Setiaji_2022_IOP_Conf._Ser.__Earth_Environ._Sci._1118_012006.pdf

by

Submission date: 05-Apr-2023 04:34PM (UTC+0700)
Submission ID: 2056473989
File name: Setiaji_2022_IOP_Conf._Ser.__Earth_Environ._Sci._1118_012006.pdf (335.05K)
Word count: 3755
Character count: 19329

IOP Conference Series: Earth and Environmental Science

Antibacterial Activity of Ethanolic Extracts from Jeringau (*Acorus calamus*)

To cite this article: J Setiaji *et al* 2022 *IOP Conf. Ser.: Earth Environ. Sci.* **1118** 012006

Antibacterial Activity of Ethanolic Extracts from Jeringau (*Acorus calamus*)

J Setiaji[1*], J Hariwitonang[1], T I Johan[1], Heriyanto[1]

[1] Department of Aquaculture, Faculty of Agriculture, Universitas Islam Riau, Pekanbaru 28284, Indonesia

*jr.setiaji@agr.uir.ac.id

Abstract. Increased resistance of pathogenic bacteria due to the use of antibiotics has become a major concern in the aquaculture industry. Environmentally friendly products are urgently needed to replace antibiotics for the treatments of fish diseases. This study aims to determine the activity of extract of Jeringau (*Acorus calamus*) as an antibacterial towards *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Vibrio alginolyticus*. The *A. calamus* extract was obtained by maceration using ethanol as a solvent. This extract was tested for its phytochemical content and its antibacterial activity was tested using the agar diffusion method. The treatments used were concentrations of *A. calamus* extract 200, 300, 400, 500 and 600 mg mL^{-1}. Phytochemical test results of *A. calamus* extract produced alkaloids, phenolic compounds, saponins, terpenoids and flavonoids. The results of the inhibition test showed that *A. calamus* extract with a concentration of 200 was able to inhibit the growth of *Aeromonas hydrophila* bacteria by 13.9 mm, 300 (14.4 mm), 400 (14.8 mm), 500 (16.2 mm), 600 (16.5 mm). On *Pseudomonas aeruginosa*, the concentrations were 200 (13.9 mm), 300 (14.5 mm), 400 (15.2 mm), 500 (15.5 mm), 600 (16.2 mm). On *Vibrio alginolyticus*, the concentrations were 200 (15.5 mm), 300 (15.7 mm), 400 (16.4 mm), 500 (18.7 mm), 600 (19 mm). In conclusion, *A. calamus* extract can be used to inhibit the growth of pathogenic bacteria in fish.

1. Introduction

Pathogen bacterial infection has become a serious problem on the fish ecosystem because it can cause mortality rate of up to 100%. Bacterial infection can also lower the quality of fish, which can have a significant economic impact [1]. A few pathogen bacteri that affect fish are *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Vibrio alginolyticus*. *A. Hydrophila* is a Gram negative, rod shaped, facultative, and anaerobic bacterium that exist in waterways. *A. Hydrophila* bacterium is the main cause of septicaemia epidemic for fresh water fish [2]. *A. Hydrophila* infects on catfish that breeds in ponds [3], salmon [4], and snapper fish [5]. *A. Hydrophila* infection results in damaged tissues to the spleen, gills, and the fish's gut [6]. *Bacillus* is a genus of bacteria which is able to produce enzymes such as proteases, cellulase and amylase.

P. aeruginosa bacterium is a Gram negative, aerobic, rod shaped, positive oxidase, and opportunistic in nature [7]. It plays an important role in the aquaculture industry. Pseudomonas is one of the fish pathogens that is most threatening and cause ulcerative syndrome and haemorrhagic septicaemia [8]. The symptoms are red spots due to haemorrhage, darkened skin colour, detached scales, fluid build-up in the stomach, protruding eyes, and fin erosions [9]. *P. Aeruginosa* can also cause strange behaviour related to the change of motor functions, such as abnormal movements to the infected fish [10]. *P. aeruginosa* bacterium has the capability to live and grow under several environmental conditions, this trait makes it possible for *P. aeruginosa* to become an infecting agent [11].

