stated that *D. maritima* extract has antibacterial activity (Higa et al. 1998; Gu et al. 2004; Nematollahi et al. 2012). Bioactive compounds produced by *Diospyros* sp. are alkanol esters, aliphatic lactone (Kuo et al. 1998), coumarins (Higa et al. 1998), benzenoids, diterpene kaurane, plumbagin-naphthoquinone (Sharma 2017), phenylpropanoid, sterols, and triterpene.

Plumbagin is a secondary metabolite derived from plants belonging to the naphthoquinone group that has benefits in pharmaceutical applications. It has several bioactivities, including antibacterial (Gu et al. 2004; Babula et al. 2009; Sharma et al. 2009), antiparasitic, antifungal, anthelminthic, antihypertensive (Bernstein et al. 2014), antidiarrheal, anticancer (Marchionatti et al. 2009), antitumor, antioxidant (Talcott et al. 1985; Trongsakul et al. 2003; Padhey et al. 2010), and anti-inflammatory (Reese et al. 2010). Plumbagin is derived from *Plumbago rosea* (Kapadia et al. 2005; Nair et al. 2016), *Plumbago indica* (Kaewbumrung and Panichayupakaranant 2014), *Plumbago zeylanica; Plumbago scandens* (de Paiva et al. 2003), *Plumbago europea*, *Diospyros* sp. (Higa et al. 1998; Gu et al. 2004; Kuete et al. 2009; Nematollahi et al. 2012; Sharma 2017), and endophytic fungi *Cladosporium delicatulum* (Venkateswarulu et al. 2018). In this study, we described the extraction, purification, and antibacterial assay of plumbagin from a new source of tropical coastal plant *D. maritima* bark that was not previously reported.

MATERIALS AND METHODS

Sample collection

Diospyros maritima bark was collected in May 2017 in Ohoi Kelanit (5°39′18.34″S;132°40′48.32″E) and Ohoi Letman (5°34′43.67″S;132°43′20.18″E), Kei Island, Southeast Maluku Regency, Maluku, Indonesia. Freshly collected bark was immediately transported to the laboratory. The bark was washed thoroughly under running tap water to remove the adhering debris. Then, it was air-dried, cut into small pieces, and coarsely ground to a powder in a mechanical mill (Hamdillah et al. 2019).

Preparation of crude extract

Powdered bark samples (200 g) were extracted with 800 mL ethanol for 24 h, and the process was repeated three times. The soluble part of the extracts was filtered using Whatman paper, evaporated on a rotary evaporator (Laborota 4000 Heidolph, German), and air-dried at room temperature for 3 days. The ethanol extracts were stored at 4°C before use.

Bacterial strain and media

Two bacteria isolates were used in the antibacterial assay, namely *S. aureus* ATCC 6538 and *A. hydrophila* BA03. These bacteria were bacterial collections of the Laboratory of Fish Health and Environmental Management, Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia. All bacteria were stored in Tryptic Soya Broth (TSB) (Oxoid, UK) medium with 20% glycerol at -80°C. The bacteria were grown on Tryptic Soy Agar (TSA) medium (Oxoid, UK). The medium was sterilized by autoclaving for 15 minutes at 121°C.

Antibacterial activity assay

All extracts were tested for antibacterial activity against *S. aureus* and *A. hydrophila* by the paper disk diffusion method on double-layer agar (Isnansetyo and Kamei 2003). The inoculum was inoculated in a TSB medium (Oxoid, UK). The cells density was determined based on the McFarland standard by the spectrophotometric method (Thermo Fisher Scientific GENESYS 10S UV Vis, USA) at 625 nm. Pathogens with a final density of 10^6 cells mL^{-1} were inoculated separately in semisolid TSA medium and overlaid on TSA agar medium. An impregnated sterile paper disk (8 mm, Advantec) was placed on the agar plate and incubated at 30°C for 24 h. Oxytetracycline (Sigma, USA) (10 µg $disk^{-1}$) and EtOH were used as the positive and negative controls, respectively. The final concentration of the extracts in this test was 1000 and 500 µg $disk^{-1}$. The antibacterial activity was measured based on the diameter of the inhibition zone.

Solvent partitioning of the crude extract

A total of 20 g of ethanol extract was dissolved in 100 mL ethanol with distilled water (1:1, v/v) and partitioned with 200 mL chloroform in a separatory funnel (Hamdillah et al. 2019). The mixture was shaken until two layers were formed. The lower layer was collected and evaporated to dryness as the chloroform extract. The remaining extract after partitioning with chloroform was further partitioned with n-hexane (1:1, v/v). The upper and lower layers were collected and evaporated as n-hexane extract and residual ethanol extract. The partitioning method for each solvent was repeated three times. All extract fractions were tested for antibacterial activity and stored at 4°C before the next purification step.

