



Effect of Galangal (*Alpinia galanga* L.) Rhizome for the Health of Cultured Fish

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Author's contribution

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

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ABSTRACT

For fish farmers, the disease are the biggest cause of loss. In an effort to control the disease is often done by using synthetic antibiotics. The continuous use of synthetic antibiotics with uncontrolled doses can lead to resistance of pathogenic microbes to these antibiotics, accumulation of antibiotics in the aquatic environment and fish bodies which is certainly dangerous for human health and other aquatic biota. The use of herbal ingredients is the right strategy for disease control in fish, because it is relatively safer and cheaper. Medicinal ingredients derived from plants have been used for thousands of years as traditional medicine by people in various parts of the world. This traditional medicine is widely used by the community not only for treatment (curative), but also as an alternative to prevent disease, to rehabilitation and also to increase immunity and health. Galangal rhizome contains several chemical compounds, namely phenols/tannins, quinones, steroids/triterpenoids, flavonoids. Based on the phytochemical content, galangal rhizome has several pharmacological property that are useful for human health, namely as antibacterial, anti-fungal, anti-inflammatory, anti-hepatotoxic, antioxidant, immunostimulant, anti-ulcerative, anti-tumor, and anti-allergenic activities. In addition, galangal (*Alpinia galanga* L.) rhizomes can improve

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growth and feed efficiency. The purpose of this article is to describe the extent to which galangal rhizomes can be used to maintain the health of cultured fish. Based on the previous studies, galangal rhizome is potential and effective for maintaining fish health, because the antibacterial properties of legkuas rhizome can treat several species of cultured fish *Cyprinus carpio*, *Oreochromis niloticus* and *Clarias gariepinus* which are infected with the pathogenic bacteria *Aeromonas hydrophilla*. In vitro test results of galangal rhizome are bacteriostatic against pathogenic bacteria in fish, namely *Edwardsiella tarda*. The antifungal properties of galangal rhizomes can treat *Oreochromis niloticus*, *Kryptoterus bicirrhis*, *Osphronemus gouramy* and *Borbonymus gonionotus* infested with fungus *Saprolegnia* sp. As an immunostimulant, galangal rhizome is able to prevent eggs and fry of *C. gariepinus* from being attacked by *Saprolegnia* sp., *Pseudomonas* sp. and *A. hydrophilla*.

Keywords: Disease; galangal (*Alpinia galanga* L.) rhizome; immunostimulant; prevention; treatment.

1. INTRODUCTION

Fish health is an indicator of success in fish farming. Fish health conditions will affect business development and increased production. Therefore the case of disease in fish is an important problem to be taken seriously. Fish can be attacked by several infectious or non-infectious diseases. Bacteria, viruses, parasites and fungi are organisms that cause infectious diseases, while the environment and unsuitable feed and malnutrition are the causes of non-infectious diseases. Several types of synthetic antibiotics and vaccines are often used to treat this infectious disease, including oxytetracyclin, erythromycin, kanamycin, and rimfamycin [1]. However, the negative impact arising from the use of these antibiotics, if used in a sustainable and uncontrolled manner, will cause bacterial resistance to these antibiotics. Meanwhile, the emergence of antibiotic-resistant pathogenic bacteria is an important problem that must be avoided in aquaculture activities. Another negative impact of using synthetic antibiotics is that they can cause residues in the aquatic environment and can accumulate in fish bodies which will certainly have an impact on reducing the quality of fishery products and harming consumers [2,3]. Another obstacle is the limited number of registered and marketed vaccines [4]. Therefore, it is necessary to find and develop alternative materials as antibacterial and immunostimulatory that are safer for the treatment of infectious and non-infectious diseases, one of which is by using natural ingredients derived from plants.

The use of natural ingredients has smaller side effects, the price is more economical and the ingredients are easily available [1,5]. One of the plants that can be used as a medicinal ingredient

is galangal rhizome which belongs to the Zingiberaceae family. Several studies have shown that the rhizome of plants from the Zingiberaceae family has several active compounds that act as antioxidants, anti-inflammatories, and antimicrobials. In addition, it can be used to increase growth, feed efficiency, increase the immune response [6,7]. Likewise, The galangal rhizome is effective for use as a therapy for several kinds of diseases, because it has an antibacterial, anti-fungal, anti-inflammatory, anti-hepatotoxic, antioxidant, immunodulator, anti-ulcerative, anti-tumor, and anti-allergic activities [8], so that it can be used for the treatment or prevention of several fish diseases caused by infectious disease agents. This article aims to describe the compounds contained in galangal and their role for fish health.