V. Alginolyticus bacterium is a Gram negative that mimics opportunistic pathogen from sea animals and humans [12]. *V. alginolyticus* causes vibriosis to fish and shrimps. This bacterium has made serious economic loss to the aquaculture industry [1]. Infections can result in exophthalmia, boils, septicaemia, and cloudy cornea to the fish [13]. Adhesion properties is a virulence trait that is of importance of *V. alginolyticus*. Epizootic disease starts with the clinical symptoms followed by death. Severe haemorrhage that affected the gastric mucosa. According to histology, gastric mucosa showed erosion and necrosis [14]. *V. Alginolyticus* caused fish's death through virulence factor and oxidative stress [15].

Generally, antibiotic is used to control bacterial diseases. However, the use of inaccurate antibiotic can cause a negative effect to the fish [16]. The long-term usage of antibiotic can increase the risk of resistance of bacteria [17]. Using natural alternative product from plants that has antibacterial activity is an effort to prevent resistance [18]. One of the alternative ways is by taking advantage of Jeringau plant (*A. calamus*).

A. calamus has a grass like shape with pointed and hard leaf, white coloured rhizome, and emits a unique scent when split apart. It thrives in a muddy soil that contains a lot of inorganic materials [19]. Rhizome extract of *A. calamus* contain saponin, flavonoid, alkaloid, tannin and astiri oil compounds [20]. Astiri oil of the *A. calamus* rhizome contain asaron, kalamenol, kalamin, kalameon, metileugenol, sineol, ascorbic acid, alpha-terpineol and eugenol [21]. Isolated *A. calamus* rhizome resulted in phenylpropanoid, sesquiterpenoid, and monoterpene compounds [22]. *A. calamus* is a well-known cure in the traditional medicine system that has proven to have anti-tumour activity [23]. This research is intended to discover the activity of Jeringau (*A. calamus*) rhizome extract as antibacterial towards *A. hydrophila*, *P. aeruginosa* and *V. Alginolyticus* bacteria.

2. Materials and Methods

2.1. Extraction of A. calamus Rhizome

A. calamus rhizome was obtained from Muaro Sentajo village, Sentajo Raya, Kuantan Singingi Regency, Riau Province, Indonesia. *A. calamus* rhizome was cut down to 2-4 mm, dried in an oven at 40ºC for 3 days. The dried species was turn indo powder using a blender. The powder rhizome was macerated with 90% ethanol solvent for 3 days. The *A. calamus* rhizome was then filtered, separating the mixture from the solids. The filtrate was steamed using rotary evaporator at 50ºC with the speed of 50 rpm to obtain the extract. The extract from the evaporation process was transferred into a 100 ml bottle and dried at room temperature.

2.2. Phytochemical Test

Phytochemical test was performed on *A. Calamus* extract that involved alkaloid, phenolic, saponin, terpenoid and flavonoid compounds. 250 µL of Mayer reagent was added to 10 mg of *A. Calamus* extract was for alkaloid test. 10 mg *A. Calamus* extract was mixed with 500 µL $FeCl_3$ 5% for the phenolic test. 5 mL of aquades was added to 10 mg *A. Calamus* extract and was shaken for 1 minute for saponin test. Then 150 µL 1N HCl and was again shaken for 1 minute. 10 drops of CH_3 COOH and 3 drops H_2SO_4 was added to 10 mg of *A. Calamus* extract for terpenoid test. 0.05 g Mg and 10 drops of 37% HCl were added to 10 mg of *A. Calamus* extract, and homogenised for 1 minute for flavonoid test.

Positive alkaloid test is shown by the forming of white sediment after Mayer reagent was added, and orange if Dragendorff reagent was added. Positive phenolic test is shown by the change of colour to blue. Positive saponin is show by forming of foam. Positive terpenoid test is shown by the forming of red colour. Positive flavonoid is shown by the colour changing to red.