Purification of the fraction extract

Based on the antibacterial activity test, n-hexane extract showed the largest zone inhibition. Therefore, a further purification step was conducted on the n-hexane extract. A total of 700 mg of n-hexane extract was dissolved in a solvent mixture containing n-hexane: ethyl acetate (5:2, v/v) and purified using silica gel column chromatography (column diameter 3 cm and length 3 cm) using silica gel 60 F_{254} (Merck, Germany) with a particle size of 0.065–0.230 mm. The column was first eluted with n-hexane:ethyl acetate (9:1, v/v) and then with a solvent mixture of n-hexane:ethyl acetate:ethanol (8:1.5:0.5, v/v); n-hexane:ethyl acetate (3:7, v/v); ethyl acetate 100% and ethanol 100%. The fractions obtained from column chromatography were checked by TLC 60 F_{254} (Merck, Germany) (Venkateswarulu et al. 2018). The fractions were tested for antibacterial assay.

Determination of MIC and MBC

Determination of MIC value was evaluated against *S. aureus* and *A. hydrophila* using the microdilution method in Mueller Hinton Broth (Conda, India) (CLSI 2010). Oxytetracycline (Sigma, Japan) and kanamycin (Wako, Japan) were used as positive controls. The initial bacterial density was 10^5 cells/mL. Plumbagin and antibiotics were diluted to obtain final doses in the range of 0.3125–40 µg/mL. The plate was incubated at 30°C for 24 h and resazurin ($C_{12}H_6NNaO_4$) (Wako, Japan) (2 mg/mL) was added after incubation. MBC was tested by inoculating 20 µL of the MIC samples from a 96-well microplate on MHA medium (Conda, India) and incubated for 24 h at 30°C.

Gas chromatography-mass spectrometry and nuclear magnetic resonance

The purified compound was analyzed by GC-MS (GC-MS 6890 N Agilent Technologies, USA) with helium as the carrier gas. Two milligrams of the active compound were dissolved in 100 µl ethanol and 1 µl injected to an HP 5 MS GC-MS 6890 N capillary column (Agilent Technologies, USA) with a length of 60 m, the thickness of 0.25 µm, and diameter of 0.25 mm. For ^1H and ^{13}C NMR analysis, a (500 MHz) spectrum was recorded on a JNM-ECZ5000R/S1 (500 MHz NMR spectrometer (JEOL, Japan) using tetramethylsilane (TMS) as an internal reference. The chemical shifts are expressed in parts per million (ppm).

RESULTS AND DISCUSSION

Screening of antibacterial activity from the bark of *Diospyros maritima*

Many people have been using traditional herbal medicine for a long time due to the presence of secondary metabolites in different parts of plants. One of the plants with great potential as a source of bioactive compounds is *D. maritima* that can be found in tropical and subtropical coastal areas. *Diospyros maritima* has been widely used as traditional medicine by the people of Taiwan, India, and Indonesia, particularly its fruit, stem, and bark. The bark of this plant contains many secondary metabolites such as lipids, aromatics, terpenoids, steroids, and naphthoquinones (Sinha and Bansal 2008; Rauf et al. 2017). The discovery of antibiotics in the early 20th century was important for preventing or curing diseases caused by bacteria. However, with the overuse and misuse of antibiotics, bacterial strains have become resistant to antibiotics. Therefore, screening for the antibacterial activity of plants is of prime importance, as plants have been used as drugs for human diseases (Ravikumar et al. 2010).

In this study, a total of 60 g of ethanol extract was obtained from 200 g of dried bark powder (30% extract yield). The extract was partitioned by n-hexane, ethanol, and chloroform and tested for antibacterial activity against *A. hydrophila* and *S. aureus* (Table 1). The results of the antibacterial activity indicated that the ethanol extract of bark samples had high antibacterial activity. The ethanol extract had the highest inhibition zone of 29.50 ± 2.00 mm against *A. hydrophila* at a dose of 1,000 µg/disk, and the lowest inhibition zone was 16.30 ± 1.75 mm that was obtained against *S. aureus* at a dose of 500 µg/disk. N-hexane extract inhibited *A. hydrophila* and *S. aureus* at doses of 1,000 µg/disk and 500 µg/disk, with the inhibition zone of 26.33-17.40 mm and 20.50-18.60 mm, respectively. Meanwhile, the chloroform extract had an antibacterial activity with zone diameters ranging from 10.40-20.00 mm. The residual ethanol extract showed no inhibitory activity against pathogenic bacteria (Figure 1).

Separation of chemical compounds in the extract

The chemical compounds in the ethanol extract were separated by Thin Layer Chromatography (TLC) and developed with a solvent mixture of n-hexane: ethyl acetate: ethanol (8: 1.5: 0.5). There were seven spots with Rf values of 0.81; 0.75; 0.45; 0.44; 0.37; 0.36; 0.19 (Figure 2.A). The n-hexane extract obtained by partitioning of ethanol extract exhibited four spots (Rf: 0.81; 0.75; 0.45; 0.19) on TLC that developed with the same solvent mixture as described above. The n-hexane extract was then purified by the gradient polarity column chromatography method (Figure 2.B-C). The purified compound was confirmed by a single spot on TLC (Figure 2.B). The TLC plate was developed in various solvent mixtures, namely n-hexane: chloroform: ethyl acetate: 2-propanol: ethanol (8: 0.5: 0.5: 0.5), n-hexane: ethyl acetate: chloroform: 2-propanol: ethanol (9: 0.25: 0.25: 0.25: 0.25), n-hexane: chloroform: 2-propanol: ethanol (9.5: 0.5: 0.25: 0.25), and n-hexane: chloroform: 2-propanol: Ethyl acetate: ethanol (9:0.5: 0.125: 0.25: 0.125). The purification step of the pure compound from *D. maritima* was shown in Figure 2.C.

