



A Mini-Review: Effect of *Pandanus amaryllifolius* Roxb Leaves for Fish Health

Rosidah^{1*}

¹*Fisheries Departement, Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Bandung, Indonesia.*

Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

In fish farming, problems often occur due to disease attacks. Disease attack can result in decreased production and quality of fish. This is very detrimental to cultivators. Disease control is an important factor to be acknowledged. The use of herbs is an alternative for disease control that is relatively safe. *Pandanus amaryllifolius* Roxb plants are known to contain several secondary metabolite compounds, namely achaloid, flavonoids, tannins and steroids which are antibacterial and antioxidant. The results of the study show that this plant species from the Pandanaceae family can be used for treatment and prevention of disease-infected fish. The purpose of this article is to describe the compounds contained in *P. amaryllifolius* and their benefits for fish health. *P. amaryllifolius* gives a positive effect on health of fish, it can be used to treat and prevent disease in fishes.

Keywords: *P. amaryllifolius roxb*; disease, treatment; prevention; immunostimulant.

1. INTRODUCTION

Fish is a fishery commodity that is developed to support food security and economic resilience,

as well as increase the welfare of the community. In fish farming, problems often occur due to disease attacks. Disease attack can result in decreased fish production and quality [1].

*Corresponding author: Email: ros_ahdi@yahoo.com;

Diseases in fish can be caused by various disease agents, such as bacteria, viruses, fungi and parasites that are pathogenic [2]. Bacteria as the cause of fish diseases which can have a decreasing impact on the amount of fish production and are economically very detrimental [3]. Some of the bacteria that often attack freshwater fish include *Aeromonas hydrophila* [4], *Streptococcus agalactiae*, *Streptococcus innae* [5].

The prevention of disease in fish is mostly carried out by fish farmers using antibiotics. However, long-term use of antibiotics can have several negative effects, including environmental pollution due to antibiotic residues and reduced effect of antibiotics due to pathogen resistance [6]. Antibiotics are used in aquaculture as a prophylactic or therapeutic measures or as feed additives and gain access to the pond environment through using human and animal wastes or integrated fish farming system. Here has been a surge in the number of foodborne infections caused by antibiotic resistant bacteria (ARB) [7]. Recently, a few studies have shown a direct relationship between antibiotic use in food animals and the emergence of antibiotic resistance in human and animal pathogens [8,9]. Therefore, the development of alternative materials that can replace antibiotics must be the main focus in disease control.

Medicinal plants are alternative materials that can be developed as a substitute for antibiotics. Apart from its abundant availability, this medicinal plant is considered to be more environmentally friendly than antibiotics [10]. According to the World Health Organization, 80% of the populations in developing countries prefer to use herbal extract and their active components as traditional medicine therapy [11]. Medicinal plants contain several bioactive compounds that can be used for the treatment of infectious diseases in the organism (host). These compounds work by disrupting the metabolism of microbes that infect the host. The medicinal properties of plants can be based on their phytochemical content, antimicrobial effects, antioxidant and antipyretic effects [12,13].

Pandanus amaryllifolius Roxb. or commonly called pandanus is a type of monocot plant from the Pandanaceae family [14,15]. This is synonym to *Pandanus hasskarlii* Merr, *Pandanus latifolius* Hask, *Pandanus odoratus* Ridley [16]. The etymologies of this plant are fragrant pandan, fragrant screw pine, pandan. *Pandanus*

amaryllifolius is a tropical plant of Southeast Asian countries including Thailand, Malaysia and Indonesia [17]. This plant is easily found in yards, gardens or grows wild on the edges of ditches that are shady and slightly humid in the tropics [18]. *P. amaryllifolius* can also grow in areas around the coast to plains as high as 500 m above sea level. The height of this plant is about 1-2 m, branching and spreading and has a supporting root. Leaf-shaped ribbons, smooth leaf surface with pointed leaf tips, parallel bones and average edges The average length of the leaves is about 40-80 cm and a width of 3-5 cm [19]. An important component and often used in Indonesia and other Asian countries is the leaves [19].