2.3. Inhibitory Activity of A. Calamus Extract

A. Calamus extract that was obtained was tested to pathogen bacteria which are *A. hydrophila, P. aeruginosa* and *V. Algynolyticus* by using agar diffusion method and 6 mm paper disc. 1 mL of pathogen bacteria inoculant (OD 600nm = 0.08-0.1) was transferred to 15 mL of liquid agar nutrient media to be inoculated at 50^{0}C, and was homogenised and poured into a petri dish. After the pathogen bacteria culture filled medium solidified, Oxytetracycline antibiotic paper was used a positive control. 30 mL of methanol was dropped onto a paper disc and was used as negative control. *A. Calamus* extract was handled and concentration of 200, 300, 400, 500, 600 mg mL^{-1} were used, diluted with methanol, then 30 µL was dropped onto paper discs, to be incubated at 30ºC for 24 hours. The inhibition activity of *A. Calamus* extract was measured from the diameter of the transparent zone formed around the disc.

2.4. Data Analysis

Data obtained were displayed in a table and analysed descriptively by comparison of of the transparent zone formed as the criteria of antibacterial activity.

3. Results and Discussion

3.1. Phytochemical Test

The phytochemical test was performed to identify active compounds that that is found in the *A. Calamus* rhizome extract. This test was to determine the main group from the compounds that are active which has antimicrobial activity. The identification of compound groups from *A. Calamus* extract can be found with the change of colour to the extract after the addition of a reagent. *A. Calamus* extract that was reacted with Mayer and Dragendroff reagent formed white and orange sediments. The formation of the two sediments showed that the sample was positive to contain alkaloid compound. The compound on the sample that contained alkaloid was to turn to white and orange if each reacted with standard reagents [24]. Plants that contained isoquinoline alkaloids, the crystals produced during the phytochemical test were white and yellowish. The colours were results of salt reaction between the sample and the reagent.

A. Calamus extract contained phenolic compound shown by the change of colour to blue black after reacting with $FeCl_3$. The colour change was due to the reduction process from phosphomolybdic acid phosphotungstate with the reagent and they formed the blue colour. The higher the phenolic content, the darker the blue colour formed. This was due to heteropoly acid (phosphomolybdic phosphotungstate) getting reduced by phenolic ions making a complex molybdenum-tungsten that thickened the colour [25]. The formation of blue-black colour was due to the formation of sugar bond in the sample.

A. Calamus extract that was mixed with HCl formed foam. The sample was positive to contain saponin if the foam formed did not disappear for around 5 minutes. The sample that reacted with he reagent was positive to contain saponin, proven by the formation of foam that was stable for around 5 minutes [26]. The foam formed on the sample on the sample after 1 drop of HCl 1N was added reached 1 to 5 cm.

A. Calamus extract that reacted with Lieber-Burchard reagent changed to red in colour. The change of colour to red was due to the acid oxidisation by sulphuric acid. The electron from the hydrogen group detached, which made the compound lengthen its conjugate that was shown by the red colour [27]. The sample colour change to red was due to terpenoid which is a dehydrogenation derivative and oxygenation from terpen compound. Terpen is a hydrocarbon group that is often produced by plants.

A. Calamus extract contained flavonoid. The extract reacted with magnesium and HCl, shaken, and the colour of the sample turned pink. The sample colour changed to pink was due to the forming of benzopirilium salt that had the colour red due to the reduction of the polyhydroxy group from flavanol by the magnesium in hydrochloric acid [28].

3.2. Inhibitory Activity

The results shown that *A. Calamus* extract was able to inhibit the growth of the three pathogen bacteria. It was shown by the formation transparent zones around the paper disc. The capability of inhibition against bacteria *A. hydrophila* produced a range from 13.9 to 16.5 mm, against *P. aeruginosa* 13.9 to 16.2 mm and against *V. alginolyticus* ranged from 15.5 to 19 mm (Table 1). The inhibitory ability of this *A. Calamus* extract belongs to the strong category [29].

Table 1. Inhibition activity of *A. calamus* extract against pathogen bacteria

A. calamus Extract concentration (mg mL^{-1})	Inhibition zone diameter (mm)		
	A. hydrophila	*P.æruginosa*	*V. alginolyticus*
200	13.9 ± 0.99	13.9 ± 0.42	15.5 ± 0.14
300	14.4 ± 0.14	14.5 ± 1.70	15.7 ± 0.28
400	14.8 ± 0.85	15.2 ± 0.14	16.4 ± 0.42
500	16.2 ± 0.28	15.5 ± 0.28	18.7 ± 0.57
600	16.5 ± 0.42	16.2 ± 0.57	19 ± 0.28