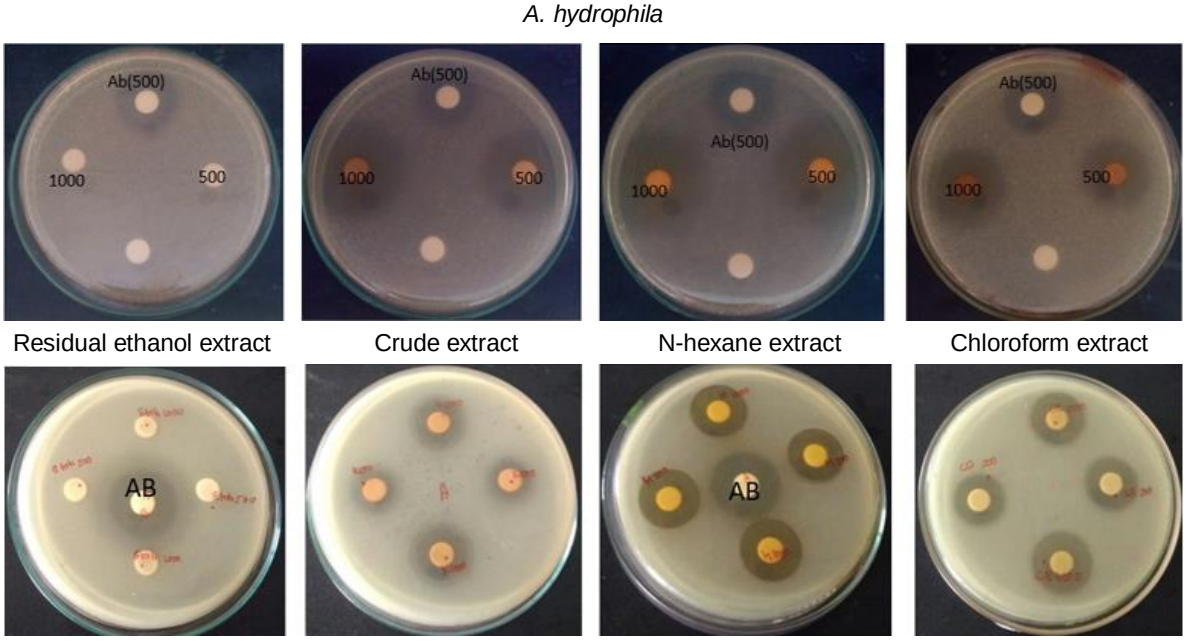
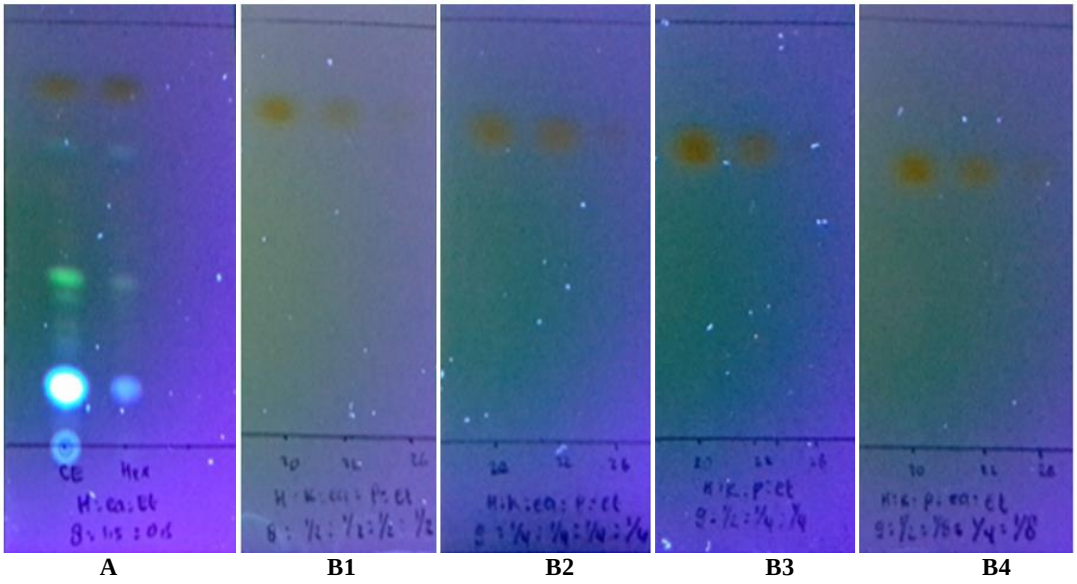
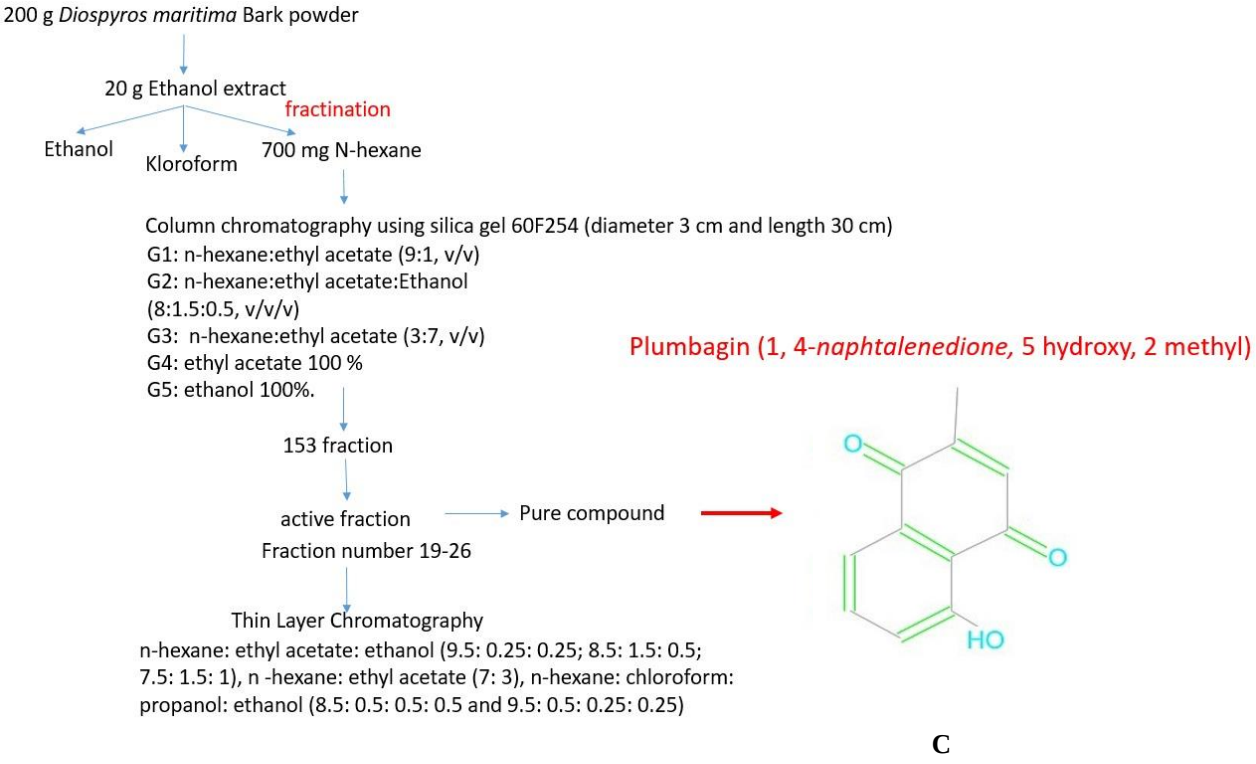