2. CLASSIFICATION AND MORPHOLOGY OF *Alpinia galanga* L.

Alpinia galanga L. Swartz (Fig. 1) belongs to the Zingiberaceae family originating from tropical Asia and it is widespread across several countries including Malaysia, Thailand, Indonesia, Egypt, Sri Lanka, Arabic Gulf areas and China. This plant is known by several regional names, including laos/laja/galangal (Indonesia), greater galangal (English), kulanjan (India), dhumarasmī (Canada), galanga (France), Kha (Thailand), hongdouku (China) and grotegalanga (Netherlands). Based on its Latin name, *Alpinia galanga* has several synonym names, namely *Alpinia pyramidata* Bl, *Alpinia officinarum* Hance, *Languas galanga* (L.) Merr., *Languas vulgare* Koenig, *Marantagalanga* L., *Amomum galanga* (L.) Lour, and *Amomum medium* Lour [9,10]. The classification of galangal as follows:

Kingdom : Plantae
 Phylum : Magnoliophyta
 Class : Liliopsida
 Order : Zingiberales
 Family : Zingiberaceae
 Subfamily : Alpinioideae
 Tribe : Alpinieae
 Genus : Alpinia
 Species : *Alpinia galanga* [11]



Fig. 1. Galangal plants and rhizomes

Source: Darsatop.lecture.ub.ac.id

Galangal is a long-lived plant, grows in open and sunny places, and it is found in forests and shrubs. This plant is about 1-2 meters high, can even reach 3.5 meters, grows in dense clumps. The stem is erect, composed of leaf sheaths which unite to form a slightly whitish green pseudo-stem. Young stems emerge as shoots from the base of the old stem. The galangal plant consists of leaves, flowers, fruit, and rhizomes. Elongated lanceolate leaves measuring 20-45 cm, 4-15 cm wide, blunt base, pointed tip, with flat leaf edges. Bell-shaped flowers include compound interest, smell good, greenish white or yellowish, 10-30 cm long and 5-7 cm wide. Galangal fruit hard, round shape with a diameter of about 1 cm. Young fruit is yellowish green, old fruit is dark red or blackish red. Galangal rhizome is quite large and thick, fleshy, cylindrical in shape, about 2-4 cm in diameter, and has many branches. The seeds are black, small and oval in shape. The outside is slightly reddish brown or pale greenish yellow, has white or reddish scales, shiny hard, while the inside is white. The structure of old rhizomes is coarse fibrous, has a sharp, pungent taste with a fragrant smell [12,13,14].

3. THE CONTENT AND BENEFITS OF GALANGAL

Based on the results of qualitative phytochemical tests, the rhizome of red and white galangal

contained several secondary metabolite compounds, including phenolics/tannins, quinones, steroids/triterpenoids, and flavonoids. However, in the process of extraction, different solvents make a difference to the results obtained. Extraction of red galangal and white galangal using n-hexane solvents only produced phenolics/tannins, quinones, steroids/triterpenoids, whereas using ethyl acetate and ethanol solvents apart from producing tannins, quinones and steroids/ triterpenoids, flavonoids were also found [15]. The results of the phytochemical test on red galangal in fresh and dry samples with the same solvent (n hexana) gave a difference in the results obtained. Extraction results from dry and fresh samples using n hexana solvent both produce alkaloid, phenolic/tannin, terpenoid and steroid compounds, among these compounds, the terpenoid compound group has the highest content. This happens because the n-hexane solvent is nonpolar, while the terpenoid compound group also has nonpolar properties, so it dissolves more easily than other groups of compounds that have nonpolar properties. In the process of extraction, different solvents also give differences in the results of quantitative phytochemical tests on total phenolic content. The total phenol content of red galangal extracted with n-hexane, ethyl acetate and ethanol were 2.60%, 18.47% and 19.61%, respectively, while the levels of these compounds in white galangal using the same solvent, respectively respectively by 3.21%, 23.38%, and 17.19%. From this it can be seen that the highest total phenol content was found in white galangal extract using ethyl acetate solvent, which was 23.381% and the largest group of phenols were flavonoids [15]. The leaves, stems and rhizomes and roots of galangal, apart from containing flavonoids, also contain essential oils/acetoxin-1,8 cineol, which are compounds that give galangal its distinctive aroma [16,17,18]. The terpenoid chemical compounds in galangal contain galanolactone, 16-dial, 12-labdione-1510.25, Galanolactone, 16-dial, 12-labdione-15 which belongs to the diterpene group and 1.8 cineol which belongs to the monoterpene group [19]. Meanwhile, the flavonoid compounds in galangal contain camphorol, galangin and alpinin [20]. 1'S-1'-acetoxychavicol acetate (ACE), is the main compound contained in galangal which has various biological activities [21].