P. amaryllifolius leaves have many benefits, including the used as a food flavoring agent in many traditional recipes [19]. In Indonesia, apart from being used as food coloring and fragrances, they are also used as room fresheners, medicines and handicraft raw materials [20]. The therapeutic potential of fragrant pandan leaves is as a nervous weakness medicine, rheumatism treatment, aches and pains, black hair, reduces hair loss, eliminates dandruff, increases appetite and treats hypertension [21], cures smallpox, headaches, fever, arthritis, etc. [22]. The leaves and other parts of this plant are also used in medications such as antidiabetic, antidiuretic and cardioprotective agents [23].

P. amaryllifolius is one of such plants with promising antibacterial potential [24]. Several secondary metabolites such as quercetin, carotenoids, tocopherols, tocotrienols, and essential oils are also contained in the leaves [25]. The ethanol extract of the leaves cultivated in Malaysia demonstrated a good scavenging activity [26]. This article aims to describe the compounds contained in *P. amaryllifolius* leaves and their role in fish health.

2. PHYTOCHEMICAL SCREENING OF LEAVES *Pandanus amaryllifolius* ROXB

Based on the results of the phytochemical test, the ethanol extract and ethyl acetate fraction of *Pandanus amaryllifolius* leaves produced almost the same secondary metabolite components, namely alkaloids, flavonoids, phenolics and saponins. Except for the terpenoid compounds identified only in ethanol extract, while steroids were identified in the ethyl acetate fraction. In the hexane solvent, the types of compounds that

were dissolved were less, namely steroids and phenolics, while alkaloids and flavonoids were not detected [27]. This is because steroids are complex fat-soluble molecules [28,29]. In addition, hexane is also a non-polar solvent, so that the components that can dissolve are less. All types of solvents show that the pandan leaf extract does not contain tannins. This is because tannins dissolve more easily in water solvents (27). As according to [30] water can dissolve tannins, alkaloids, and polyphenols. Similar to research conducted by [31] extraction with water as a solvent, besides producing alkaloids, flavonoids, saponins and phenols, it also produces tannins. However, based on the results of a phytochemical screening conducted by [15], it was shown that the results of pandan leaf extraction using water, chloroform and ethanol solvents apart from containing terpenoids, alkaloids, saponins, steroids and flavonoids, were also detected to contain tannins and anthraquinone compounds. The results of phytochemical screening of pandan wangi leaves carried out by [32] using 96% ethanol solvent produced alkaloids, saponins, polyphenolic flavonoids and tannins. (Table 1). In addition, pandan leaves also contain essential oils and aromatic compounds that are volatile, so that pandanus has a strong distinctive aroma. The distinctive aroma of pandan leaves is thought to be derived from the amino acid phenyl alanine derivative compound, 2-acetyl-1-pyrroline [18].

The type of solvent determines the screening results of secondary metabolite compounds contained in a plant, this is because of the different polarity of each solvent [33]. Water as a polar solvent generally dissolves sugar, amino acids, proteins, polyglycosides, tannins, alkaloid salts, and polyphenols [30]. Ethanol, with a lower polarity than water, can dissolve alkaloids, diglycosides, phenolics, flavonoids, and small amounts of essential oils [34,35]. Ethyl acetate with the relatively lowest polarity is able to dissolve alkaloid, aglycone, monoglycoside, terpenoid, and steroid class compounds [36].

The above descriptions are seen with the same solvent, resulting in different secondary metabolite compounds. According to [37] chemical compounds in a plant are strongly influenced by several factors, including plant age or leaf age, harvest time, and the environment in which it grows (ecophysiology). Plant age and harvest time are one of the stages in the process of cultivating medicinal plants. Harvest time is usually related to plant age (maturation level)

and this is a critical phase because it determines the quality and quantity of yields, therefore each type of plant has a different time and method of harvesting [37]. As according to [38] the phytochemical content of an extracted plant depends on the nature of the material and plant origin, processing level, moisture content and particle size.