Figure 1. Inhibition zones of residual ethanol extract, crude extract, n-hexane, and chloroform extract (1000 µg disk^{-1} and 500 µg disk^{-1}) against *Aeromonas hydrophila* (upper) and *Staphylococcus aureus* (lower). Commercial antibiotic as the positive control (Oxytetracycline, 500 µg disk^{-1})



A **B1** **B2** **B3** **B4**

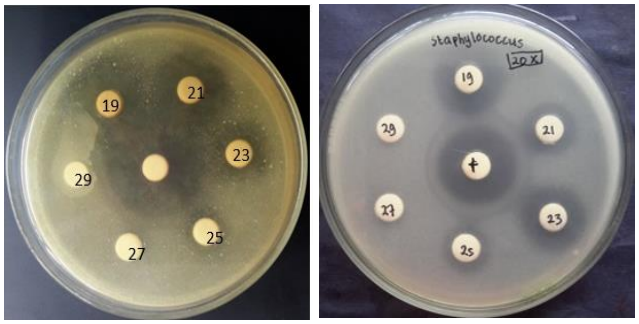


C

Figure 2. (A). TLC chromatogram of crude extract and n-hexane was observed at a wavelength of 254 nm with a mobile phase of n-hexane: Ethyl Acetate: Ethanol (8:1.5;0.5, v/v/v). Seven spots were detected in crude extracts while in the n-hexane fraction were 4 spots. (B) Pure compound obtained from column chromatography was observed on the TLC at UV wavelengths of 254 nm with a mobile phase of n-hexane: chloroform: ethyl acetate: 2-propanol: ethanol (8: 0.5: 0.5: 0.5) (B1), n-hexane: ethyl acetate: chloroform: 2-propanol: ethanol (9: 0.25: 0.25: 0.25: 0.25) (B2), n-hexane: chloroform: 2-propanol: ethanol (9.5: 0.5: 0.25: 0.25) (B3), and n-hexane: chloroform: 2-propanol: Ethyl acetate: ethanol (9:0.5: 0.125: 0.25: 0.125) (B4). (C) Flow chart of plumbagin isolation from *Diospyros maritima* bark

Table 1. Antibacterial activity of the crude extract, n-hexane, chloroform, and residual ethanol extract from *Diospyros maritima* bark