Galangal contains several useful compounds. The traditionally galangal rhizome has long been

used as a spice for food products and also as a good source of essential oil [9,22]. Traditionally, galangal is often used as a medicine for stomach pain, carminative, fever, rheumatism, liver, diabetes, anti-inflammatory, hypo-allergenic and anti-hypoglycemic, ulcers, cholera and even HIV [23,24,19,9]. The pharmacological activity of the galangal plant it has an effect as antibacterial, antifungal, antiviral, antiprotozoal [25,26,27], immunomodulator, antioxidant, antidiabetic, antiplatelet, hypolipidemic, antitumor, antihelmintic, antidiuretic and many other pharmaceutical function [20,24].

4. EFFECT OF GALANGAL RHIZOME ON FISH HEALTH

4.1 Antibacterial Activity

It has been stated above that galangal has activity as an antibacterial. In vitro test results, 96% ethanol extract of red galangal rhizome (*Alpinia purpurata*) at concentrations of 250, 500, 750 and 1000 ppm can inhibit the growth of pathogenic bacteria that cause disease in cultivated fish, namely *Aeromonas hydrophila* (density 10^8 CFU/mL) by producing an inhibition zone different. The largest inhibition zone was obtained at a concentration of 1000 ppm, which was 9.7625 mm. The diameter of the inhibition zone formed was categorized as moderate sensitivity as an antibacterial. Another study showed the same results, that galangal rhizomes have antibacterial activity which can inhibit the growth of *A. hydrophila* bacteria. The results obtained show that galangal rhizome juice has inhibitory and lethal power against *A. hydrophila* bacteria. The minimum concentration of galangal rhizome juice which has killing power against *A. hydrophila* at a density of 10^8 CFU/ml is 50% (0.835 g/mL). Other bacteria such as *Escherichia coli*, *Salmonella bacillus subtilis* and *Staphylococcus aureus* can be inhibited by using galangal rhizomes. The in vivo test results showed that 8 grams of galangal dissolved in ethanol could inhibit the growth of these bacteria by producing an inhibition zone diameter of between 7 mm and 10 mm [28]. It was reported that one of the secondary metabolite compounds contained in galangal rhizome that has antibacterial activity are flavonoids, which are phenolic compounds. At low concentrations, phenols work by damaging cell membranes, causing cell leakage. At high concentrations, phenol can coagulate with cellular proteins (protein denaturation) and cause cell membranes to become thin. This activity is very effective

when the bacteria are in the division stage, where the phospholipid layer around the cell is very thin so that phenol can penetrate optimally and damage the microbial cell membrane and denatures microbial cell proteins.