The biggest result of *A. Calamus* extract inhibition activity was to *V. alginolyticus* bacterium. The difference in the inhibition zone diameter was due to the capability of pathogen bacteria to defend against antibacterial compound produced by *A. Calamus* extract. The capability of an antibacterial compound to inhibit microorganism growth deepened on the concentration and the type of antimicrobial material produced. This was due the higher concentration given, which also increased the amount of active substance contained inside it. The effectivity of bacteria inhibition increased and resulted in the wider transparent zone [30]. The formation of inhibition zone on the pathogen bacteria culture medium indicated that *A. Calamus* extract contained antibacterial compound such as alkaloid, phenolic, saponin, terpenoid, and flavonoid.

Alkaloid can inhibit activity from several enzymes such as esterase, DNA-polymerase, RNA-polymerase, and as able to prevent cellular respiration [31]. The working mechanism of alkaloid as antibacterial was by interfering with the peptidoglycan constituent components of bacterial cells which resulted in cell walls to not form fully and caused cell deaths. Alkaloid component is known as DNA intercalator and inhibits bacterial cell topoisomerase enzyme.

Phenolic compound has aromatic ring that has one or two hydroxyl groups and has a strong antimicrobial trait. Phenolic acted as protein denatures and prevented bacteria ingestion. Bacterial cell membrane was destroyed by dissolving the fat on the cell walls [32].

Saponin has antibacterial and antifungal traits. The antibacterial property is that it interferes with membrane permeability, so that membrane permeability increases and causes leakage of certain proteins and enzymes in bacteria [33]. Saponins that interacted with sterol membranes can damage the cell wall structure and cause important components in bacterial cells to come out.

Terpenoid compound as antibacterial substance was suspected by causing damage to the membrane by lipophilic compound [34]. Terpenoid was able to react with porin (protein transmembrane) with the outer wall of bacterial cell membrane which formed strong polymer bond and damaged porin. It resulted in permeability reduction of the bacterial cell wall to a point where the bacterial cells lacked nutrition and bacterial growth was inhibited or stopped completely.

Flavonoids was able to form complex compounds that contained traits that were able to damage bacterial cell walls. The compound interacted with bacterial extracellular protein that caused intracellular components to come out. Flavonoid was also able to release transduction energy toward cytoplasmic bacteria and inhibited bacterial motility [35]. Flavonoid compounds inhibit bacterial growth by binding to adhesins, damaging membranes, cell walls and inactivating enzymes. Beta rings and OH groups in flavonoid was suspected as the structure that was involved in the antibacterial activity. Flavonoid was able to stop nucleic acid synthesis, cytoplasm membrane functions by interfering the formation of biofilm, porin, permeability and interacted with several crucial enzymes [36].

4. Conclusion

Acorus calamus rhizome extract was positive to have contained alkaloids, flavonoids, phenolics, saponins and terpenoids. Acorus calamus extract concentration of 200, 300, 400, 500, 600 mg mL^{-1} can strongly inhibit the growth of bacteria *A. hydrophila, P. aeruginosa* and *V. alginolyticus.*

Acknowledgments

Author would like to thank laboratory assistants who have supported this research.

References

[1] Cai S, Cheng H, Pang H, Jian J and Wu Z 2018 *Veterinary Microbiology* 213: 35-41 dio: 10.1016/j.vetmic.2017.11.016
[2] Xia H, Tang Y, Lu F, Luo Y, Yang P, Wang W, Jiang J, Li N, Han Q, Liu F and Liu L 2017 *Journal Immunology* 42(3): 239-243 doi: 10.5114/ceji.2017.70965
[3] Jubirt M M, Hanson L A, Hanson-Dorr K C, Ford L, Lemmons S P and Cunningham F L 2015 *Journal of Wildlife Diseases*. 51(3): 634-639 doi: 10.7589/2014-06-156
[4] Citterio B, and Biavasco F 2015 *Virulence*. 6 (5): 417-418 doi: 10.1080/21505594.2015.1058479
[5] Hamid R, Ahmad A and Usup G 2016 *Environmental Science and Pollution Research*. 1-8. doi: 10.1007/s11356-016-6655-8
[6] Hamed A H, Ibrahim I, Baumgartner W, Lawrence M L and Karsi A 2017 *Frontiers in Microbiology* 8: 1519 doi.org/10.3389/fmicb.2017.01519