Pathogen	Crude extract (mm)		n-hexane (mm)		Chloroform (mm)		Residual ethanol (mm)	
	1000 µg/disc	500 µg/disc	1000 µg/disc	500 µg/disc	1000 µg/disc	500 µg/disc	1000 µg/disc	500 µg/disc
Aeromonas hydrophila	29.50±2.00	22.27±1.81	26.33±2.51	17.40±2.15	20.00±1.73	13.80±1.44	0	0
Staphylococcus aureus	21.50±0.81	16.30±1.75	20.50±0.50	18.60±0.53	13.60±2.13	10.40±1.87	0	0



A. hydrophila *S. aureus*

Figure 3. Antibacterial activity of eight active fractions against *Aeromonas hydrophila* (*left*) and *Staphylococcus aureus* (*right*)

Antibacterial activity of chromatography column fractions

A total of 153 fractions were obtained from this purification step. Antibacterial test of fractions obtained from column chromatography showed that eight fractions were active against *S. aureus* and *A. hydrophila* (fraction number 19-26) (Figure 3).

GC-MS and NMR

The purified compound obtained by column chromatography was analyzed by GC-MS and NMR. The GC chromatogram is shown in Figure 4.A, with a single major peak at a retention time of 9.2 min. The ^{1}H NMR spectrum detected two protons was a multiplet at 7.68-7.59 ppm, one proton was a singlet at 6.87 ppm, and one proton was a doublet at 7.26 ppm, which corresponded to four aromatic protons. The ^{13}C NMR spectrum showed eleven carbon signals, eight signals for the aromatic ring (186.06, 162.38, 151.17, 137.41, 134.49, 133.76, 17.75, and 16.51 ppm), two signals at 191.91 and 191.05 ppm indicating the presence of carbonyl group, and a peak was detected at 116.48 ppm corresponding to a methyl group (Figure 4.B). The purified compound which was active as an antibacterial compound was identified as plumbagin (1, 4-*naphthalene dione*, 5- hydroxy, 2- methyl). Plumbagin is classified as hydrophobic and insoluble in water (Kapadia et al. 2005). Plumbagin of *D. maritima* bark exhibited higher antibacterial activity against *S. aureus* than against *A. hydrophila*. Naphthoquinone plumbagin isolated from *D. maritima* bark accounted for more than 0.11% of the dry weight of plant material.

MIC and MBC

The MIC values of plumbagin, oxytetracycline, and kanamycin against *S. aureus* were 0.625 µg/mL, 0.15625 µg/mL, and 0.625 µg/mL, respectively. The MIC values of plumbagin, oxytetracycline, and kanamycin against *A. hydrophila* were 2.5 µg/mL, 0.15625 µg/mL, and 2.5 µg/mL, respectively (Table 2).

No studies report yet on the MIC value of plumbagin against *A. hydrophila*. Hence, this is the first report of its antibacterial activity against this pathogen. Surprisingly, the MIC of plumbagin in this study showed much higher activity against *S. aureus* than in previous work (de Paiva et al. 2003; Hamdillah et al. 2019). The MBC of plumbagin against *S. aureus* was higher than that of oxytetracycline and kanamycin. A similar MBC pattern of plumbagin was shown against *A. hydrophila* (Table 2). Plumbagin has been reported as an antibacterial substance against *Mycobacterium tuberculosis* (Nematollahi et al. 2012) and *S. aureus* (Abdul et al. 1995). The growth of *S. aureus* and *Candida albicans* is completely inhibited by plumbagin, but ineffective against *Escherichia coli* and *Salmonella typhimurium* (de Paiva et al. 2003). However, it shows different bioactivity compared to Gram-negative. Completely different results were found in the present study, since the plumbagin in this study is also active against Gram-negative bacterium, *A. hydrophila*, with a MIC of 0.5 g/mL. In addition, the plumbagin purified from *D. maritima* shows an activity comparable to that of de Paiva et al. (2003) with MIC 1.56 g/mL against *S. aureus*. Hamdillah et al. (2019) also found that the MIC value of a partially purified antibacterial compound from *D. maritima* against *S. aureus* was 3.125 g/mL. The antibacterial activity of plumbagin is higher against *S. aureus* than against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Nair et al. 2016).

Plumbagin had higher activity against Gram-positive bacteria. It can be due to the different microorganism cell membrane permeability and the variation in the bacterial cell wall composition (Nikaido 1998). Gram-negative bacteria are also able to restrict the influx of many antibiotics. Multidrug efflux pumps at the trans-membrane are also responsible for a higher intrinsic resistance in Gram-negative bacteria (Mohammad et al. 2017). Gram-negative pathogens are particularly problematic because they are resistant to several commercial antibiotics and reminiscent of the pre-antibiotic era (Rossolini et al. 2014). *Staphylococcus aureus* is a species of Gram-positive bacteria. It is a pathogen that contaminates fishery products and causes food poisoning (Jay et al. 2005; Vaiyapuri et al. 2019). So far it has been a safety concern for the food industry. Most *S. aureus* is resistant to several commercial antibiotics. It is often found in water, in the environment, and even in food (Ludwig et al. 2009). The antibacterial activity of plumbagin against Gram-positive and Gram-negative pathogens suggests that plumbagin can be used as a lead compound to develop new antibiotics to overcome bacterial resistance.