The type of solvent not only affects the quality of the secondary metabolite screening results, but also affects quantity of these results. The results of [35] showed differences in the number of different phenolic and flavonoid levels with different extract ingredients. The total phenolic content of water extract of pandan wangi leaves is 24,0004 mg / g of extract; with 96% ethanol extract of 478.7629 mg / g extract; with 96% water and ethanol extract with a ratio of 0.5: 0.5 of 308.9702 mg / g extract. While the total flavonoid content of the water extract of *P. amaryllifolius* leaves was 4.6102 mg / g of extract; with 96% ethanol extract of 99.4086 mg / g extract; with 96% water and ethanol extract with a ratio of 0.5: 0.5 of 33.6216 mg / g extract. The optimization results showed that the maximum extraction solution for extracting phenolic and flavonoid compounds was 96% ethanol with a total phenolic content of 478.7629 mg / g and a total flavonoid level of 99.4086 mg / g. The content of phenolic compounds is much higher than flavonoid compounds, this shows that phenolic compounds are the largest compounds in fragrant pandan leaves. High phenolic compounds will increase the pharmacological activity of *P. amaryllifolius* leaves as antimicrobial, antioxidant, anti-inflammatory, anti-allergic, anti-carcinogenic, antihypertensive, cardioprotective, and anti-arthritic activities [39-44].

3. ANTIBACTERIAL EFFECT OF *Pandanus amaryllifolius* ROXB. LEAVES

Pandanus amaryllifolius leaves are generally used as a colorant and flavoring agent for food. It will be very strategic if it is developed as a medicinal ingredient to treat disorders / diseases caused by bacteria. The high use of synthetic medicinal ingredients needs to be balanced with efforts to develop natural medicinal ingredients that are relatively safer and applicable, through their ability to suppress bacterial growth, especially those that attack cultured fish, among others, *Aeromonas hydrophila*, *Escherichia coli* and *Staphylococcus aureus*, because bacterial

Table 1. Phytochemical screening of *Pandanus amaryllifolius*

Extract solvent	Alkaloids	Flavonoids	Tannins	Saponins	Steroids	Phenols	Terpenoids	Ref
Ethanol	+	+	-	+	-	+	+	27
Ethyl acetat	+	+	-	+	+	+	-	27
Hexane	-	-	-	-	+	+	-	27
water	+	+	+	-	-	+	-	30
water	+	+	+	+	-	-	+	15
Chloroform	+	+	+	-	-	-	+	15
Ethanol	+	+	+	-	-	-	+	15
Ethanol	+	+	+	+	-	+	-	32
Water	+	+	+	+	-	+	-	31

diseases are an obstacle for fish cultivators in the world. besides *E. coli* and *S. aureus* are also bacteria that indicate food safety [18]. Based on the results of phytochemical tests, *P. amaryllifolius* leaves contain several secondary metabolite compounds including flavonoids, alkaloids, saponins, tannins, polyphenols, and dyes, which have a contribution to antibacterial activity [45].

Flavonoids have antibacterial activity because they are able to combine with bacterial cell membranes and extracellular proteins [46]. Flavonoids as antimicrobe agent work to inhibit nucleic acid synthesis, to inhibit the function of the cell membrane, and to inhibit the energy metabolism [47] while saponins are cytotoxic because they can change the permeability of microbial cytoplasm, causing microbial cells to undergo lysis [46]. Steroids can inhibit bacterial growth by interacting with cell membrane phospholipids which are permeable to lipophilic compounds, causing decreased membrane integrity and altered cell membrane morphology which causes brittle cells and lysis [48]. Terpenoids can inhibit bacteria through a mechanism of action that involves damage to the membrane by lipophilic compounds in which bacterial growth is inhibited or dies [12]. Phenolic compounds in killing microorganisms by denaturing cell proteins [49]. Alkaloid compounds inhibit bacterial growth by disrupting the peptidoglycan constituent components in bacterial cells so that the cell wall layer is not

formed completely and causes cell death [50].