[7] Souza C F, Baldissera M, Descovi S N, Zeppenfeld C C, Verdi C M, Santos R C V, da Silva A S and Baldisserotto B 2019 *Microbial Pathogenesis* 129: 271-276 doi: 10.1016/j.micpath.2019.02.024
[8] Algammal A M, Mabrok M, Sivaramasamy E, Youssef F M, Atwa M H, El-kholy A W, Hetta H F and Hozzein W N 2020 *Scientific Reports* 10 (15961) doi: 10.1038/s41598-020-72264-4
[9] Altinok I, Kayis S and Capkin E 2006 *Aquaculture* 261: 850-855 doi:10.1016/j.aquaculture.2006.09.009
[10] Baldissera M D, Souza C F, Santos R C V, da Rosa L V C, Rosemberg D B and Baldisserotto B 2017 *Aquaculture* 473: 74-79 doi:10.1016/j.aquaculture.2017.02.004
[11] Lee J and Zhang L 2015 *Protein and cell* 6(1): 2641 doi: 10.1007/s13238-014-0100-x
[12] Zhao Z, Liu J, Deng Y, Huang W, Ren C, Call D R and Hu C 2018 *Virulence* 9(1): 318-330 doi:10.1080/21505594.2017.1414134
[13] Wang Y D, Wang Y H, Hui C F and Chen J Y 2016 *Fish Shellfish Immunology* 52: 31-43 doi: 10.1016/j.fsi.2016.03.013
[14] Rameshkumar P, Nazar A K A, Pradeep M A, Kalidas C, Jayakumar R, Tamilmani G, Sakthivel M, Samal A K, Sirajudeen S, Venkatesan V and Nazeera B M 2017 *Letters in Applied Microbiology* 65(5): 423-430 doi: 10.1111/lam.12800
[15] Gong Q, Yang M, Yang L, Chen Z, Jiang M and Peng B 2020 *Microbial Biotechnology* 13 (3): 796-812 doi: 10.1111/1751-7915.13553
[16] Rodriguez-Mozaz S, Vaz-Moreira I, Giustina S V D, Llorca M, Barcelo D, Schubert S, Berendonk T U, Michael-Kordatou I, Fatta-Kassinos D, Martinez J L, Elpers C, Henriques I, Jaeger T, Schwartz T, Paulshus E, O'Sullivan K, Parnanen K M M, Virta M, Do T T, Walsh F and Manaia C M 2020 *Environment International*. 140(105733): 1-11 doi.org/10.1016/j.envint.2020.105733
[17] Rasul M G and Majumdar B C 2017 *The Saudi Journal of Life Sciences*. 2(3): 81–88. doi: 10.21276/haya
[18] Mutiawati V K 2016 *Jurnal Kedokteran Syiah Kuala* 16(1): 53-63 http://jurnal.unsyiah.ac.id/JKS/article/view/5013
[19] Utari C S, Widyarini T, Sutarmiadji D, Dharmawan R and Darukutni 2016 *Jurnal Biofarmasi* 4(1):1-3 doi:10.13057/biofar/f040101
[20] Purwanti N U and Susanti R 2022 *Jurnal Sains dan Kesehatan* 4(1): 56-61 https://doi.org/10.25026/jsk.v4i1.1092
[21] Mainawati D, Brahmana E M and Mubarrak J 2017 *Jurnal FMIPA UR* 1 (1): 1-6 https://www.neliti.com/id/publications/189221/
[22] Nasir O 2021 *Saudi Journal Biology Science* 28(5): 2701-2708 doi: 10.1016/j.sjbs.2021.02.033
[23] Das B K, Swamy A H M V, Koti B C and Gadad P C 2019 *Eliyon* 5(5): e01585 doi: 10.1016/j.heliyon.2019.e01585
[24] Khadijah, Jayali A M, Umar S and Sasmita I 2017 *Jurnal Kimia Mulawarman* 15(1): 11-18 https://doi.org/10.30872/jkm.v15i1.495
[25] Tahir M, Muflihunna A and Syfrianti 2017 *Jurnal Fitofarmaka Indonesia* 4(1): 215-218 https://doi.org/10.33096/jffi.v4i1.231
[26] Kumalasari M L F and Andiarna F 2020 *Indonesian Journal for Health Sciences* 4(1): 39-44 doi: 10.24269/ijhs.v4i1.2279
[27] Masadi Y I, Lestari T and Dewi I K 2018 *Jurnal Kebidanan dan Kesehatan Tradisional* 3(1): 32-40 http://jurnal.poltekkes-solo.ac.id/index.php/JKK/article/view/ 373/326
[28] Aribowo A I, Lubis C F, Urbaningrum L M, Rahmawati N D and Anggraini S 2021 *Jurnal Health Sains* 2(6): 751-757 https://doi.org/10.46799/jhs.v2i6.188
[29] Davis W W and Stout T R 1971 Applied and Environmental Microbiology 22(4): 666-670 https://doi.org/10.1128/am.22.4.666-670.1971
[30] Rahmah R P A, Bahar M and Harjono Y 2017 *Biogenesis* 5(1): 34-41 https://doi.org/10.24252/bio.v5i1.3431
[31] Hastuti U S, Rahmawati D, Sari R Y, Fitri R D and Al Asna P M 2019 *AIP Conference Proceedings* 050002 https://doi.org/10.1063/1.5061895
[32] Hardiansi F, Afriliana D, Munteira A and Wijayanti E D 2020 *Pharmacy Medical Journal* 3(1): 16-22 https://doi.org/10.35799/pmj.3.1.2020.28959