Table 2. MIC and MBC value of plumbagin, oxytetracycline (OTC), and kanamycin (Kana) against pathogenic bacteria

Pathogen	MIC (µg/mL)			MBC (µg/mL)		
	Plumbagin	OTC	Kana	Plumbagin	OTC	Kana
Aeromonas hydrophila	5	0.15625	2.5	20	2.5	10
Staphylococcus aureus	0.625	0.15625	0.625	5	1.25	1.25

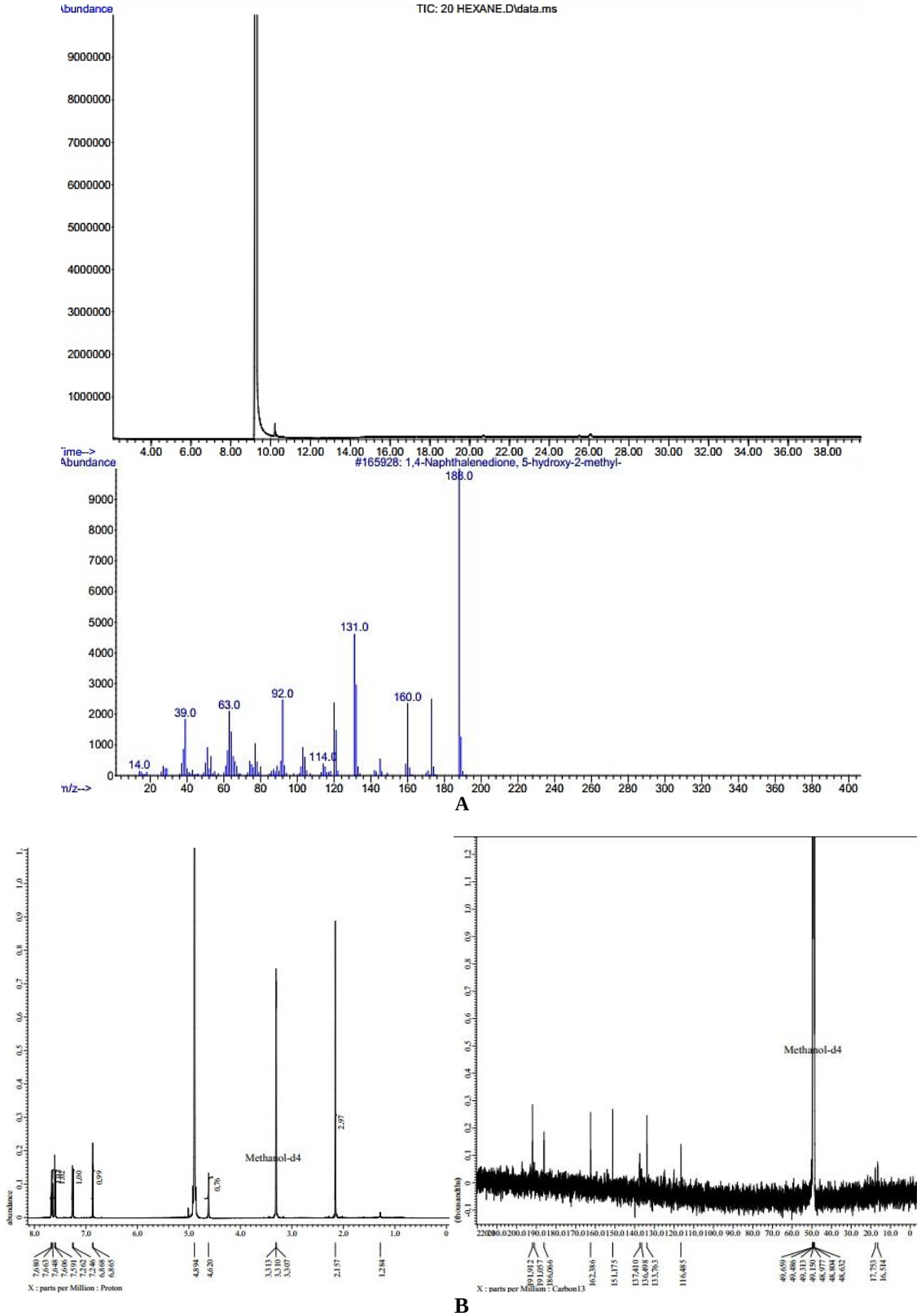


Figure 4. A. The mass spectrum of the pure compound, identified as plumbagin (1, 4 napthalenedione, 5 hydroxy, 2 methyl); B. Plumbagin spectrum by ^{1}H NMR spectrum of plumbagin (*left*) and ^{13}C NMR spectrum of plumbagin (*right*)

Research on the antibacterial activity of plumbagin against *A. hydrophila* is still rare. Plumbagin is widely used as an antibacterial in humans and rarely used to treat fish diseases. This study is the first report of the antibacterial activity of plumbagin against *A. hydrophila*. *Aeromonas* have broad impacts on aquatic animal health. *Aeromonas hydrophila* is distributed worldwide and is the most common bacterium in freshwater habitats. It often causes

disease in farmed and wild fish (Austin and Austin 2007). Janda and Abbot (2010) have reported that *Aeromonas* is isolated from marine waters, rivers, lakes, swamps, sediments, chlorinated water, water distribution systems, drinking water, and residual water. The number of isolates from drinking water is generally small compared to the amounts found in food. *Aeromonas* strains have been found in various types of foods, such as meat, fish, seafood, vegetables, and processed foods (Tomas 2012). The emergence of antibiotic-resistant bacteria involves the urgent discovery of new antibiotic sources. Hence, plumbagin isolated from *D. maritima* bark extract can be used as a new natural antibiotic to treat *A. hydrophila* infection in aquaculture. It is important to carry out the upscaling of the active ingredient, the determination of the cytotoxicity, and the therapeutic effectiveness of plumbagin against bacterial pathogens in humans and fish in our further study.