Several studies have proven that fragrant pandan leaf extract is antibacterial for pathogenic bacteria that infect cultured fish, namely *Streptococcus agalactiae*, and other *Streptococcus* species, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. The sensitivity effect of *P. amaryllifolius* leaves as antibacterial depends on the type of solvent used in making the extract, the concentration of the extract and the type of bacteria. The ethanol extract of *P. amaryllifolius* leaves at a concentration of 1200 ppm produces an inhibition zone of 14.30 mm against *Aeromonas hydrophila* bacteria [51], while for *S. aureus* bacteria produces an inhibition zone of 13 mm at a concentration of 14000 ppm (40%) [24]. The 10% (10,000 ppm) ethanol extract produced an inhibition zone of 13.23 for *S. aureus* bacteria [32]. Ethyl acetate extract of 5 mg / disc resulted in an inhibition zone of 11.3 ± 0.6 mm for *S. aureus* and 26.0 ± 0 mm for *E. coli* bacteria [33]. *P. amaryllifolius* leaf extract with a mixture of ethanol and ethyl acetate solvents at a concentration of 5 mg / disc produces an inhibition zone of 15.7 ± 0.6 for *Staphylococcus aureus* bacteria, while for *E. coli* bacteria produces an inhibition zone of 17.7 ± 1.2 mm [33]. Here, it can be seen that the extract of *P. amaryllifolius* leaves with a mixture of ethanol and ethyl acetate solvent produced greater inhibition than ethanol or ethyl acetate solvent alone (Table 2).

Table 2. Antibacterial activity of *P. amaryllifolius* leaves

Extract	Concentration Extract	Types of bacteria	Inhibition zone(mm)	Ref.
Ethanol	1200 ppm	<i>Aeromonas hydrophila</i>	14.30	51
Ethanol	40%	<i>Staphylococcus aureus</i> .	13	24
Ethanolic	10%	<i>Escherichia coli</i>	13.13	32
Ethanolic	10%	<i>Streptococcus aureus</i>	13.23	32
Ethyl acetat	5 mg/disc	<i>Staphylococcus aureus</i> .	11.3 ± 0.6	33
Ethyl acetat	5 mg/disc	<i>E. coli</i>	26.0 ± 0	33
Ethanol-etil acetate	5 mg/disc	<i>Staphylococcus aureus</i> .	15.7 ± 0.6	33
Ethanol-etil acetate	5 mg/disc	<i>E. coli</i>	17.7 ± 1.2	33

4. ANTIBACTERIAL OF *Pandanus amaryllifolius* Roxb LEAVES

Based on the results of phytochemical screening, *Pandanus amaryllifolius* leaves contain several secondary metabolite compounds including alkaloids, flavonoids, tannins, saponins, steroids, phenols and terpenoids [15,30]. Some of these compounds are known to have antioxidant activity [51,52]. The antioxidant activity in the water extract of pandan wangi leaves was 66.82% [31].

Based on the results of research conducted by [53] showed 1 mg of ethanol extract of *P. amaryllifolius* contains 57.02 ± 0.02 mg/gm GAE (Gallic acid equivalent) phenolic compounds. Then the antioxidant test was carried out on the ethanol extract of *P. amaryllifolius* using the DPPH (diphenyl-picryl hydrazyl) method, using vitamin C as a positive control and BHT (butylated hydroxytoluene) as a negative control. The results show *P. amaryllifolius* ethanol leaf extract exhibited significant DPPH (diphenyl-picryl hydrazyl) radical scavenging activity with IC50 values 110.57 ± 36.42 $\mu\text{g} / \text{mL}$ compared to vitamin C IC50 values 7.79 ± 1.25 $\mu\text{g} / \text{mL}$ and BHxyT (butylated hydroxytoluene) IC50 values 93.16 ± 10.46 $\mu\text{g} / \text{mL}$. Here it is seen that there is a correlation between the total phenolic content and DPPH radical scavenging activity. This shows that phenolic compounds from plant extracts contribute more than 50% to antioxidant activity. The results showed that the index antioxidant activities of *Pandanus amaryllifolius* by IC50 (110.57 ± 36.42 $\mu\text{g} / \text{mL}$) was categorized as moderate scale [54]. The levels of antioxidant power is divided into 4 levels, namely very strong (IC50 <50 $\mu\text{g} / \text{mL}$), strong (IC50 50-100 $\mu\text{g} / \text{mL}$), moderate (IC50 100-250 $\mu\text{g} / \text{mL}$), and weak (IC50 250- 500 $\mu\text{g} / \text{mL}$) [55].