[33] Ravi L, Manasvi V and Praveena L B 2016 *Asian Journal of Pharmaceutical and Clinical Research* 9(2): 344- 347 https://doi.org/10.22159/ajpcr.2016.v9s3.15064
[34] Xiu P, Liu R, Zhang D and Sun C 2017 *Applied and Environmental Microbiology*. 83(12): 1-14. doi:10.1128/AEM.00450-17
[35] Manik D F, Hertiani T dan Anshory H 2016 *Jurnal Khazanah* 6(2): 1-11 doi:10.20885/khazanah.vol6.iss2.art1
[36] Gorniak I, Bartoszewski R and Kroliczewski J 2019 *Phytochemistry Reviews* 18(2) :241–272. doi:10.1007/s11101-018-9591-z

Setiaji_2022_IOP_Conf._Ser.__Earth_Environ._Sci._1118_0120...

ORIGINALITY REPORT

13%	9%	12%	7%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Universitas Brawijaya Student Paper	6%
2	Frans Selfanay, Syahran Wael, Theopilus Wilhelmus Watuguly. "Inhibition of methanol extract snail gonggong (Strombus urturella) to pathogenic bacteria Vibrio alginolyticus", BIOEDUPAT: Pattimura Journal of Biology and Learning, 2022 Publication	1%
3	N Nursyirwani, D Yoswaty, D A Oktavia. "Growth of Bacillus toyonensis in Tofu Wastewater", IOP Conference Series: Earth and Environmental Science, 2021 Publication	1%
4	eprints.uniska-bjm.ac.id Internet Source	1%
5	pearl.plymouth.ac.uk Internet Source	1%
6	Arga Setyo Adji, Nabila Atika, Yemima Billyana Kusbijantoro, Atiyatum Billah, Astrid Putri, Fitri	1%

Handajani. "A review of Leaves and Seeds Moringa oleifera Extract: The potential Moringa oleifera as Antibacterial, Anti-Inflammatory, Antidiarrhoeal, And Antiulcer Approaches To Bacterial Gastroenteritis", Open Access Macedonian Journal of Medical Sciences, 2022
Publication

7	www.science.gov Internet Source	1 %
8	repo.untag-banyuwangi.ac.id Internet Source	1 %
9	iopscience.iop.org Internet Source	1 %
10	Febri Doni, Nurul Shamsinah Mohd Suhaimi, Muhamad Shakirin Mispan, F Fathurrahman et al. "Microbial Contributions for Rice Production: From Conventional Crop Management to the Use of 'Omics' Technologies", International Journal of Molecular Sciences, 2022 Publication	1 %

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		