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REFERENCES

Abdul KM, Ramchender RP. 1995. Modulatory effect of plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) on macrophage functions in BALB/c mice potentiation of macrophage bactericidal activity. Immunopharmacology 30: 231-236. DOI: 10.1016/0162-3109(95)00027-Q.

Austin B, Austin DA. 2007. Bacterial fish pathogens: Diseases of farmed and wild fish, 4th Edition. Springer-Praxis, England.

Babula P, Adam V, Havel L, Kizek R. 2009. Noteworthy secondary metabolites naphthoquinones – their occurrence, pharmacological properties and analysis. Curr Pharm Anal 5: 47-68. DOI: 10.2174/157341209787314936.

Bernstein KE, Gonzalez-Villalobos RA, Giani JF, Shah K, Bernstein E, Janjulia T, Koronyo Y, Shi P D, Koronyo-Hamaoui M, Fuchs S, Shen XZ. 2014. Angiotensin-converting enzyme overexpression in myelocytes enhances the immune response. Biol Chem 395: 1173-1178. DOI: 10.1515/hsz-2013-0295.

CLSI. 2010. Performance standards for antimicrobial susceptibility testing; 20th informational supplement. CLSI Document M100, S20. CLSI, Wayne, PA. Clinical and Laboratory Standards Institute.

de Paiva SR, Figueiredo MR, Aragão TV, Kaplan MAC. 2003. Antimicrobial activity in vitro of plumbagin isolated from *Plumbago* species. Memorias do Instituto Oswaldo Cruz 98: 959-961. DOI: 10.1590/s0074-02762003000700017.

Gu JQ, Graf TN, Lee D, Chai HB, Mi Q, Kardono LB, Setyowati FM, Ismail R, Riswan S, Farnsworth NR, Cordell GA. 2004. Cytotoxic and antimicrobial constituents of the bark of *Diospyros maritima* collected in two geographical locations in Indonesia. J Nat Prod 67: 1156-1161. DOI: 10.1021/np040027m.

Hamdillah A, Isnansetyo A, Istiqomah I, Puspita ID, Handayani DP, Kaneko T. 2019. Antibacterial activity of coastal plants and marine sponges from Kei Island Indonesia against bacterial fish pathogens. Pharmacognosy J 11: 1-6. DOI: 10.5530/pj.2019.11.130.

Higa M, Ogihara K, Yogi S.1998. Bioactive naphthoquinone derivatives from *Diospyros maritima* BLUME. Chem Pharm Bull 46: 1189-1193. DOI: 10.1248/cpb.46.1189.

Isnansetyo A, Kamei Y. 2003. MC21-A, a bactericidal antibiotic produced by a new marine bacterium, *Pseudoalteromonas phenolica* sp. Nov. O-BC30T, against methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 47: 480-488. DOI: 10.1128/AAC.47.2.480-488.2003.

Janda JM, Abbott SL. 2010. The genus *Aeromonas*: taxonomy, pathogenicity, and infection. Clin Microbiol Rev 23: 35-73. DOI: 10.1128/CMR.00039-09.

Jay MJ, Loessner JM, Golden AD. 2005. Foodborne diseases, modern food microbiology. Springer, Germany.

Kaewbumrung S, Panichayupakaranant P. 2014. Antibacterial activity of plumbagin derivative-rich *Plumbago indica* root extracts and chemical stability. Nat Prod Res 28: 835-837. DOI: 10.1080/14786419.2013.879585.

Kapadia NS, Isarani SA, Shah MB. 2005. A simple method for isolation of plumbagin from roots of *Plumbago rosea*. Pharm Biol 43: 551-553. DOI: 10.1080/13880200500220888.

Kuete V, Tangmouo JG, Meyer JJ, Lall N. 2009. Diospyrone, crassiflorone and plumbagin: three antimycobacterial and antigonorrhoeal naphthoquinones from two *Diospyros* spp. Intl J Antimicrob Agents 34: 322-325. DOI: 10.1016/j.ijantimicag.2009.04.008.

Kuo YH, Huang SL, Chang CI. 1998. A phenolic and an aliphatic lactone from *Diospyros maritima*. Phytochemistry 49: 2505-2507. DOI: 10.1016/S0031-9422(98)00360-4.

Ludwig W, Schleifer KH, Whitman WB. 2009. Revised road map to the phylum Firmicutes. Bergey's manual of systematic bacteriology. Springer, New York. DOI: 10.1007/978-0-387-68489-5_1.