This causes cell metabolic activity to stop which results in the death of bacterial cells [29,30]. Antimicrobial activity depends on the concentration of bioactive compounds it contains [31]. Based on the results of the inhibition test, the 96% ethanol extract of red galangal (*Alpinia purpurata*) showed antibacterial activity in the case of some fish diseases, namely *Edwardsiella tarda* after 48 hours of incubation. A dose of 500 ppm provides the highest effectiveness with an inhibition zone diameter of $7.46 + 0.09$ mm, the diameter of which is 32 is categorized as a very strong inhibition zone [32]. The resulting inhibitory effect is 54.85%. SEM (Scanning Electron Microscope) test showed that red galangal extract could lyse *E. tarda* bacteria [33]. This proved that red galangal had anti-bacterial compounds including flavonoids, tannins and alkaloids. Red galangal as an antibacterial works by inhibiting nucleic acid synthesis and disrupting the function of cell membranes. Flavonoids form complex compounds with extracellular proteins, the cytoplasmic membrane of microbial cells being damaged and microbial metabolism it is inhibited afterwards [34]. Alkaloids work by inhibiting the work of enzymes that play a role in DNA replication, resulting in bacteria being unable to divide and grow [35]. Another opinion explains that alkaloids as antibacterial work by interfering with the formation of cross bridges that form the peptidoglycan component of bacterial cells, as a result the bacterial cell wall layer is lysed [36]. Tannins work by has deactivating adhesins, enzymes, and cell envelope proteins. Besides that tannins inhibit bacterial growth by forming hydrogen bonds with proteins in bacterial cells, as a result the denatured protein in bacterial cells and bacterial metabolism will be disrupted [37]. In vivo test results from several researchers showed that galangal rhizome has beneficial effect with several types of cultivated consumption fish, such as common carp (*Cyprinus carpio*), Nile tilapia and north African catfish (*Clarias gariepinus*). Infected with the pathogen *A. hydrophila* 96% ethanol extract of red galangal (*A. purpurata*) rhizome at a concentration of 1000 ppm can significantly treat Nile tilapia infected with *A. hydrophila* bacteria at a density of 10^8 CFU/mL by soaking for 5 minutes. Healing was seen after seven days of treatment [5].

Another study showed that catfish (*Clarias gariepinus*) fry infected with *A. hydrophila* bacteria with a density of 10^8 CFU/mL experienced healing after being treated with 96% ethanol extract of galangal rhizome by soaking for 24 hours. The concentration of 385.88 ppm is an effective concentration [38].

4.2 Antifungal Activity

Galangal rhizome also has activity as an antifungal. Secondary metabolites contained in galangal, namely flavonoids, alkaloids, saponins, tannins, triterpenoids, hydroquinone phenolic compounds and steroids have potential as antimicrobials, including as antifungal drugs [39,40]. The alkaloid content inhibits the development of *Candida albicans* by means of nucleic acid biosynthesis, alkaloids bind to cell DNA and interfere with cell function in microbes or fungi. Meanwhile, flavonoids inhibit the growth of these fungi through the formation of pseudohyphae [40], and by denaturing microbial cell proteins and can remodel and damage microbial membranes irreparably. Tannins work as antifungals by damaging cell membranes so that microbial growth can be inhibited, while saponins by damaging the cytoplasmic membrane, resulting in the death of fungal/microbe cells [41]. Based on in vitro test results, galangal extract using methanol ethyl acetate and hexane solvents produced different inhibition on the growth of *Candida albicans*. The greatest inhibition occurs by using methanol solvents. Ethyl acetate solvent produces little inhibition, even with hexane solvent the fungus cannot inhibit its growth. This happens because the methanol solvent produces a fraction of 4.70%, while 1.70% ethyl acetate and 0% hexane solvent [19].

In vivo tests regarding the effectiveness of galangal as an antifungal have been carried out by several researchers to control fungi that often attack cultivated fish species, namely the *Saprolegnia* fungus. Tilapia infected with the fungus *Saprolegnia* sp. experienced healing after treatment with galangal extract, with an optimal dose of 90 ppm and a cure percentage of 73.3% [42]. Whereas for healing of Asian glass catfish was obtained at a lower dose of 30.29 mg/l with a cure percentage of 90.30% [43]. Galangal extract concentration of 75 ppm significantly ($P > 5\%$) could cure gourami fry (*Osphronemus goramy*) 5 – 8 cm in size which were infected with *Saprolegnia* sp. through immersion for 12 days, with a healing percentage of $67.14 \pm$

0.58% [44]. Another study showed that a 100 ppm concentration of galangal rhizome could significantly treat silver barb (*Borbonymus gonionotus*) fry infected with the fungus *Saprolegnia* sp., characterized by reduced hypa, wounds caused by the fungal infection began to heal and fish survival reached 90.00% [45].