The temperature and time of pandan leaf extraction determine the extract yield and levels of phenolic compounds or Total Phenolic Content (TPC) and indirectly affect the antioxidant activity. As the results of research conducted by [14] showed that the best antioxidant activity was obtained from ethanol extract of 96% *P. amaryllifolius* leaves at an extraction temperature of 50°C with an extraction time of 5.5 hours, with a scavenging activity of 93.21%, while at the extract temperature. 70°C, the percentage of scavenging activity obtained was only 90.74%. This happened because with an extraction time of 5.5 hours at a temperature of 50 ° C, the TPC value was greater than that of 70°C, at high

temperatures (70°C) the fragrant pandanus extract began to deteriorate.

The results of research conducted by [27] showed that the extract solvent had an effect on the antioxidant content contained in the extract of *P. amaryllifolius* leaves. The antioxidant activity of *P. amaryllifolius* extract from various types of extract solutions was tested by observing the reduction power with the ferritocyanate (FTC) method and the DPPH radical scavenging capacity and the EC50 value.

The results of the reduction power test showed that the extract of *P. amaryllifolius* leaves with ethyl acetate fraction solvent had greater reducing ability than the ethanol extract. Meanwhile, the hexane fraction has the lowest reducing ability.

Based on the results of the DPPH radical scavenging power test, the ethanol extract of *P. amaryllifolius* leaves and its fractions has the ability to scavenge radicals higher than commercial vitamin E, but lower than BHT (butylated hydroxytoluene). This can be seen from the value of RSA (Radical Scavenging Activity), where vitamin E has an RSA value of 24.15%, while ethanol extract is 69.96%, hexane fraction 54.81%, ethyl acetate fraction 54.60% and BHT 84.80. %. The difference in the antioxidant ability against DPPH free radicals is due to the ability to transfer different hydrogen atoms [56]. Other factors that influence the ability to scavenge free radicals are the polarity of the reagent medium, the chemical structure of the radical scavenger, and the pH of the reaction mixture [57].

Based on the calculation of the EC50 value of *P. amaryllifolius* extract from each solvent is different. The EC50 value is the concentration level of the test compound that can cause antioxidant activity by 50%, the EC50 value (Effective Concentration Value) correlates with the level of effectiveness of antioxidant activity [58]. The EC50 value is inversely related to antioxidant activity. The smaller the EC50 value, the greater the antioxidant activity. The ethanol extract of *P. amaryllifolius* leaves has an EC50 value of 4.51 mg / mL, while the ethyl acetate fraction is 0.90 mg / mL, and the hexane fraction is 8.66 mg / mL. The three of them have a smaller EC50 value than vitamin E, namely 11.76 mg / mL, so that when viewed from the reducing power, the ethanol extract of *P. amaryllifolius* leaves and its fractions has higher antioxidant