Marchionatti AM, Picotto G, Narvaez CJ, Welsh J, Tolosa de Talamoni NG. 2009. Synergistic anticancer activity of 1,25-dihydroxy vitamin D (3) and immune cytokines: the involvement of reactive oxygen species. J Steroid Biochem Mol 113: 227-232. DOI: 10.1016/j.jsbmb.2009.01.004.

Mohammad NS, Geneto M, Abateneh DD, Salahuddin M, Manzar MD, Rao UM. 2017. Extraction of secondary metabolites from roots of *Acanthus ilicifolius* L and screening for antioxidant and antibacterial activity. Intl J Pharm Sci Invent 6: 31-36.

Nair SV, Baranwal G, Chatterjee M, Sachu A, Vasudevan AK, Bose C, Banerji A, Biswas R. 2016. Antimicrobial activity of plumbagin, a naturally occurring naphthoquinone from *Plumbago rosea*, against *Staphylococcus aureus* and *Candida albicans*. Intl J Med Microbiol 306: 237-248. DOI: 10.1016/j.ijmm.2016.05.004.

Nematollahi A, Aminimoghadamfarouja N, Wiart C. 2012. Reviews on 1,4-naphthoquinones from *Diospyros* L. J Asian Nat Prod Res 14: 80-88. DOI: 10.1080/10286020.2011.633515.

Nikaido H. 1998. Antibiotic resistance caused by gram-negative multidrug efflux pumps. Clin Infect Dis 27: 32-41. DOI: 10.1086/514920.

Padhey S, Dandawate P, Yusufi M, Ahmad A, Wiley FH. 2010. Perspectives on medicinal properties of plumbagin and its analogs. Med Res Rev 32 (6): 1131-1158. DOI: 10.1002/med.20235.

Rauf A, Uddin G, Patel S, Khan A, Halim SA, Bawazeer S, Ahmad K, Muhammad N, Mubarak MS. 2017. Diospyros, an under-utilized, multi-purpose plant genus: A review. Biomed Pharmacother 91: 714-730. DOI: 10.1016/j.biopha.2017.05.012.

Ravikumar S, Muthuraja M, Sivaperumal P, Gnanadesigan M. 2010. Antibacterial activity of the mangrove leaves *Exoecaria agallocha* against selected fish pathogens. Asian J Med Sci 2: 211-213.

Reese S, Vidyasagar A, Jacobson L, Acun Z, Esnault S, Hullett D, Malter JS, Djamali A. 2010. The Pin 1 inhibitor juglone attenuates kidney fibrogenesis via Pin 1-independent mechanisms in the unilateral ureteral occlusion model. Fibrogenesis Tissue Repair 3 (1): 1-8. DOI: 10.1186/1755-1536-3-1.

Rossolini GM, Arena F, Pecile P, Pollini S. 2014. Update on the antibiotic resistance crisis. Curr Opin Pharmacol 18: 56-60. DOI: 10.1016/j.coph.2014.09.006.

Santos L, Ramos F. 2018. Antimicrobial resistance in aquaculture: Current knowledge and alternatives to tackle the problem. Intl J Antimicrob Agents 52: 135-143. DOI: 10.1016/j.ijantimicag.2018.03.010.

Sharma S, Sharma BK, Prabhakar YS. 2009. Juglone derivatives as antitubercular agents: a rationale for the activity profile. Eur J Med Chem 44: 2847-2853. DOI: 10.1016/j.ejmech.2008.12.015.

Sharma V. 2017. Brief review on the genus *Diospyros*: a rich source of naphthoquinones. Asian J Adv Basic Sci 5: 34-53.

Sinha BN, Bansal SK. 2008. A review of phytochemical and biological studies of *Diospyros* species used in folklore medicine of Jharkhand. J Nat Remed 8: 11-17. DOI: 10.18311/jnr/2008/289.

Talcott RE, Smith MT, Giannini DD. 1985. Inhibition of microsomal lipid peroxidation by naphthoquinones: structure-activity relationships and

possible mechanisms of action. Arch Biochem Biophys 241: 88-94. DOI: 10.1016/0003-9861(85)90365-0.

Tomas JM. 2012. The main *Aeromonas* pathogenic factors. SRN Microbiol 12: 1-22. DOI: 10.5402/2012/256261.

Trongsakul S, Panthong A, Kanjanapothi D, Taesotikul T. 2003. The analgesic, antipyretic and anti-inflammatory activity of *Diospyros variegata* Kruz. J Ethnopharmacol 85: 221-225. DOI: 10.1016/s0378-8741(03)00020-5.

Vaiyapuri M, Joseph TC, Rao BM, Lalitha KV, Prasad MM. 2019. Methicillin-Resistant *Staphylococcus aureus* in Seafood: Prevalence, laboratory detection, clonal nature, and control in seafood chain. J Food Sci 84: 3341-3351. DOI: 10.1111/1750-3841.14915.

Venkateswarulu N, Shameer S, Bramhachari PV, Basha SKT, Nagaraju C, Vijaya T. 2018. Isolation and characterization of plumbagin (5-hydroxyl- 2- methylnaptalene-1,4-dione) producing endophytic fungi *Cladosporium delicatulum* from endemic medicinal plants. Biotechnol Rep 20: e00282. DOI: 10.1016/j.btre.2018.e00282.