4.3 Immunostimulant Activity

Galangal not only has antibacterial and antifungal activity, it also has activity as an immunostimulant which can boost the body's immune system. The class of flavonoid compounds, namely quercetin compounds play a role in increasing the body's immunity and controlling the performance of the body's immunity. Aside from being an immunostimulant, quercetin also exhibits strong antioxidant effects. The results of the study proved that significantly the glycosides in quercetin in galangal could significantly increase the titer or the number of antibodies in rat test animals. Another role of glycosides is to clean carbon and reduce hypersensitivity responses. Apart from quercetin, another class of flavonoids contained in galangal, which has the effect of increasing the body's immunity, is galangin. The immunity-boosting effect on galangal rhizomes is also shown by the content of polysaccharides which dissolve in hot water [46]. hot water polysaccharide extract from *A. galanga* L. has immunostimulant activity, shown by an increase in the number of peritoneal exudate (PEC) cells, and rat spleen cells. The mechanism of action of galangal on the body's immunity is through macrophage activation and increasing the proliferation of T lymphocyte cells which play a role in the body's defense system [47].

The in vivo test results showed that galangal extract could not only increase the resistance of catfish larvaeto pathogenic bacteria, but also increase the resistance of catfish eggs to pathogenic bacteria and hatching rate of eggs. Fertilized catfish eggs were treated with ethanol extract of galangal rhizome by immersion at the time of hatching with concentrations of 600, 800 and 1,000 ppm respectively, and each treatment was infested with *Saprolegnia* spp., *A. hydrophyla* and *Pseudomonas* sp., resulting in a higher hatchability compared to the control, which was 76.30 -97.16%. Eggs immersed in 800 ppm extract solution and infested with *Saprolegnia* sp. produced the best hatchability in all treatments, followed by *A. hydrophyla* and

Pseudomonas sp., with respective values of 97.21 ± 1.93 , 89.54 ± 3.12 and 82.84 ± 3.76 %, while the control respectively 52.63 ± 2.98 , 60.19 ± 8.49 and 58.41 ± 1.77 %. The larval viability test was carried out using ethanol extract of galangal with a concentration of 800 and 1,000 ppm by immersion for 30 minutes, then each treatment was challenge tested with *A. hydrophyla*, *Pseudomonas* sp. (density 10^6 CFU/mL), and *Saprolegnia* spp., then the larvae were reared for 3 weeks. The results obtained showed the same results as egg hatchability, where the best larval viability was also obtained from those challenged with *Saprolegnia* spp., following *A. hydrophyla* and *Pseudomonas* spp. [48]. *A. galanga* extract is more effective in preventing the fungus *Saprolegnia* sp. compared to preventing bacteria in catfish larvae. However, the viability of catfish larvae challenged with *A. hydrophyla* was higher than that challenged with *Pseudomonas* sp. The best viability of catfish larvae was successively using the ethanol extract of *A. galanga* rhizome, then the rhizome water extract of *A. galanga*, the ethanol extract of *A. galanga* leaves, and the lowest viability using the aqueous extract of *A. galanga* leaves [48]. The ethanol extract of *A. galanga* rhizome produced a zone of inhibition of 12.33 mm for *A. hydrophyla*, for *Pseudomonas* sp. of 12.00 mm, and for *Saprolegnia* spp. produced the largest diameter of the inhibition zone, which was 12.67 mm [49]. The ethanol extract of *A. galanga* showed the strongest inhibitory effect against *Staphylococcus aureus* [50]. Observation of clinical symptoms after administration of the extract and before the challenge test showed that treatment using *A. galanga* was safe and did not cause death and specific clinical symptoms. Anatomical pathological observations were made on negative controls after being infected with microbes it was revealed that the larvae looked weakened and lethargic, hemorrhagic on the head, with tail deformity. Catfish larvae infected with *Saprolegnia* sp. on the surface of the skin and tail it looks like white or gray cotton sticks [48].

5. CONCLUSION

Based on the above studies, galangal rhizome is potential and effective for maintaining fish health, because the antibacterial properties of galangal rhizome can treat several types of as *Cyprinus carpio*, *Oreochromis niloticus* and *Clarias gariepinus* which are infected with the pathogenic bacteria *Aeromonas hydrophila*. In vitro test results of galangal rhizome are bacteriostatic

against pathogenic bacteria in fish, namely *Edwardsiella tarda*. The antifungal properties of galangal rhizomes can treat *Oreochromis niloticus*, *Kryptopterus bicirrhis*, *Osphronemus gouramy* and *Borbonymus gonionotus* fish from attack by the fungus *Saprolegnia* sp. As an immunostimulant, galangal rhizome is able to prevent eggs and larva of catfish from being attacked by bacteria *Saprolegnia* sp., *pseudomonas* sp. and *A. hydrophila*.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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