activity than commercial vitamin E. The EC50 value of the extract / fraction was still lower than the EC50 BHT (butylated hydroxytoluene) value of 0.59 mg / mL [59]. From this it can be seen that the ethyl acetate fraction has the largest antioxidant activity, followed by ethanol extract and the lowest is the hexane fraction. This occurs in relation to the quantity and type of compound extracted. Ethyl acetate fraction had the highest phenolic and flavonoid levels, followed by ethanol extract and the lowest was the hexane fraction. The types of compounds extracted in the ethyl acetate fraction include alkaloids, flavonoids, phenolics, steroids and saponins, the ethanol extract is almost the same, namely alkaloids, flavonoids, phenolics, terpenoids and saponins, while the hexane fraction is only phenolic and steroid only. The types of compounds extracted in the hexane fraction were phenolic and steroid only. The difference in the extracted compounds from each solvent is due to differences in the polarity of the solvent. Ethanol has a lower polarity than water so that it can dissolve alkaloid compounds, diglycosides, phenolics, flavonoids, and small amounts of essential oils [33,34]. While ethyl acetate is a semipolar solvent so that it can still dissolve polar and non-polar components and hexane is a non-polar solvent so that only a few components of the ethanol extract can dissolve [60]. From the description above, it shows that the ethyl acetate fraction of the ethanol extract of *P. amaryllifolius* leaves has the potential as a source of natural antioxidants.

5. TREATMENT EFFECTS OF *Pandanus amaryllifolius* ROXB LEAVES EXTRACT ON FISH HEALTH

The use of *Pandanus amaryllifolius* leaf extract as medicine in fish infected with bacteria is based on the ability of several chemical compounds contained in the extract to be antibacterial. This is in accordance with the results of phytochemical tests that have been carried out by [61], which states that *P. amaryllifolius* leaves have chemical content including alkaloid compounds, flavonoids, anthraquinones and tannins which are antibacterial compounds. Research conducted by [51] proved that Sangkuriang catfish *Clarias gariepinus* (Burchell 1822) juvenile infected with *Aeromonas hydrophila* bacteria experienced healing after being treated with fragrant pandan leaf extract through immersion for 24 hours. A concentration of 800 ppm is the best concentration, indicated by a faster healing

process, namely on the 5th day the wound on the body's surface has started to decrease and on the 10th day there is a complete healing and results in the highest survival compared to other treatments (400, 600 and 1000 ppm) which is equal to 86.67%.

6. IMMUNOSTIMULANT EFFECTS OF *P. amaryllifolius* (ROXB.) LEAVES EXTRACT ON FISH HEALTH

The use of *Pandanus amaryllifolius* extract as an immunostimulant to increase the resistance of fish bodies against pathogenic bacteria has not been studied. However, several studies have shown that *P. amaryllifolius* extract contains flavonoids and saponins which act as antioxidants [34,36] because they are able to stop free radical chain reactions. Meanwhile, antioxidants have an immunostimulating effect that can increase non-specific resistance [62]. Like other types of the Pandanaceae family, *P. tectorius* also contains antioxidants [63,64] and has been shown to have an immunostimulating effect that can prevent the attack of *Yersinia ruckeri* bacteria on rainbow trout [65]. The results showed that the activity of lysozyme and serum total protein as humoral immune (non-specific immunity) increased in the rainbow trout group after being fed with *P. tectorius* extract with a dose of 0.5% and 2% for 2 weeks, the highest increase occurred at the dose. 2% [65]. After the challenge test was carried out with the *Yersinia ruckeri* bacteria, the test fish that were fed with 2% *P. tectorius* extract added the highest survival. Lysozymes are non-specific immune which plays an important role in the defense mechanism of fish against disease, by activating the complement system and phagocytes [66]. Likewise serum protein is an important parameter in the humoral immune system in fish, it plays an important role in maintaining fish health. Moreover, the most important role played by serum proteins in the acute phase is to limit the spread of infectious agents through repairing tissue damage and killing micro-organisms [67,68]. Alkaloids include secondary metabolite compounds contained in fragrant pandan leaves and are found in almost all types of plants [69], with levels ranging from 10-15% [70] as well as a strong activator for immune cells that can destroy bacteria, viruses, fungi, and cancer cells [37].

7. CONCLUSION

Pandanus amaryllifolius Roxb contains various compounds that are antibacterial, antioxidant and

immunotimulant, so that they have a positive effect on fish health, which can be used to treat and prevent fish diseases.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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